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ORIGINAL INVESTIGATION

Cannabidiol potentiates Δ^9-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats

Charlotte Klein · Emily Karanges · Adena Spiro · Alexander Wong · Jarrah Spencer · Thanh Huynh · Nathan Gunasekaran · Tim Karl · Leonora E. Long · Xu-Feng Huang · Kelly Liu · Jonathon C. Arnold · Iain S. McGregor

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Abstract
Rationale The interactions between Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) during chronic treatment, and at equivalent doses, are not well characterised in animal models.
Objectives The aim of this study is to examine whether the behavioural effects of THC, and blood and brain THC levels are affected by pre-treatment with equivalent CBD doses.
Methods Adolescent rats were treated with ascending daily THC doses over 21 days (1 then 3 then 10 mg/kg). Some rats were given equivalent CBD doses 20 min prior to each THC injection to allow examination of possible antagonistic effects of CBD. During dosing, rats were assessed for THC and CBD/THC effects on anxiety-like behaviour, social interaction and place conditioning. At the end of dosing, blood and brain levels of THC, and CB_1 and 5-HT_{1A} receptor binding were assessed.
Results CBD potentiated an inhibition of body weight gain caused by chronic THC, and mildly augmented the anxiogenic effects, locomotor suppressant effects and decreased social interaction seen with THC. A trend towards place preference was observed in adolescent rats given CBD/THC but not those given THC alone. With both acute and chronic administration, CBD pre-treatment potentiated blood and brain THC levels, and lowered levels of THC metabolites (THC-COOH and 11-OH-THC). CBD co-administration did not alter the THC-induced decreases in CB_1 receptor binding and no drug effects on 5-HT_{1A} receptor binding were observed.
Conclusions CBD can potentiate the psychoactive and physiological effects of THC in rats, most likely by delaying the metabolism and elimination of THC through an action on the CYP450 enzymes that metabolise both drugs.

Keywords THC · Cannabidiol · Cannabis · Adolescent · Anxiety · Reward · Pharmacokinetics

C. Klein · E. Karanges · A. Spiro · A. Wong · J. Spencer · T. Huynh · N. Gunasekaran · J. C. Arnold · I. S. McGregor (✉)
Schools of Psychology and Department of Pharmacology,
University of Sydney,
Brennan MacCullum Building,
Sydney, NSW 2006, Australia
e-mail: iain.mcgregor@sydney.edu.au

T. Karl · L. E. Long
Neuroscience Research Australia,
University of New South Wales,
Hospital Road,
Randwick, NSW 2031, Australia

X.-F. Huang · K. Liu
Centre for Translational Neuroscience, University of Wollongong,
Northfield Avenue,
Wollongong, NSW 2522, Australia

Introduction

Cannabis sativa has been used as a medicine, for religious ceremonies and recreational purposes for many thousands of years (Mechoulam 1986). At least 80 compounds deriving from the cannabis plant are recognised as cannabinoids, although the psychotropic effects of the plant are thought to be largely due to Δ^9-tetrahydrocannabinol (THC) activating cannabinoid (CB_1) receptors in the brain. As well as being the world's most popular illicit recreational drug, THC has been used clinically since the 1980s for suppression of nausea and vomiting during chemotherapy and stimulation of appetite in AIDS patients (Robson 2005; Pertwee and Thomas 2007).

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Another important cannabis constituent, cannabidiol (CBD), lacks significant psychoactive effects and has low affinity for CB_1 receptors. However, CBD has complex actions at other cannabinoid-related targets: it is an inverse agonist at cannabinoid CB_2 receptors, an antagonist at the putative cannabinoid receptor GPR55 and also inhibits the inactivation of the endocannabinoid anandamide (Pertwee 2008; Izzo et al. 2009). Recent work indicates that antidepressant, anxiolytic and neuroprotective effects of CBD can be blocked by the 5-HT_{1A} receptor antagonist WAY100635 (Mishima et al. 2005; Hayakawa et al. 2007; Campos and Guimaraes 2008; Resstel et al. 2009; Alves et al. 2010; Zanelati et al. 2010).

As well as having therapeutic potential in its own right (Pertwee and Thomas 2007), CBD can modulate the functional effects of THC with a general consensus that CBD antagonises THC actions when given at appropriate doses (Thomas et al. 2007; Pertwee 2008; Izzo et al. 2009). Such antagonistic effects are evident in rodent models; thus, CBD attenuated the effects of THC on operant behaviour (Zuardi et al. 1981), conditioned place aversion (Vann et al. 2008) and social interaction (Malone et al. 2009), and produced anxiolytic effects when given alone (Guimaraes et al. 1990; Moreira et al. 2006; Long et al. 2010). CBD also reduced THC-induced anxiety in humans (Karniol et al. 1974; Zuardi et al. 1982), enhanced its pleasurable effects (Karniol et al. 1974) and attenuated the induction of psychotic-like symptoms by THC (Bhattacharyya et al. 2010). *C. sativa* plants can vary enormously in their relative CBD/THC content with high potency varieties generally showing high THC and low, or negligible, CBD content (Potter et al. 2008; Morgan et al. 2010a). Interestingly, individuals showing only THC in their hair (rather than THC in combination with CBD) showed higher levels of positive schizophrenia-like symptoms (Morgan and Curran 2008) and those smoking varieties with higher CBD content appear to show less cognitive impairment when intoxicated (Morgan et al. 2010b). Such results suggest that CBD can reduce adverse THC-induced events and perhaps augment its clinical efficacy (Pertwee 2004; Russo and Guy 2006). It is notable that Sativex® (GW Pharmaceuticals, UK), a cannabis-based therapeutic approved for the treatment of pain and spasticity (Kmietowicz 2010), is specifically formulated to contain equivalent concentrations of CBD and THC.

However, studies examining the interactions between THC and CBD do not always show antagonism. In rats, an augmented depression of motility as well as an intensified short-term decrease in food and water intake and body temperature was seen when CBD was added to THC (Fernandes et al. 1974). In other more recent studies, CBD either had few effects on THC actions in rodents (Varvel et al. 2006) or dose-dependently exacerbated THC effects on locomotor activity, rectal temperature and spatial memory (Reid and Bornheim 2001; Hayakawa et al. 2008). Prolongation and enhancement of THC psychological and cardiovascular effects by CBD was also reported in one human study (Hollister and Gillespie 1975), while similarity of THC and Sativex® (CBD/THC) cardiovascular and subjective effects were reported in a recent human study (Karschner et al. 2011a, b).

Part of this ambiguity may reflect widely disparate dose ratios of THC/CBD used in different studies, dose ordering effects, differences in chronicity of treatment (e.g. acute versus repeated administration) as well as the specific behavioural or physiological endpoints of interest (Karniol and Carlini 1973; Reid and Bornheim 2001). CBD may inhibit hepatic microsomal drug metabolism (Fernandes et al. 1973; Bornheim and Correia 1989), and this may lead to increased circulating THC levels in the blood of rodents (Jones and Pertwee 1972; Bornheim et al. 1995; Reid and Bornheim 2001; Varvel et al. 2006) and humans (Nadulski et al. 2005). This provides a mechanism for the possible exacerbation of THC effects by CBD.

To further investigate this issue, the current study treated male adolescent rats chronically with increasing doses of THC, with or without equivalent CBD pre-treatment 20 min before the THC. Over the 21-day drug administration period, anxiety-like behaviours were examined using the emergence test and elevated plus maze (EPM), social behaviours using the social interaction test and drug reward/aversion using a place conditioning paradigm. Pharmacokinetic interactions were probed by assessing brain and blood cannabinoid concentrations after acute and chronic treatment. CB_1 and 5-HT_{1A} receptor binding were also assessed to determine whether neuroadaptations in these receptors resulting from chronic THC treatment (Breivogel et al. 1999; Zavitsanou et al. 2010) were modulated by CBD. We believe this may be the first study in which THC has been given chronically with or without CBD in rats, and this may provide some improved insight into real-life situations such as heavy cannabis use in juveniles or chronic medicinal treatment with cannabis extracts such as Sativex®.

Materials and methods

Subjects

A total of 48 experimentally naïve male Australian Albino Wistar (AAW) rats (Animal Resource Centre, Perth, Australia) were given chronic adolescent cannabinoid treatment. At the start of the experiment, the rats were postnatal day (PND) 33 to 39 with a weight range from 130 to 200 g. This age range corresponds to early adolescence in the rat (Spear 2000).

A further 16 male AAW rats (Animal Resource Centre) were used to assess the effects of acute CBD pre-treatment on blood THC levels. On testing day, they were aged 54–60 days and weighed between 270 and 360 g, matching the age at which half of the 48 chronically treated rats above were sacrificed for blood and brain THC determination after chronic THC or CBD/THC treatment.

