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### A behavioural comparison of acute and chronic $\Delta^9$ -tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice

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## A behavioural comparison of acute and chronic $\Delta^9$ -tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice

### Abstract

Cannabis contains over 70 unique compounds and its abuse is linked to an increased risk of developing schizophrenia. The behavioural profiles of the psychotropic cannabis constituent  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and the non-psychotomimetic constituent cannabidiol (CBD) were investigated with a battery of behavioural tests relevant to anxiety and positive, negative and cognitive symptoms of schizophrenia. Male adult C57BL/6JArc mice were given 21 daily intraperitoneal injections of vehicle,  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg).  $\Delta^9$ -THC produced the classic cannabinoid CB<sub>1</sub> receptor-mediated tetrad of hypolocomotion, analgesia, catalepsy and hypothermia while CBD had modest hyperthermic effects. While sedative at this dose,  $\Delta^9$ -THC (10 mg/kg) produced locomotor-independent anxiogenic effects in the open-field and light–dark tests. Chronic CBD produced moderate anxiolytic-like effects in the open-field test at 50 mg/kg and in the light–dark test at a low dose (1 mg/kg). Acute and chronic  $\Delta^9$ -THC (10 mg/kg) decreased the startle response while CBD had no effect. Prepulse inhibition was increased by acute treatment with  $\Delta^9$ -THC (0.3, 3 and 10 mg/kg) or CBD (1, 5 and 50 mg/kg) and by chronic CBD (1 mg/kg). Chronic CBD (50 mg/kg) attenuated dexamphetamine (5 mg/kg)-induced hyperlocomotion, suggesting an antipsychotic-like action for this cannabinoid. Chronic  $\Delta^9$ -THC decreased locomotor activity before and after dexamphetamine administration suggesting functional antagonism of the locomotor stimulant effect. These data provide the first evidence of anxiolytic- and antipsychotic-like effects of chronic but not acute CBD in C57BL/6JArc mice, extending findings from acute studies in other inbred mouse strains and rats.

### Keywords

behavioural, comparison, acute, chronic, tetrahydrocannabinol, cannabidiol, C57BL, 6JArc, mice

### Disciplines

Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

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# A behavioural comparison of acute and chronic $\Delta^9$ -tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice

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## Abstract

Cannabis contains over 70 unique compounds and its abuse is linked to an increased risk of developing schizophrenia. The behavioural profiles of the psychotropic cannabis constituent  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and the non-psychotomimetic constituent cannabidiol (CBD) were investigated with a battery of behavioural tests relevant to anxiety and positive, negative and cognitive symptoms of schizophrenia. Male adult C57BL/6JArc mice were given 21 daily intraperitoneal injections of vehicle,  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg).  $\Delta^9$ -THC produced the classic cannabinoid CB<sub>1</sub> receptor-mediated tetrad of hypolocomotion, analgesia, catalepsy and hypothermia while CBD had modest hyperthermic effects. While sedative at this dose,  $\Delta^9$ -THC (10 mg/kg) produced locomotor-independent anxiogenic effects in the open-field and light–dark tests. Chronic CBD produced moderate anxiolytic-like effects in the open-field test at 50 mg/kg and in the light–dark test at a low dose (1 mg/kg). Acute and chronic  $\Delta^9$ -THC (10 mg/kg) decreased the startle response while CBD had no effect. Prepulse inhibition was increased by acute treatment with  $\Delta^9$ -THC (0.3, 3 and 10 mg/kg) or CBD (1, 5 and 50 mg/kg) and by chronic CBD (1 mg/kg). Chronic CBD (50 mg/kg) attenuated dexamphetamine (5 mg/kg)-induced hyperlocomotion, suggesting an antipsychotic-like action for this cannabinoid. Chronic  $\Delta^9$ -THC decreased locomotor activity before and after dexamphetamine administration suggesting functional antagonism of the locomotor stimulant effect. These data provide the first evidence of anxiolytic- and antipsychotic-like effects of chronic but not acute CBD in C57BL/6JArc mice, extending findings from acute studies in other inbred mouse strains and rats.

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**Key words:** Antipsychotic, anxiolytic, cannabidiol, mouse,  $\Delta^9$ -tetrahydrocannabinol.