All rats were group-housed with eight rats per cage in a temperature- and humidity-controlled colony room and maintained on a reverse 12-h light/12-h dark cycle. Experimental sessions took place during the dark cycle. Rats had ad libitum access to water and rodent lab chow. All procedures were approved by the University of Sydney Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Drug preparation and administration

Δ^9-Tetrahydrocannabinol and cannabidiol (THC Pharm GmbH, Frankfurt/Main, Germany) were dissolved in absolute ethanol before being added to an equal amount of Tween 80 and diluted in 0.9% saline to give a final stock of ethanol/Tween 80/saline (1:1:18). THC and CBD were administered intraperitoneally (i.p.) at doses of 1, 3 and 10 mg/kg. All drugs were freshly prepared before being used at an injection volume of 1 ml/kg.

The adolescent rats were treated daily with vehicle+vehicle (VEH group, n=16), vehicle+THC (THC group, n=16) or CBD+THC (CBD/THC group, n=16) for 21 consecutive days. The starting dose of both drugs was 1 mg/kg, which was increased to 3 mg/kg after 7 days and to 10 mg/kg after a further 7 days (see Table 1). A 20-min interval separated the injections of CBD and THC to better assess possible antagonistic effects of CBD.

The separate cohort of adult rats used to examine acute cannabinoid treatment received either VEH (n=8) or CBD (3 mg/kg, n=8) followed 20 min later by THC (3 mg/kg).

Experimental procedures

The behavioural testing sequence is shown in Table 1 and used procedures derived from our previous study of chronic THC effects in adolescent rats (Quinn et al. 2008). Rats were tested for anxiety-like behaviours during week 1 (daily 1 mg/kg dosing) and week 2 (daily 3 mg/kg dosing) using the emergence, elevated plus maze (EPM) and social interaction tests. Place conditioning was undertaken in week 3 during which rats received daily 10 mg/kg doses. All behavioural testing took place during the dark cycle between the hours of 10.00 and 16.00 h.

On each day, rats were given two injections, the first one either CBD or VEH, and the second one either THC or VEH, which was given 20 min later. Rats usually underwent behavioural testing 20 min after the second injection, except during the place conditioning phase when rats were either injected prior to testing or afterwards (see detailed description below). Body weights of the rats were recorded daily throughout the drug administration period.

After 21 days of dosing and a further 24-h washout period, 24 of the 48 chronically treated rats were decapitated and trunk blood was collected. The remaining 24 chronically treated rats were maintained in their home cages for a further 21 weeks to assess possible residual effects of chronic cannabinoid treatment on body weight.

The acutely dosed adult rats (n=16) were given a single injection of either CBD or VEH followed by a second injection of THC 20 min later. After a further 30 min, these rats were decapitated and their trunk blood collected.

Behavioural testing

Emergence test On their first (1 mg/kg) and eighth (3 mg/kg) day of treatment, rats were tested in the emergence test (see Table 1) 20 min after THC or VEH treatment (corresponding to 40 min after CBD or VEH pre-treatment). The emergence test involved a 120×120×60 cm wooden arena with three white melamine walls and one black wall. A wooden hide box (40×24×17 cm), with a hinged red Perspex lid and an opening in the front, was placed centrally against the black wall. Two spotlights with 150-W PAR-78 globes illuminated the open field. Each trial was recorded using a video camera mounted above the arena. Behaviour was automatically tracked and scored using TRACKMATE version 1.0 software (MotMen Ltd, Cooks Hill, NSW, Australia). Scored behaviours included time spent in the open field, time spent in the hide box, risk assessment (defined as front paws, head and back protruding from the hide box) and distance travelled. The trials spanned 5 min, with the rat placed in the hide box at the onset of testing. The open field and hide box were wiped between trials with 30% ethanol.

Elevated plus maze On their second (1 mg/kg) and ninth (3 mg/kg) treatment day, rats were placed in the EPM for 5 min (see Table 1), again 20 min after THC or VEH treatment. The apparatus consisted of two open and two closed 50×11 cm arms arranged in a cross-elevated position, 60 cm off the ground. All arms were constructed from red Perspex with black plywood floor. The closed arms had 41-cm-high red Perspex walls on three sides. The test was performed in a dark room illuminated by infrared light emitting diodes (LEDs). A video camera mounted above the apparatus was connected to a computer for

Table 1 Behavioural testing sequence

Treatment day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
(Dose)	1 mg/kg							3 mg/kg							10 mg/kg						
Emergence	√							√													
Elevated plus maze		√							√												
Social interaction					√	√						√	√								
Injections only			√	√			√			√	√										
CPP baseline[a]														√							
CPP conditioning (drug)															√		√		√		
CPP conditioning (no drug)[a]																√		√		√	
CPP test[a]																					√

[a] On these days, rats (n=48; 16 per group) were injected 1 h after being run in the CPP boxes. On all other days, rats received their two injections at 40 and 20 min prior to testing

automatic scoring by TRACKMATE software. Scored behaviours included percentage of time spent in the open arms, total number of entries into the open arm, risk assessment (defined as when the head of the rat protruded into the open arm area while the body of the rat remained in the closed arm) and the time spent engaged in activity (time active). The arms and walls were wiped down between trials with 30% ethanol.

Social interaction On days 5–6 (1 mg/kg) and 12–13 (3 mg/kg) of treatment, rats were assessed in the social interaction test (Table 1). The experimental chamber was a 120×120×60 cm arena with four black walls. The testing room was illuminated by a 40-W red lamp. A video camera was mounted above the apparatus and connected to a computer for automatic scoring of social behaviour by TRACKMATE SOCIAL software (MotMen Ltd). The program automatically scored a variety of social behaviours including following, adjacent lying, anogenital sniffing and head-to-head interactions. These separate components were accumulated to produce an overall social interaction score. The program also produces a measure of distance travelled that reflected the average distance travelled by the two rats. Each rat was tested for 10 min with a treatment-matched novel conspecific, of approximately the same body weight, but from a different home cage. There were eight pairs for each treatment condition, and each rat was run on two consecutive days for each dose (with a different conspecific), to give 16 data points per treatment condition for each dose. Between trials, the arena was wiped with 30% ethanol.

Place conditioning The place conditioning paradigm involved equipment as previously described (Quinn et al. 2008), which consisted of four identical chambers (Med Associates, St Albans, VT, USA), each with two side compartments (28×21×21 cm) and a centre compartment (12×21×21 cm). Guillotine doors were placed at both entries to the centre to restrict access when required. The two side compartments differed texturally and visually. The left compartment had black and white striped walls, a grid mesh floor and had additionally 0.5 ml of white vinegar (No Frills, Australia) in the waste pan below the floor. The right side had black walls and a metal rod floor but no olfactory cue. Two infrared-sensitive miniature video cameras with infrared illuminating LEDs (Jaycar Ltd, Australia, model QC3468) were suspended above the left and right sides of each box. Cameras were connected via a quad splitter to a PC running TRACKMATE software, which recorded time spent in each chamber as well as distance travelled during the experimental session.

All testing was performed in a dark room illuminated by a 60-W red light. The experiment had three phases: baseline (day 1), conditioning (days 2–7) and test (day 8). For baseline, rats were allowed to freely explore all compartments of the chamber for 20 min, with time spent in each compartment recorded. On that day, the rats were not injected with drugs until the baseline session was completed. Half of the rats in each treatment condition were randomly allocated to the striped compartment for drug conditioning and the other half in the black compartment.

During the conditioning phase, rats were first injected and placed into the designated drug-associated compartment after 20 min on days 2, 4 and 6 (‘drug days’). On days 3, 5 and 7 (‘no drug days’), rats were placed in the non-drug-associated compartment and were injected 60 and 80 min after (rather than 40 and 20 min before) the conditioning session. During the conditioning sessions, the connecting doors were closed and rats could not move to the other compartments. Between trials, the waste pans were washed with hot water and each time the olfactory cue was re-applied onto the waste pans of the striped compartment. On test day, rats were allowed to freely move inside the apparatus for 20 min, with time spent in each compartment recorded. They did not receive drug injections that day until the test session was completed.

Euthanasia and tissue collection

At 24 h following the final drug dosing, half (n=24) of the chronically treated rats were decapitated via guillotine. The remaining rats (n=24) were monitored for recovery from body weight loss for a further 21 weeks. For the 16 acutely treated rats, decapitation took place 30 min following their second (THC) injection.

Immediately after decapitation, trunk blood was collected in ice-chilled EDTA coated vacutainer tubes and stored at −20°C for later analysis. Brains were rapidly removed, placed in plastic jars and snap frozen in liquid nitrogen before being stored in a −80°C freezer prior to sectioning for autoradiography.

Detection and quantification of THC and THC-COOH in whole blood and brain samples

The methods used for determining blood and plasma THC, THC-COOH, 11-OH-THC and CBD were as described previously with slight modifications (Nadulski et al. 2005; Quinn et al. 2008; Gunasekaran et al. 2009; Karschner et al. 2011a). A total of 50 μl of (deuterated) D3-CBD/D3-THC/D3-11-OH-THC/D3-THC-COOH (0.25:0.25:0.5:0.5 mg/L) internal standard solution was added to each 0.5 ml sample of whole blood or brain homogenate. Calibration standards were prepared by spiking drug-free sheep blood or drug-free brain homogenates for blood and brain analyses, respectively, at concentrations of 2.5–100 ng/ml for CBD and THC and 5–200 ng/ml for 11-OH-THC and THC-COOH. The standards were vortexed and treated identically to other samples.

For brain analyses, half brains were homogenised in 0.9% saline at a 1:2 ratio (w/v). Brain cannabinoids were extracted using 0.2 M sodium hydroxide (pH 13) and acetonitrile. All samples were then placed on a blood roller for 20 min and centrifuged at 3,000 rpm for 5 min. Brain samples were dried under nitrogen stream for 90 min. Following this, brain and blood samples were treated identically, where blood and brain cannabinoids were extracted using 0.1M pH 4.0 Acetate buffer and 1-chlorobutane, placed on a blood roller for 20 minutes, then centrifuged at 3,000 rpm for 2 min and dried under a nitrogen gas stream. The samples underwent derivatisation of the polar functional groups (COOH, OH) with bis(trimethylsilyl)trifluroacetamide (BSTFA). Quantification (2.5 ng/ml limit of quantification) of the derivatised extract was performed by gas chromatography–mass spectrometry (GC–MS) (Shimadzu 2010Plus system) using electron impact ionisation in selective ion mode.

Detection and quantification of CB_1 and 5-HT_{1A} receptor density

Coronal sections (20 μm) of the forebrain were cut at −16°C on a Microm cryostat and mounted onto polysine-coated slides (LabServ, Australia) which were then stored at −80°C. Cannabinoid CB_1 receptor binding was performed as previously described (South and Huang 2008). Briefly, slides were air-dried, then pre-incubated for 30 min in 50 mM Tris–HCl buffer (pH 7.4) containing 5% bovine serum albumin (BSA) at room temperature. Sections were incubated for 120 min with 10 nM [H^3]CP-55940 (168 Ci/mmol; Perkin Elmer, Boston, MA, USA) in 50 mM Tris–HCl buffer (pH 7.4) containing 5% BSA to determine total binding, and non-specific binding was determined by incubating subsequent sections in 10 nM [H^3]CP-55940 in the presence of 10 μM CP-55940. Sections were washed in 50 mM Tris–HCl buffer (pH 7.4) containing 1% BSA at 4°C for 60 min, repeated in fresh buffer for a further 180 min. Sections were then washed in 50 mM Tris–HCl buffer for 5 min at 4°C, dipped in cold milliQ H_2O to remove buffer salts and gently dried in a stream of cool air.

5-HT_{1A} receptor binding was performed based on that described previously (Han et al. 2009). Brain sections were warmed to room temperature and pre-incubated in 50 mM [H^3]WAY-100635 (specific activity 83.0 Ci/mmol; Amersham Biosciences UK Limited) at room temperature for 2.5 h in 50 mM Tris–HCl (pH 7.4) containing 10 μM pargyline (Sigma). Non-specific binding was determined by incubating consecutive sections exposed to 10 μM 5-HT. All sections were washed for 2 min and then 3 min in ice-cold 50 mM Tris–HCl buffer. After a brief rinse in ice-cold distilled water, the slides were rapidly dried under a stream of cold air. The slides were exposed to Kodak BioMax MR films, which were then developed and quantified using standard procedures. All films were analysed using a computer-assisted image analysis system, 'Multi-Analysis', connected to a GS-690 imaging Densitometer (Bio-Rad, USA).

Statistical analysis

A mixed-model ANOVA was used for analysis of body weights over the 21 drug treatment days with treatment as the between-subjects factor and day as the within-subjects factor. Body weight on individual days, test data obtained from anxiety, social interaction and place conditioning tests and receptor density were analysed by one-way ANOVA with treatment as the between-subjects factor. When a significant overall effect of treatment was observed, pairwise comparisons using Bonferroni tests directly compared the treatment groups, either alone or combined, against the VEH group. For analysis of locomotor activity during the conditioning phase of the place conditioning paradigm, a two-way repeated measures ANOVA was used, with treatment and day as the between-subjects factors. An unpaired t test was used to compare THC and THC-COOH concentrations in blood and THC and 11-OH-THC in brains between the THC and CBD/THC treatment groups. For autoradiography, binding density in each delineated

brain area was analysed using a one-way ANOVA followed by post hoc Bonferroni tests where applicable. All analysis was conducted using SPSS 14.0 for Windows with the level of significance set at 0.05.

Results

Body weight

The body weight data are shown in Fig. 1. ANOVA revealed an overall treatment by time effect on body weight over the 21-day drug administration period [$F(2,42)=14.98$, $p<0.001$]. Pairwise comparison of groups 24 h following the final drug injection (day 22) revealed that both the CBD/THC and THC-treated rats weighed less than controls on this day (Fig. 1). Pairwise comparisons also showed that body weight loss relative to controls became significant on day 10 of treatment in the CBD/THC condition ($p<0.05$) and day 14 of treatment ($p<0.05$) in the THC condition.

For the 24 chronically treated rats given a washout of 21 weeks after drug treatment, pairwise comparisons showed that CBD/THC-treated rats still weighed significantly less than VEH rats at the end of this washout ($p<0.05$). CBD/THC-treated rats also showed a non-significant trend towards reduced body weight compared to THC only treated rats ($p=0.09$) (Fig. 1, insert).

Emergence test

The effects of THC and CBD/THC treatment in the emergence test are shown in Fig. 2. ANOVA revealed an overall effect of treatment on hide time at both 1 mg/kg [$F(2,45)=3.40$, $p<0.05$] and 3 mg/kg doses [$F(2,45)=4.64$, $p<0.05$]. Both doses of CBD/THC significantly prolonged the time rats stayed in the hide box (1 mg/kg, $p<0.05$; 3 mg/kg, $p<0.05$) compared to VEH rats, but not compared to THC rats.

The 1 mg/kg drug treatments had no overall effect on risk assessment [$F(2,45)=1.79$; $p=0.178$] or open field time [$F(2,45)=2.41$; $p=0.10$]. However, the 3 mg/kg treatments had a significant overall effect on risk assessment [$F(2,45)=6.50$; $p<0.01$] and open field time [$F(2,45)=4.40$, $p<0.05$]. Bonferroni tests showed that an effect on risk assessment was apparent in both treatment groups at 3 mg/kg [THC ($p<0.01$) and CBD/THC ($p<0.05$)] relative to VEH treatment, but only CBD-pre-treated rats spent significant less time in the open field ($p<0.05$).

An overall effect of both doses was observed in travelled activity [1 mg/kg—$F(2,45)=3.55$, $p<0.05$; 3 mg/kg—$F(2,45)=4.18$, $p<0.05$], with pairwise comparisons showing only rats from the CBD/THC group travelling less distance than VEH rats (1 mg/kg—$p<0.05$; 3 mg/kg—$p<0.05$).

Elevated plus maze

The EPM results are shown in Fig. 3. A one-way ANOVA showed an overall treatment effect on percent open arm time for both 1 mg/kg [$F(2,45)=3.26$, $p<0.05$] and 3 mg/kg doses [$F(2,45)=7.42$, $p<0.01$]. At the 1 mg/kg dose, Bonferroni tests revealed a trend towards a difference between CBD/THC group and VEH rats ($p=0.052$). At the 3 mg/kg dose, both THC-treated ($p<0.05$) and CBD/THC-treated ($p<0.01$) rats spent significantly less time on the open arm compared to VEH controls.