## Introduction

The population risk for schizophrenia is increased by cannabis use (Henquet *et al.* 2005; Moore *et al.* 2007). This increase is reported to be greater in people with a predisposition to psychosis (Henquet *et al.* 2005).  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the most abundant

of the >70 cannabis constituents, produces the euphoric effects sought by recreational users, and produces psychotomimetic symptoms such as altered perception and disrupted working memory. Schizophrenia patients show increased susceptibility to these effects (D'Souza *et al.* 2005).  $\Delta^9$ -THC has partial agonist efficacy at cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors and exerts many of its central effects via inhibition of neurotransmitter release by presynaptic CB<sub>1</sub> receptors. Cannabidiol (CBD), another cannabis constituent, does not produce the psychotropic effects of  $\Delta^9$ -THC and antagonizes the effects of CB<sub>1</sub>/CB<sub>2</sub> receptor agonists (Pertwee, 2008). Interestingly, consumption of

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cannabis with little or no CBD content is associated with increased incidence of psychotic symptoms (Morgan & Curran, 2008; Rottanburg *et al.* 1982), suggesting that CBD may partially attenuate the psychotomimetic effects of  $\Delta^9$ -THC or other cannabis constituents. The therapeutic potential for CBD is supported by observations of its antipsychotic-like (Leweke *et al.* 2000; Zuardi *et al.* 2006a) and anxiolytic-like effects (Crippa *et al.* 2004, 2009) after acute oral administration in healthy volunteers. While preliminary data in treatment-resistant schizophrenia patients suggest that CBD monotherapy is not effective (Zuardi *et al.* 2006b), regular oral CBD administration reduces psychotic symptoms in Parkinson's disease sufferers (Zuardi *et al.* 2008).

Reports of CBD effects in rodents are currently restricted to acute studies: CBD exerts anxiolytic-like (Campos & Guimaraes, 2008; Guimaraes *et al.* 1994; Moreira *et al.* 2006; Onaivi *et al.* 1990; Resstel *et al.* 2006) and antipsychotic-like (Long *et al.* 2006; Moreira & Guimaraes, 2005; Zuardi *et al.* 1991) effects in mice (Swiss, ICR) and rats (Wistar). Interestingly, CBD induces *c-fos* expression in the rat nucleus accumbens but not the dorsal striatum, in a pattern similar to that of clozapine (Guimaraes *et al.* 2004). To accurately model the effects of regular cannabis use in humans and to determine the potential for lasting clinical efficacy of cannabinoids such as CBD, chronic rodent behavioural studies are necessary. Furthermore, since a majority of mutant mouse models are generated on a C57BL/6J or mixed C57BL/6J  $\times$  129SvJ genetic background, studies in C57BL/6J mice are crucial for further research.

This study therefore aimed to directly compare the acute and chronic effects of  $\Delta^9$ -THC and CBD in C57BL/6J mice using a multi-tiered battery of schizophrenia- and anxiety-relevant behavioural tests. CBD effects were compared to the well-characterized effects of  $\Delta^9$ -THC in the classic cannabinoid CB<sub>1</sub> receptor agonist 'tetrad'. Following this, mice were assessed using a comprehensive multi-tiered battery of behavioural models relevant to positive (spontaneous hyperactivity), negative (social withdrawal, anxiety) and cognitive (disrupted learning and working memory, impaired sensorimotor gating) symptoms of schizophrenia during repeated treatment with  $\Delta^9$ -THC or CBD. The antipsychotic effect of chronic CBD on drug-induced psychotomimetic behaviour was assessed using acute challenges with the non-competitive NMDA antagonist MK-801 and the catecholaminergic stimulant dexamphetamine (Dex). To enable comparison of the effects of repeated treatment, a follow-up study on acute anxiolytic and potentially anti-

psychotic-like effects of CBD was conducted, using doses that were behaviourally active in the chronic study.

## Methods

### Animals

Test animals were 119 male C57BL/6JArc mice (aged 12–14 wk). Standard social interaction opponents were 12 male A/JArc mice (aged 9–10 wk) (Animal Resources Centre, Australia). Mice were maintained under a 12-h light/red light cycle (lights on 07:00 hours) and pair-housed in Macrolon cages (1144B: Tecniplast, Australia) containing paper tissues (Kimwipes<sup>®</sup>, Kimberley-Clark, Australia) as nesting material with *ad libitum* access to water and standard irradiated mouse feed (Gordon's Specialty Stockfeeds, Australia). Chronic experiments were performed in four sets of 22–24 mice over 8 wk, with treatment randomized and counterbalanced across sets. Follow-up acute experiments with CBD were performed in a single set of 29 mice. Research and animal care procedures were approved by the Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

### Drug treatment

$\Delta^9$ -THC and CBD (THC Pharm GmbH, Germany) were suspended in a 1:1:18 mixture of ethanol: Tween-80:saline. Dex and MK-801 (Sigma, Australia) were dissolved in saline. Dose ranges of  $\Delta^9$ -THC and CBD were based on previous studies (Boucher *et al.* 2007; Long *et al.* 2006). Drugs were injected at a volume of 10 ml/kg.