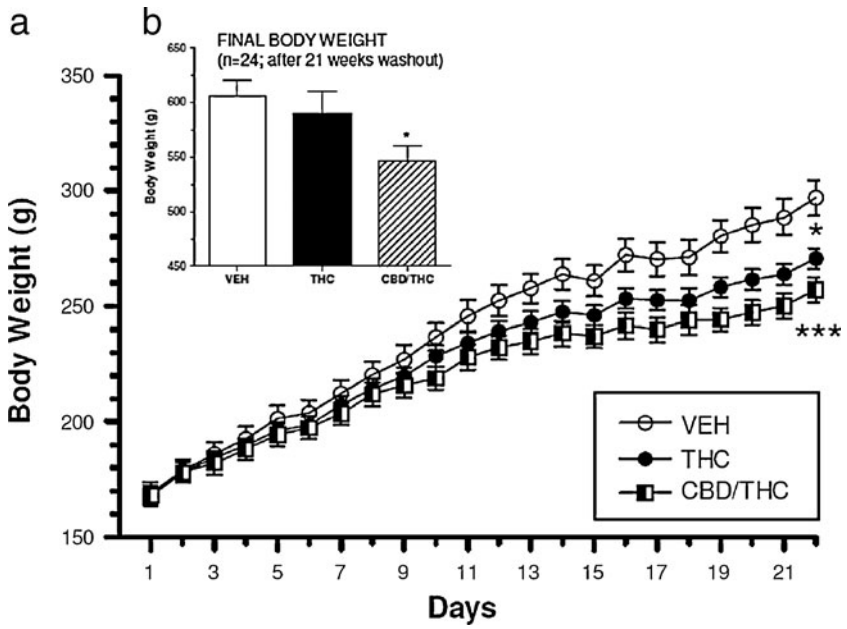

Fig. 1 *Main graph* (**a**) body weight (g±SEM) of the treatment groups ($n=16$ per condition) across the 21 days of drug administration days plus 24-h washout. On the washout day, both the CBD/THC and THC groups showed significantly lower body weight than the VEH group. *Insert graph* (**b**) final body weight (g±SEM) of the treatment groups ($n=8$ per group) after 21 weeks of washout: at this stage, only the CBD/THC group had significantly lower body weight than the VEH group. *$p<0.05$, ***$p<0.001$ relative to VEH group

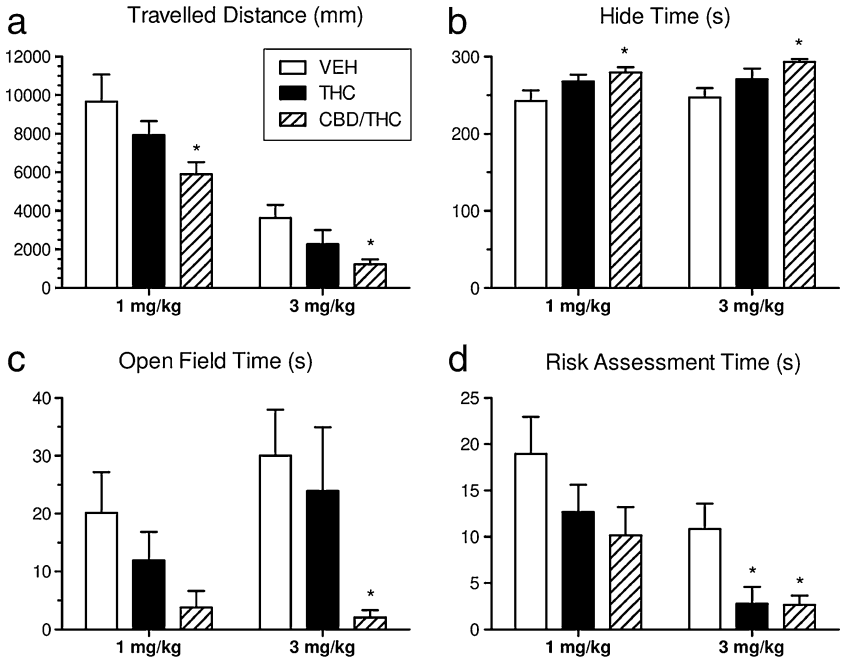

Fig. 2 Results from the emergence test (mean±SEM), showing for each treatment group (n=16): **a** travelled distance, **b** time spent in hide box, **c** time spent in open field and **d** time spent in risk assessment. The 1 mg/kg test was conducted on day 1 of the experiment while the 3 mg/kg test was conducted on day 8. *p<0.05 relative to VEH group

An overall treatment effect was seen with the higher (3 mg/kg) doses in time spent in risk assessment [F(2,45)= 12.94, p<0.001] as well as in number of open arm entries [F(2,45)=11.38, p<0.001]. Rats of both treatment groups significantly differed from the VEH group in time they spent in risk assessment (THC, p<0.01; CBD/THC, p< 0.001) and in number of open arm entries (THC, p<0.01; CBD/THC, p<0.001), with generally more pronounced effects in CBD/THC rats. The 1 mg/kg dose did not affect risk assessment overall [F(2,45)=2.13; p=0.13] or the number of open arm entries [F(2,45)=2.529, p=0.091]. An overall effect on time active was only observed at 3 mg/kg [F(2,45)=5.40, p<0.01], with both CBD/THC (p<0.05) and THC rats (p<0.05) spent less time active than controls.

Social interaction test

Results from the social interaction test are presented in Fig. 4. The 3 mg/kg [F(2,45)=12.73, p<0.001] but not the

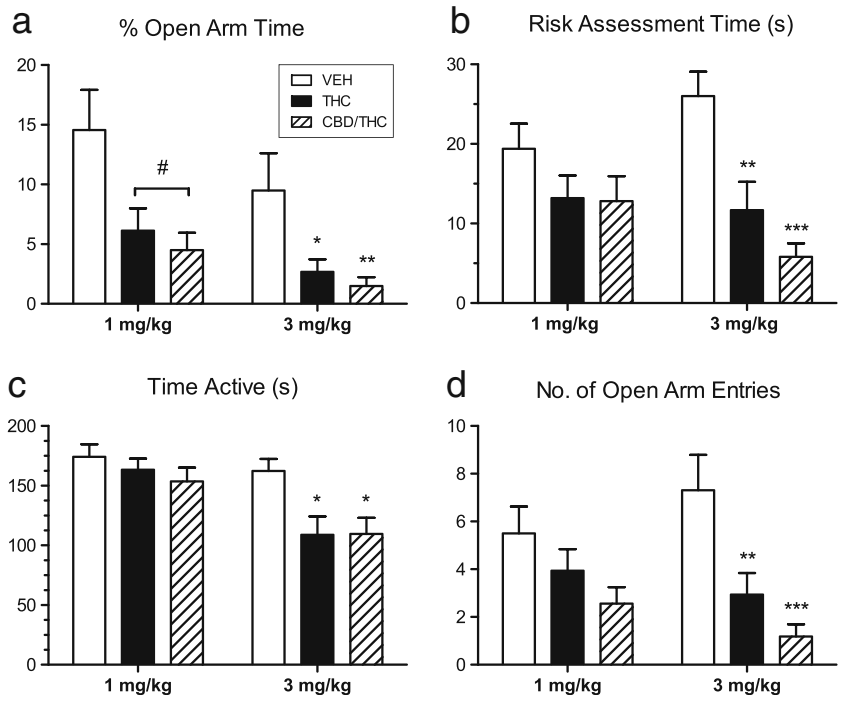

Fig. 3 Results from the elevated plus maze (mean±SEM), showing for each treatment group (n=16): **a** time spent on open arm as percentage of total time, **b** time spent in risk assessment, **c** time active and **d** number of open arm entries. The 1 mg/kg test was conducted on day 2 of the experiment while the 3 mg/kg test was conducted on day 9. #p<0.05, overall drug effect with combined CBD/THC and THC groups compared to VEH group, *p<0.05, **p<0.01, ***p< 0.001 for THC or CBD/THC group relative to VEH group

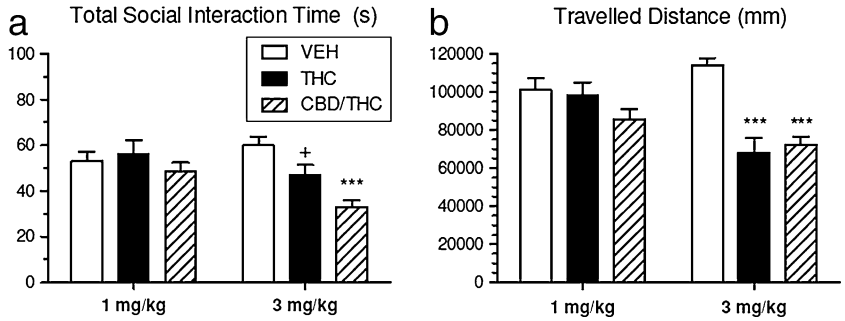

Fig. 4 Results from the social interaction test (mean±SEM), showing for each treatment group (n=16): **a** time spent in total social interaction and **b** distance travelled. +p<0.05, THC compared to CBD/THC group, ***p<0.001, THC and CBD/THC compared to VEH group. Data represent two social interaction tests for each rat (with a different conspecific) on two consecutive days for each dose (1 mg/kg tested on days 5–6 and 3 mg/kg tested on days 12–13)

1 mg/kg [F(2,45)=0.665, p=0.52] dose had an overall effect on social interaction. Pairwise comparisons revealed a significant reduction in social interaction in the CBD/THC group (p<0.001), but only a trend towards this effect in THC-treated rats (p=0.057) at 3 mg/kg. There was also an overall effect of treatment on distance travelled in the drug-treated rats at 3 mg/kg [F(2,45)=22.55, p<0.001], with both THC (p<0.001) and CBD/THC (p<0.001) reducing the travelled activity during social interaction compared to controls.

Place conditioning

The results from the place conditioning paradigm are shown in Fig. 5. On the 'drug days' during conditioning (days 2, 4 and 6—when drug was given before the rats were confined to the drug-paired compartment), there was an overall effect of treatment [F(2,45)=10.05, p<0.001] and day [F(2,45)=10.71, p<0.0001] on distance travelled. Pairwise comparisons revealed significantly reduced travelled distance in both the CBD/THC (p<0.001; p<0.001) and THC-treated groups (p<0.01, p<0.05) on days 2 and 4, but not day 6 (Fig. 5a).

On the 'no drug' days during conditioning (days 3, 5 and 7—when drug was given after the conditioning session), ANOVA showed an overall effect of treatment [F(2,45)= 5.73, p<0.01] and day [F(2,45)=19.05, p<0.0001] on distance travelled (Fig. 5b). Pairwise comparisons revealed that CBD/THC but not THC-treated rats displayed decreased travelled distance on days 3, 5 and 7 (p<0.01, p<0.05, p<0.01), 24 h after drug treatment.

On the place conditioning test day (Fig. 5d), only a trend was evident towards increased preference for the drug paired side in CBD/THC-treated rats relative to THC-treated rats (p=0.07, unpaired t test) with no significant overall treatment effect. There was an overall effect of treatment on locomotor activity on the test day [F(2,45)= 3.38, p<0.05) with CBD/THC, but not THC, rats showing a trend towards decreased travelled activity (p=0.056).

Cannabinoid levels in blood and brain

Results from the GC–MS analysis of bloods taken from acutely and chronically dosed rats are presented in Table 2. Rats acutely treated with CBD (3 mg/kg) prior to acute THC (3 mg/kg) showed higher blood THC [t(1,12)=2.46, p<0.05] and lower THC-COOH levels [t(1,13)=3.80, p< 0.01] than those treated with vehicle prior to THC. There was also a strong trend towards higher brain THC [t(1,13)= 2.01, p=0.065] and significantly lower 11-OH-THC levels [t(1,13)=3.33, p<0.01] in those pre-treated with CBD rather than with vehicle.

In the chronically treated adolescent rats, 24 h after the final dose, there was a strong trend towards higher THC [t(1,14)=1.98, p=0.068] and significantly lower THC-COOH [t(1,14)=2.41, p<0.05] blood levels in the CBD/THC group relative to the THC group. The CBD/THC rats also showed higher brain THC levels [t(1,11)=2.48, p< 0.05] compared to the THC group. There was no difference in 11-OH-THC levels between the two chronic treatments.

Brain analysis of CB_1 and 5-HT_{1A} receptor density

Table 3 presents the results for CB_1 autoradiography including results from hippocampus, substantia nigra (SN), caudate putamen (CPU) and cingulate gyrus (CG). One-way ANOVAs revealed an overall treatment effect on CB_1 receptor binding in hippocampus [F(2,16)=45.85, p< 0.0001], SN [F(2,18)=6.13, p<0.01], CPU [F(2,20)=9.92, p<0.001] and CG [F(2,20)=29.01, p<0.0001] with pairwise comparisons showing significantly decreased amounts of CB_1 receptors per square millimetre in brains of THC-treated rats compared to the VEH group (hippocampus, p< 0.0001; SN, p<0.05; CPU, p<0.01; CG, p<0.0001), and also in the CBD/THC group when compared to VEH-treated rats (hippocampus, p<0.0001; SN, p<0.05; CPU, p<0.01; CG, p<0.0001). There were no significant differences between the CBD/THC and THC groups in any region.

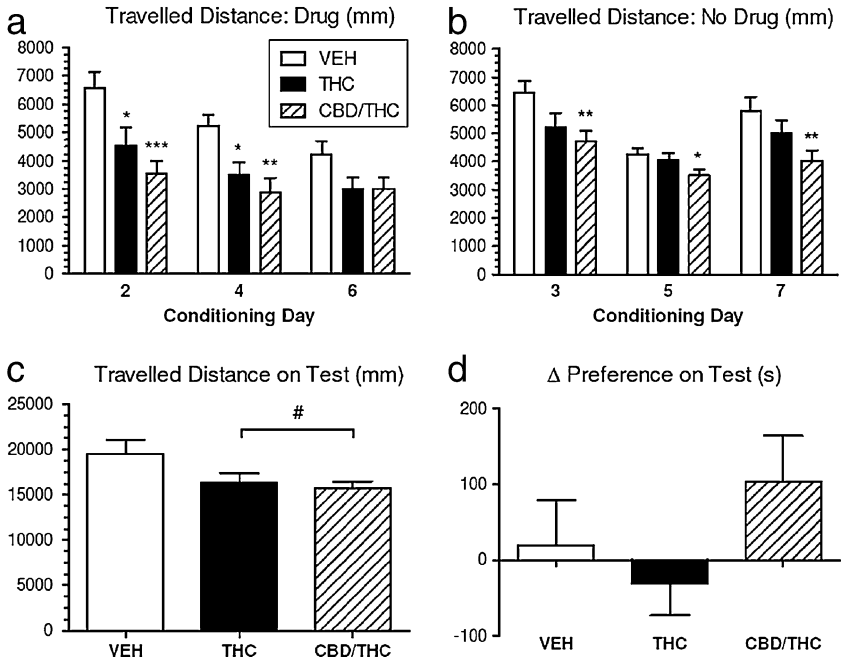

Fig. 5 Results from the place conditioning test (mean±SEM), showing for each treatment group (n=16): **a** distance travelled on conditioning days (2, 4 and 6) when drug injections (10 mg/kg THC or CBD/THC) were given prior to conditioning, **b** distance travelled on conditioning days (3, 5 and 7) when drug injections were given 1 h after conditioning ('no-drug' days), **c** distance travelled on test day (drug given after test) and **d** change in the time spent on the conditioned side from baseline to test day: a positive value represents a preference for the conditioned side and a negative value an aversion. #p< 0.05, overall drug treatment effect with combined CBD/THC and THC groups compared to VEH group; *p<0.05, **p<0.01, ***p<0.001, THC or CBD/THC relative to VEH group

In Table 4, the results from the analysis of the 5-HT_{1A} receptor binding study in hippocampus, lateral septal nucleus intermediate (LSI), lateral septal nucleus dorsal (LSD) and cingulated gyrus (CG) are summarised. No changes in receptor binding were observed in hippocampus, LSI and CG. There was a trend towards a treatment effect on 5-HT_{1A} receptor density in the LSD [F(2,19)= 2.93, p=0.078].

Discussion

The present study was designed to address some perceived ambiguity in the literature with respect to behavioural and pharmacokinetic interactions between CBD and THC and to examine how these interactions were manifested in rats exposed to chronic, incremental CBD/THC treatment. The interactions between THC and CBD are relevant given

Table 2 Blood and brain cannabinoid levels (ng/ml)

		Single treatment		21-Day treatment	
		THC	CBD/THC	THC	CBD/THC
Blood	THC	12.13 (0.94)*	24.77 (5.54)	8.61 (2.61)	16.52 (4.15)
	THC-COOH	9.79 (1.57)**	2.6 (0.97)	24.13 (9.36)*	6.92 (3.36)
	CBD	–	n/a	–	9.04 (0.86)
Brain	THC	19.56 (1.84)	29.16 (4.67)	8.9 (2.21)*	23.19 (4.95)
	11-OH-THC	29.79 (2.86)**	15.45 (3.25)	5.49 (1.25)	9.28 (3.99)
	CBD	–	20.42 (3.71)	–	1.79 (0.51)

Data represent means (SEM)

Single treatment rats (n=8 per group) acutely dosed with THC or CBD/THC (30 min before brain removal and blood collection), *21-Day treatment* rats (n=8 per group) given 21 days of THC or CBD/THC treatment and 24-h washout, *n/a* not available

*p<0.05, **p<0.01, THC compared to CBD/THC group

Table 3 CB_1 receptor binding (OD/mm^2) in specific brain regions

Condition	CB_1 receptor density (OD/mm^2)		
	VEH	THC	CBD/THC
Hippocampus	31.89 (0.34)	26.92 (0.37)***	26.97 (0.58)***
Substantia nigra	103.51 (1.6)	93.13 (1.67)*	90.31 (4.7)*
Caudate putamen	31.13 (0.45)	28.29 (0.29)**	28.78 (0.61)**
Cingulate gyrus	28.08 (0.25)	24.96 (0.3)***	24.98 (0.43)***

Data represent means (SEM) for eight rats per condition chronically treated with VEH, THC or CBD/THC

OD optical density

*$p<0.05$, **$p<0.01$, ***$p<0.001$, relative to VEH group

current concerns that widespread use of high potency hydroponic varieties of cannabis with high THC and negligible CBD (Potter et al. 2008) pose greater health risks to users than traditional varieties of cannabis that contain lower THC and substantial levels of CBD (McLaren et al. 2008; Morgan and Curran 2008; Di Forti et al. 2009; Bhattacharyya et al. 2010; Morgan et al. 2010b). Moreover, the clinical use of cannabis extracts that contain 1:1 CBD and THC ratios (Sativex®) for pain and spasticity are partly predicated on the maximal therapeutic benefits and minimal adverse psychological effects that such mixtures produce (Russo and Guy 2006). Somewhat surprisingly then, the current results suggest that pre-treatment with equivalent CBD 20 min prior to THC treatment leads to increased blood and brain THC levels and intensification of THC behavioural effects. This was seen with both acute and chronic administration of THC and CBD to rats, with moderate augmentation of THC effects by CBD evident in a range of behavioural measures as well as on body weight.