For chronic treatment, mice received 21 consecutive daily intraperitoneal (i.p.) injections of vehicle (1:1:18 ethanol:Tween-80:saline),  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg) ( $n=10$ ). On day 7 all mice were injected with MK-801 (0.5 mg/kg i.p.) 35 min after cannabinoid injection. On day 21 all mice were injected with Dex (5 mg/kg i.p.) 45 min after cannabinoid injection.

### Follow-up experiment

After observing key effects of chronic CBD we investigated the acute behavioural effects of CBD at relevant doses. Separate groups of age-matched male C57BL/6JArc mice received an i.p. injection of vehicle or CBD (1 or 50 mg/kg) ( $n=9-10$ ). Mice were tested in the elevated plus maze (EPM) (5 min) followed by

**Table 1.** Chronic test biography of C57BL/6J Arc mice

Day	Test	Time
-2	Prepulse inhibition acclimatization	07:00–10:00 16:00–19:00
-1	Prepulse inhibition acclimatization	07:00–10:00 16:00–19:00
0	Prepulse inhibition (baseline)	07:00–10:00 16:00–19:00
1	Body temperature, catalepsy, open field, tail flick, prepulse inhibition	07:00–11:00 15:00–19:00
3	Body temperature, catalepsy	07:00–10:00
5	Body temperature, catalepsy	07:00–10:00
7	Body temperature, catalepsy, MK-801 and prepulse inhibition	07:00–11:00 15:00–19:00
12	Body temperature, catalepsy	07:00–10:00
14	Body temperature, catalepsy, tail flick	07:00–10:00
15	Open field	07:00–10:00
16	Y-maze	07:00–12:00
17	Light–dark, elevated plus maze	07:00–13:00
18	Social interaction, prepulse inhibition	07:00–11:00 15:00–19:00
19	Passive avoidance training	07:00–11:00
20	Passive avoidance retention test, tail flick	07:00–11:00
21	Dex and open field	07:00–15:00

$\Delta^9$ -THC,  $\Delta^9$ -tetrahydrocannabinol; CBD cannabidiol, Dex, dexamphetamine.

Ninety mice were tested over a period of 8 wk. Mice were injected with either vehicle or  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg body weight) or CBD (1, 5, 10 or 50 mg/kg) once daily for 21 d ( $n=10$ ). On day 7, all mice were injected with MK-801 (0.5 mg/kg) and on day 21 all mice were injected with Dex (5 mg/kg). The test schedule shows the order in which tests were completed. Test order and age at commencement of testing was standardized to avoid differential impact of repeated testing on the behavioural parameters investigated. Tests involving an aversive stimulus (i.e. electric footshock in passive avoidance) were performed after tests of anxiety-like behaviour such as the elevated plus maze to avoid confounds related to the stress response of the mice.

open-field (OF) testing of Dex-induced locomotor activity (45 min), in which all mice received an injection of Dex (5 mg/kg i.p.) 45 min after vehicle or CBD injection (in a manner identical to the Dex-induced OF testing after chronic CBD).

### Behavioural testing

Injections commenced at the start of the light cycle (07:00 hours). On behavioural testing days injections were staggered within the light cycle to standardize intervals between injection and testing (Table 1). Mice

were returned to the home cage following injection and behavioural testing. Environmental odours were removed from test apparatus between trials with 70% ethanol.

In conducting behavioural testing for schizophrenia, experiments need to target the range of behavioural domains that are affected in the disorder (Powell & Miyakawa, 2006). Furthermore, behavioural testing batteries over one or more days have previously yielded informative data on anxiety-related behaviour (Karl *et al.* 2008), sensory and motor function (Metz & Schwab, 2004) and defensive behaviour (Griebel *et al.* 1999). Ethical considerations prompted us to investigate more than one behavioural domain in each group of mice, and during repeated treatment, the order of behavioural testing proceeded from least to most aversive, such that tests involving an aversive stimulus (i.e. electric footshock in passive avoidance) were performed after tests of anxiety-like behaviour such as the EPM to avoid confounds related to the stress response of the mice.