Our observations that chronic THC exposure inhibits body weight gain in adolescent rats is in accord with various reports that cannabinoid receptor agonists can inhibit consumption of moderate palatability foods (e.g. lab chow) and lead to body weight loss in rats (Sjoden et al. 1973; McGregor et al. 1996). Of interest here is the observation that pre-treatment with CBD potentiates the reduction in body weight seen with THC. The body weight loss with THC was far more evident during weeks 2 and 3 when higher THC doses were administered (3 and 10 mg/kg, respectively) so exacerbation of body weight loss by CBD may be a result of the higher and longer lasting functional amounts of THC produced in blood as a result of CBD pre-treatment. Such effects recapitulate the augmentation by CBD of THC appetite suppression in rats originally reported by Fernandes et al. (1974). Human cannabis smokers of high CBD content varieties showed reduced attentional bias to food stimuli when intoxicated relative to smokers of low CBD content cannabis, suggesting that CBD may also modulate the characteristic appetite-stimulatory effects of THC independently of pharmacokinetic factors (Morgan et al. 2010a). In addition, very recent preclinical research indicates that chronic daily treatment with CBD (2.5–5 mg/kg) alone inhibits body weight gain in Wistar rats via an action on CB_2 receptors (Ignatowska-Jankowska et al. 2011), suggesting that the effects reported here might reflect an independent action of CBD above and beyond the anorexic action of THC.

Table 4 $5\text{-}HT_{1A}$ receptor binding (OD/mm^2) in specific brain regions

Condition	$5\text{-}HT_{1A}$ receptor density (OD/mm^2)		
	VEH	THC	CBD/THC
Hippocampus	34.32 (0.76)	33.53 (0.83)	33.16 (0.47)
LS (intermediate)	32.17 (0.74)	33.29 (0.58)	33.65 (0.6)
LS (dorsal)	34.88 (0.72)	37.10 (0.5)	36.73 (0.77)
Cingulate gyrus	26.30 (0.3)	26.96 (0.31)	26.57 (0.33)

Data represent means (SEM) of eight rats per condition chronically treated with VEH, THC or CBD/THC

OD optical density, *LS* lateral septal nucleus

Also of interest in the present study is the observation that CBD/THC pre-exposure during adolescence produced rats that were significantly lower in body weight well into adulthood, 21 weeks later. This indicates a notable long-lasting residual effect of chronic adolescent CBD/THC exposure on growth and development. We have preliminary data (Gunasekaran et al., unpublished observations), in agreement with in vitro studies (Deveaux et al. 2009; Teixeira et al. 2010), that weight loss in rats given THC is accompanied by fundamental changes in the number and morphology of adipocytes and macrophage infiltration, and this may be a primary mechanism underlying the lasting body weight loss reported here.

With respect to behaviour, pre-treatment with CBD tended to increase THC-induced anxiety-like behaviours in both the emergence test and EPM, although in many cases the CBD/THC and THC groups did not differ significantly when directly compared. However, in the emergence test, only rats

pre-treated with CBD prior to THC showed significantly increased hide time (both 1 and 3 mg/kg doses) and decreased open field time (3 mg/kg dose only) relative to the VEH group. Some augmentation of THC effects by CBD was seen on the very first day of drug treatment (1 mg/kg emergence test) indicating that this augmentation does not require prolonged exposure to the drug combination, or neuroadaptations emerging during chronic treatment.

On the EPM, THC was anxiogenic both with and without CBD pre-treatment, but overall the effects on open arm time and risk assessment were numerically, if not statistically, greater when CBD was also given. The observed anxiogenic effects of THC in the emergence test and EPM are in accordance with a number of previous studies with THC (Onaivi et al. 1990; Quinn et al. 2008) or with other cannabinoid agonists such as CP 55,940 (Genn et al. 2004) and HU-210 (Guilani et al. 2000). The present results provide a notable contrast to studies in rodents and humans, where CBD itself had anxiolytic properties (Guimaraes et al. 1990; Moreira et al. 2006; Long et al. 2010) or where it reduced the anxiety associated with THC (Karniol et al. 1974; Zuardi et al. 1982).

Significant exacerbation of THC effects by CBD pre-treatment was evident in the social interaction test where significantly greater decreases in social interaction were obtained with THC (3 mg/kg) when CBD was given 20 min earlier. The observed reduction in social interaction by THC agrees with previous studies (Genn et al. 2004; Quinn et al. 2008; Malone et al. 2009), although the augmenting effect of CBD is opposite to the antagonistic effect reported with Malone et al (2009), albeit with much higher CBD/THC ratios (20:1). A modest reduction in distance travelled was also apparent in rats treated with THC (3 mg/kg) in the social interaction test. Such locomotor hypoactivity was also seen in the emergence and EPM tests in which CBD pre-treated rats exhibited markedly greater locomotor suppressant effects of THC. This agrees with some previous findings in rodents (e.g. Hayakawa et al. 2007) but differs from some previous investigations in which no effect of CBD on THC-induced hypolocomotion was evident (e.g. Varvel et al. 2006).

Since no obvious signs of catalepsy were evident in the rats tested on these models, it is unlikely that the observed results on anxiety and social behaviour models were confounded by gross motor impairment, although a confounding role of the modest hypolocomotor effects of THC on anxiety measures cannot be completely ruled out. It is also important to note that although there was no CBD only control group in the current study, other investigations suggest that it has little or no effect on its own on social interaction in rats (Malone et al. 2009), or on locomotor activity in mice (Varvel et al. 2006; Hayakawa et al. 2008; Long et al. 2010) despite clear hypolocomotor effects of THC in these studies.

Cannabinoid agonists are often found to be aversive to adult rats, as shown by the formation of a conditioned place aversion (McGregor et al. 1996; Mallet and Beninger 1998; Quinn et al. 2008). However, the current work confirms our previous findings that repeated THC causes little by way of place aversion in adolescent rats (Quinn et al. 2008). Interestingly, there was a trend towards CBD pre-treatment shifting rats towards a place preference with THC, but further work will be required to conclusively demonstrate that such an effect reliably exists in rats. This might mimic effects reported in adult mice where CBD was capable of reversing THC-induced place aversion (Vann et al. 2008). It is also worth noting that CBD given alone, in other studies, appears to have little effect on place conditioning (Parker et al. 2004; Vann et al. 2008).

At the high 10 mg/kg dose used in place conditioning, THC and CBD/THC affected the motility of the rats. Impaired locomotor activity was clearly stronger on days when the rats were injected prior to conditioning, but was still notably present in the CBD/THC treated rats on 'drug-free' days, when they were tested approximately 24 h after their previous 10 mg/kg dose of THC. Thus, CBD potentiated the THC reduction in locomotor activity both acutely, but also 24 h later. The reason for this is evident from Table 2, which shows that rats given CBD/THC combinations produced higher THC levels in both blood and brain after a 24-h washout than rats given THC alone. Indeed, blood levels 24 h after 10 mg/kg CBD/THC were similar to those seen 30 min after a single 3 mg/kg acute dose of THC to drug-naïve rats. The longevity of THC in blood following chronic administration is evident in our previous studies with rats (Gunasekaran et al. 2009), and reflects the high lipophilicity of the drug and its slow release from fat stores back into the circulation. The fact that THC was still present in substantial amounts on the 'no-drug' conditioning days may therefore have affected our ability to produce place preference or aversion: in effect rats were being asked whether they prefer an environment paired with high or low blood levels of THC. Similarly, the reduced locomotor activity seen in THC and CBD/THC rats on the final place conditioning test day may reflect substantial blood THC levels on this day and could have interfered with the expression of a place avoidance or preference on this day.

To confirm that CBD potentiates THC effects through a pharmacokinetic interaction, we acutely injected 16 rats with either THC or CBD/THC and analysed levels of THC and its major metabolites in blood and brain using GC–MS. The results clearly indicate that pre-treatment with CBD potentiates THC-induced effects in rats by delaying THC metabolism, as previously shown in mice (Reid and Bornheim 2001).

Rats acutely pre-treated with CBD (3 mg/kg) prior to THC (3 mg/kg) had higher blood THC levels and lower levels of THC-COOH than those pre-treated with vehicle. Augmenting effects of CBD pre-treatment on brain THC levels fell just short of statistical significance, but brain 11-OH-THC levels were significantly lower in the CBD/THC group. Overall, our acute results agree with the increased brain levels of THC reported in mice with CBD pre-treatment (Reid and Bornheim 2001).

Similar effects of CBD pre-treatment on THC levels were evident in the chronically treated rats and although the effect on THC in blood fell just short of significance, the effect on THC-COOH was significant. In the brain, rats repeatedly dosed with THC had significantly higher THC levels when pre-treated with CBD. Low residual levels of CBD were also detected in the brains of these chronically treated rats while no group differences in 11-OH-THC levels were detected. As the primary THC metabolite with a relatively short half-life, the 11-OH-THC was most likely already further metabolised to THC-COOH after 24 h in these rats. The fact that CBD augmentation persists even after a 24-h washout in chronically treated rats indicates a cumulative long-lasting effect of CBD on THC metabolism, rather than a temporary spike in blood and brain THC immediately after CBD injection. Presumably this reflects a long-lasting effect of CBD (or its metabolites) on hepatic enzymes responsible for THC metabolism.

CBD is known to impair hepatic microsomal drug metabolism in rodents in vitro via inactivation of specific cytochrome P450s (CYP) belonging to the CYP2C and CYP3A subfamilies (Bornheim and Correia 1990, 1991). These enzymes are responsible for phase I metabolism of cannabinoids, in which THC undergoes hydroxylation to its primary metabolite, 11-OH-THC (which retains THC-like pharmacological activity), before being further oxidised to non-psychoactive THC-COOH. Clearly, interpretation of the present results would have been improved by including groups where CBD and THC were given simultaneously, although this was not possible due to limited supplies of both drugs and our wish to use an unambiguous 'antagonist' methodology in which the proposed antagonist was administered prior to the agonist. At least one previous study has indicated that interposing a delay (15–60 min) between CBD and subsequent THC administration leads to greater intensification of THC levels than simultaneous administration (Reid and Bornheim 2001). This perhaps indicates that it is a metabolite of CBD, rather than CBD itself, that is responsible for the pharmacokinetic interaction that inhibits THC metabolism. Identification of this metabolite would clearly be a worthy aim for future studies.

To our knowledge, the present study is the first experiment to link enhancement of THC behavioural effects to elevated blood and brain THC levels after CBD pre-treatment in rats. Although we propose a hepatic, pharmacokinetic explanation for this phenomenon, we cannot exclude the involvement of other interactions. Hayakawa et al. (2008) report an exacerbation of THC-behavioural effects by CBD in mice and an enhancement of CB_1 receptor expression in the hippocampus and hypothalamus in rats given acute CBD/THC combinations. These authors suggested that CBD also potentiated pharmacological effects of THC via a CB_1 receptor-dependent mechanism. Several autoradiographic binding studies found a dose-dependent downregulation of CB_1 receptors after chronic and even after acute treatment with cannabinoids such as THC, WIN 55212-2, CP-55,940 and HU210 (Oviedo et al. 1993; Breivogel et al. 1999; Dalton et al. 2005). This effect is replicated here with repeated, incremental doses of THC. Interestingly, Oviedo et al. (1993) reported no effect of repeated daily treatment with 10 mg/kg CBD on CB_1 receptor binding, which is consistent with our present finding of no significant difference between THC and CBD/THC-treated rats in the extent of decreased CB_1 receptor binding. The fact that the chronically treated rats still had substantial THC levels in their brains at the time of sacrifice necessarily clouds the interpretation of CB_1 receptor binding results, given that the THC might compete with [H^3] CP-55,940 for CB_1 receptor occupancy. This issue could be definitively addressed in future studies by the use of B_{max} measures in a receptor binding assay, rather than by autoradiography (Oviedo et al. 1993).

While a recent study (Zavitsanou et al. 2010) noted region-specific upregulation of 5-HT_{1A} receptor binding after chronic cannabinoid treatment in rats, the current study failed to detect any such effect. This may reflect our use of adolescent rats, which appear to have higher basal 5-HT_{1A} receptor density and may be resistant to chronic cannabinoid effects on this receptor (Zavitsanou et al. 2010). Given recent indications that CBD can have potent interactions with the 5-HT_{1A} receptor (Mishima et al. 2005; Hayakawa et al. 2007; Campos and Guimaraes 2008; Resstel et al. 2009; Zanelati et al. 2010; Gomes et al. 2011), we predicted some modulation of this receptor in CBD/THC-treated rats, but this did not eventuate.

Very recent work in human participants (Karschner et al. 2011a, b) indicates few differences between orally administered THC and buccal Sativex® (CBD/THC) in terms of subjective effects, cardiovascular effects or THC pharmacokinetics observed during acute administration of these drugs. This casts some doubt on the relevance of CBD as a modulator of THC effects with respect to Sativex®. However, it is worth bearing in mind that these studies involved relatively small numbers of participants and only oral and/or buccal administration. Future studies might usefully study whether the very different kinetics observed with smoked cannabis, or injections of THC and CBD, lend themselves to more

antagonistic effects, and whether chronic administration, or delays between CBD and THC administration, are relevant to such interactions. Clearly, the emerging clinical literature suggests differences in mental health and cognitive outcomes in smokers of high CBD versus low CBD cannabis (Di Forti et al. 2009; Morgan et al. 2010a, b), and this makes further pharmacokinetic investigations of great relevance.

Overall, then, it is clear that the existing literature on CBD and THC is complex and requires further clarification. The present results in some ways add to that complexity by reinforcing earlier findings that CBD is not always a functional antagonist of THC, and can modestly potentiate the psychoactive and physiological effects of THC in rats. This potentiation most likely reflects CBD augmenting brain THC levels through a pharmacokinetic interaction involving common CYP450 metabolic pathways. Modulation by CBD of the transport systems for THC into the brain, liver or adipose tissue may be other avenues for CBD–THC interactions (Reid and Bornheim 2001). Further studies will hopefully further illuminate the range of mechanisms through which CBD modulates THC effects and their exact relevance to human use of cannabis and cannabis-based therapeutics such as Sativex®.

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References

Alves FH, Crestani CC, Gomes FV, Guimaraes FS, Correa FM, Resstel LB (2010) Cannabidiol injected into the bed nucleus of the stria terminalis modulates baroreflex activity through 5-HT1A receptors. Pharmacol Res 62:228–236

Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T, Nosarti C, O'Connel CM, Seal M, Allen P, Mehta MA, Stone JM, Tunstall N, Giampietro V, Kapur S, Murray RM, Zuardi AW, Crippa JA, Atkan Z, McGuire PK (2010) Opposite effect of delta9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology 35:764–774

Bornheim LM, Correia MA (1989) Effect of cannabidiol on cytochrome P-450 isoenzymes. Biochem Pharmacol 38:2789–2794

Bornheim LM, Correia MA (1990) Selective inactivation of mouse liver cytochrome P-450IIIA by cannabidiol. Mol Pharmacol 38:319–326

Bornheim LM, Correia MA (1991) Purification and characterization of the major hepatic cannabinoid hydroxylase in the mouse: a possible member of the cytochrome P-450IIC subfamily. Mol Pharmacol 40:228–234

Bornheim LM, Kim K, Beatrice J, Perotti Y, Benet L (1995) Effect of cannabidiol pretreatment on the kinetic of tetrahydrocannabinol metabolites in mouse brain. Drug Metab Dispos 23:825–831

Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Vogt LJ, Sim-Selley LJ (1999) Chronic delta9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. J Neurochem 73:2447–2459

Campos AC, Guimaraes FS (2008) Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology (Berl) 199:223–230

Dalton GD, Smith FL, Smith PA, Dewey WL (2005) Chronic delta9-tetrahydrocannabinol treatment produces antinociceptive tolerance in mice without altering protein kinase A activity in mouse brain and spinal cord. Biochem Pharmacol 70:152–160

Deveaux V, Cadoudal T, Ichigotani Y, Teixeira-Clerc F, Louvet A, Manin S, Nhieu JT, Belot MP, Zimmer A, Even P, Cani PD, Knauf C, Burcelin R, Bertola A, Le Marchand-Brustel Y, Gual P, Mallat A, Lotersztajn S (2009) Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. PLoS ONE 4:e5844

Di Forti M, Morgan C, Dazzan P, Pariante C, Mondelli V, Marques TR, Handley R, Luzi S, Russo M, Paparelli A, Butt A, Stilo SA, Wiffen B, Powell J, Murray RM (2009) High-potency cannabis and the risk of psychosis. Br J Psychiatry 195:488–491

Fernandes M, Warning N, Christ W, Hill R (1973) Interactions of several cannabinoids with the hepatic drug metabolizing system. Biochem Pharmacol 22:2981–2987

Fernandes M, Schabarek A, Cooper H, Hill R (1974) Modification of delta9-THC actions by cannabinol and cannabidiol in the rat. Psychopharmacologia 38:329–338

Genn RF, Tucci S, Marco EM, Viveros MP, File SE (2004) Unconditioned and conditioned anxiogenic effects of the cannabinoid receptor agonist CP 55,940 in the social interaction test. Pharmacol Biochem Behav 77:567–573

Gomes FV, Resstel LB, Guimaraes FS (2011) The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. Psychopharmacology (Berl) 213:465–473

Guilani D, Ferrari F, Ottani A (2000) The cannabinoid agonist HU 210 modifies rat behavioural responses to novelty and stress. Pharmacol Res 41:47–53

Guimaraes FS, Chiaretti TM, Graeff FG, Zuardi AW (1990) Antianxiety effect of cannabidiol in the elevated plus-maze. Psychopharmacology (Berl) 100:558–559

Gunasekaran N, Long L, Arnold JC, McGregor IS (2009) Reintoxication: the release of fat-stored delta-9-tetrahydrocannabinol (THC) into blood is enhanced by food deprivation or ACTH exposure. British Journal of Pharmacology 158:1330–1337

Han M, Huang XF, du Bois TM, Deng C (2009) The effects of antipsychotic drugs administration on 5-HT1A receptor expression in the limbic system of the rat brain. Neuroscience 164:1754–1763

Hayakawa K, Mishima K, Nozako M, Ogata A, Hazekawa M, Liu A-X, Fujioka M, Abe K, Hasebe N, Egashira N, Iwasaki K, Fujiwara M (2007) Repeated treatment with cannabidiol but not delta9-tetrahydrocannabinol has a neuroprotective effect without the development of tolerance. Neuropharmacology 52:1079–1087

Hayakawa K, Mishima K, Hazekawa M, Sano K, Irie K, Orito K, Egawa T, Kitamura Y, Uchida N, Nishimura R, Egashira N, Iwasaki K, Fujiwara M (2008) Cannabidiol potentiates pharmacological effects of delta9-tetrahydrocannabinol via a CB1 receptor-dependent mechanism. Brain Res 1188:157–164

Hollister LE, Gillespie H (1975) Interactions in man of delta9-tetrahydrocannabinol, H-cannabinol and cannabidiol. Clin Pharmacol 18:329–338

Ignatowska-Jankowska B, Jankowski MM, Swiergiel AH (2011) Cannabidiol decreases body weight gain in rats: involvement of CB2 receptors. Neurosci Lett 490:82–84

Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci 30:515–527

Jones G, Pertwee RG (1972) A metabolic interaction in vivo between cannabidiol and 1-tetrahydrocannabinol. Br J Pharmacol 45:375–377

Karniol IG, Carlini EA (1973) Pharmacological interaction between cannabidiol and delta9-tetrahydrocannabinol. Psychopharmacologia 33:53–70

Karniol IG, Shirakawa I, Kasinaki N, Carlini EA (1974) Cannabidiol interferes with the effects of delta9-tetrahydrocannabinol in man. Eur J Pharmacol 28:172–177

Karschner EL, Darwin WD, McMahon RP, Liu F, Wright S, Goodwin RS, Huestis MA (2011a) Subjective and physiological effects after controlled Sativex and oral THC administration. Clin Pharmacol Ther 89:400–407

Karschner EL, Darwin WD, Goodwin RS, Wright S, Huestis MA (2011b) Plasma cannabinoid pharmacokinetics following controlled oral delta9-tetrahydrocannabinol and oromucosal cannabis extract administration. Clin Chem 57:66–75

Kmietowicz Z (2010) Cannabis based drug is licensed for spasticity in patients with MS. BMJ 340:c3363

Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T (2010) A behavioural comparison of acute and chronic delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. Int J Neuropsychopharmacol 13:861–876

Mallet PE, Beninger PJ (1998) Delta9-tetrahydrocannabinol, but not the endogenous cannabinoid receptor ligand anandamide, produces conditioned place avoidance. Life Sci 62:2431–2434

Malone DT, Jongejan D, Taylor DA (2009) Cannabidiol reverses the reduction in social interaction produced by low dose of delta9-tetrahydrocannabinol in rats. Pharmacol Biochem Behav 93:91–96

McGregor IS, Issakidis CN, Prior G (1996) Aversive effects of the synthetic cannabinoid CP 55,940 in rats. Pharmacol Biochem Behav 53:657–664

McLaren J, Swift W, Dillon P, Allsop S (2008) Cannabis potency and contamination: a review of the literature. Addiction 103:1100–1109

Mechoulam R (1986) The pharmacohistory of *Cannabis sativa*. In: Mechoulam R (ed) Cannabinoids as therapeutic agents. CRC, Boca Raton, p 19

Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K, Fujiwara M (2005) Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. Stroke 36:1071–1076

Moreira FA, Aguiar DC, Guimaraes FS (2006) Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. Prog Neuropsychopharmacol Biol Psychiatry 30:1466–1471

Morgan CJ, Curran HV (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. Br J Psychiatry 192:306–307

Morgan CJ, Freeman TP, Schafer GL, Curran HV (2010a) Cannabidiol attenuates the appetitive effects of delta(9)-tetrahydrocannabinol in humans smoking their chosen cannabis. Neuropsychopharmacology 35:1879–1885

Morgan CJ, Schafer G, Freeman TP, Curran HV (2010b) Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study. Br J Psychiatry 197:285–290

Nadulski T, Pragst F, Weinberg G, Roser P, Schnelle M, Fronk EM, Stadelmann AM (2005) Randomized, double-blind, placebo-controlled study about the effects of cannabidiol (CBD) on the pharmacokinetics of delta9-tetrahydrocannabinol (THC) after oral application of THC verses standardized cannabis extract. Ther Drug Monit 27:799–810

Onaivi ES, Green MR, Martin BR (1990) Pharmacological effects of cannabinoids in the elevated plus-maze. J Pharmacol Exp Ther 253:1002–1009

Oviedo A, Glowa J, Herkenham M (1993) Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: a quantitative autoradiographic study. Brain Res 616:293–302

Parker LA, Burton P, Sorge RE, Yakiwchuk C, Mechoulam R (2004) Effect of low doses of delta9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. Psychopharmacology (Berl) 175:360–366

Pertwee RG (2004) The pharmacology and therapeutic potential of cannabidiol. In: Marzo VD (ed) Cannabinoids. Kluwer/Plenum, New York, p 51

Pertwee RG (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol 153:199–215

Pertwee RG, Thomas A (2007) Therapeutic applications for agents that act at CB1 and CB2 receptor. In: Reggio PH (ed) The cannabinoid receptors. Humana, Totowa

Potter DJ, Clark P, Brown MB (2008) Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. J Forensic Sci 53:90–94

Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, Thompson MR, Dawson B, Mallet PE, Kashem MA, Matsuda-Matsumoto H, Iwazaki T, McGregor IS (2008) Adolescent rats find repeated delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology 33:1113–1126

Reid MJ, Bornheim LM (2001) Cannabinoid-induced alterations in brain disposition of drugs of abuse. Biochem Pharmacol 61:1357–1367

Resstel LB, Tavares RF, Lisboa SF, Joca SR, Correa FM, Guimaraes FS (2009) 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. Br J Pharmacol 156:181–188

Robson P (2005) Human studies of cannabinoids and medicinal cannabis. In: Pertwee RG (ed) Cannabinoids. Handbook of experimental pharmacology. Springer, Heidelberg, p 37

Russo E, Guy GW (2006) A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses 66:234–246

Sjoden PO, Jarbe TU, Henriksson BG (1973) Influence of tetrahydrocannabinols (delta8-THC and delta9-THC) on body weight, food, and water intake in rats. Pharmacol Biochem Behav 1:395–399

South T, Huang XF (2008) Temporal and site-specific brain alterations in CB1 receptor binding in high fat diet-induced obesity in C57Bl/6 mice. J Neuroendocrinol 20:1288–1294

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463

Teixeira D, Pestana D, Faria A, Calhau C, Azevedo I, Monteiro R (2010) Modulation of adipocyte biology by delta(9)-tetrahydrocannabinol. Obesity (Silver Spring) 18:2077–2085

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007) Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol 150:613–623

Vann RE, Gamage TF, Warner JA, Marshall EM, Taylor NL, Martin BR, Wiley JL (2008) Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of delta(9)-tetrahydrocannabinol. Drug Alcohol Depend 94:191–198

Varvel SA, Wiley JL, Yang R, Bridgen DT, Long K, Lichtman AH, Martin BR (2006) Interactions between THC and cannabidiol in mouse models of cannabinoid activity. Psychopharmacology (Berl) 186:226–234

Zanelati TV, Biojone C, Moreira FA, Guimaraes FS, Joca SR (2010) Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. Br J Pharmacol 159:122–128

Zavitsanou K, Wang H, Dalton VS, Nguyen V (2010) Cannabinoid administration increases 5HT1A receptor binding and mRNA expression in the hippocampus of adult but not adolescent rats. Neuroscience 169:315–324

Zuardi AW, Finkelfarb E, Bueno OF, Musty RE, Karniol IG (1981) Characteristics of the stimulus produced by the mixture of cannabidiol with delta9-tetrahydrocannabinol. Arch Int Pharmacodyn Thér 249:137–146

Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG (1982) Action of cannabidiol on the anxiety and other effects produced by delta9-THC in normal subjects. Psychopharmacology (Berl) 76:245–250