### Cannabinoid tetrad A: body temperature, catalepsy and nociception

Mice were assessed for the classical cannabinoid behavioural tetrad of hypothermia, catalepsy, hypolocomotion and nociception (Compton *et al.* 1993). Body temperature was measured 5 min before and 30 min after injection on days 1, 3, 5, 7, 12 and 14 using a lubricated rectal thermometer (SDR Clinical Technology, Australia). Catalepsy was measured 20 min after injection on days 1, 3, 5, 7, 12 and 14. The hindpaws of the mouse were placed on the bench and the forepaws were placed on a bar (0.2 cm diameter) raised 8 cm off the bench surface. The latency for the mouse to place both forepaws on the bench was recorded. Nociception was measured with the hot-water tail-flick test 45 min after injection on days 1, 14 and 20. Mice were gently wrapped in a towel leaving the tail unrestrained. The tail tip (1.5 cm) was placed in a beaker of water at room temperature (25 °C) for 10 s then placed immediately in a water bath at 52 °C. The latency for the mouse to flick its tail was recorded. Data are reported as the mean of three consecutive trials performed for each mouse, with 2-min inter-trial intervals during which mice were returned to the home cage.

### Cannabinoid tetrad B: spontaneous and Dex-induced locomotor activity

Locomotor activity was measured by placing mice into an OF activity chamber (Med Associates Inc., USA). Horizontal (distance travelled) and vertical activity

(rearing) in central and peripheral zones were measured (described previously; Karl *et al.* 2007). The ratio of central to total distance travelled (distance ratio) and time spent in the central zone were taken as measures of anxiety (Denenberg, 1969). On days 1 and 15 mice were placed in the OF 35 min after injection and allowed to explore freely for 10 min. On day 21 of the chronic study, and in the follow-up study, mice were placed in the OF 30 min after injection and allowed to explore freely for 15 min before they were removed, injected with Dex and immediately returned to the chamber for a further 30 min.

#### *Light-dark (LD) test*

Mice were placed into the opening of the dark compartment of the LD test apparatus (described previously; Karl *et al.* 2007) and allowed to explore freely for 10 min. The ratio of distance travelled in the light compartment to total distance travelled (distance ratio) and time spent in the light compartment were taken as measures of anxiety. Mice were tested with the LD test 30 min after injection on day 17.

#### *EPM*

Mice were placed onto the central platform of the EPM (described previously; Karl *et al.* 2007) facing an enclosed arm and allowed to explore freely for 5 min. Arm entries, time spent in arms and in the central platform, rearing and head dipping were scored. Arm entries were recorded when mice entered the arm with all four paws. Anxiety-related behaviour was measured by entries into the open arm from the centre, percentage of time spent on open arms (divided into inner and outer halves) and the percentage of open-arm entries (open-arm entry ratio) (Hogg, 1996; Pellow *et al.* 1985). Mice were tested in the EPM 45 min after injection on day 17 and 25 min after injection in the acute study.

#### *Social interaction (SI)*

SI between pairs of rodents is used to measure anxiety-like behaviours (File & Seth, 2003; Kask *et al.* 2001). Furthermore, reduction in SI models aspects of social withdrawal observed in schizophrenia patients (Ellenbroek & Cools, 2000; Rung *et al.* 2005). Test mice and weight-matched A/JArc standard opponents, with no prior exposure to either the test arena or to each other, were placed in opposite corners of a grey perspex arena (35 × 35 × 30 cm; illumination 50 lx) and allowed to explore freely for 10 min. Frequency and total duration of the active socio-positive behaviours general sniffing, anogenital sniffing, allogrooming,

following and climbing over/under in each test mouse were recorded manually. Mice were tested for SI 35 min after injection on day 18.

#### *Prepulse inhibition (PPI)*

PPI, an operational measure of sensorimotor gating, is impaired in schizophrenia patients (Braff *et al.* 2001; Ludewig *et al.* 2003). Startle reactivity was measured using SR-Lab startle chambers (San Diego Instruments, USA). Mice were habituated to this apparatus for 30 min on two consecutive days. The next day, mice were tested for baseline PPI, and on the following day chronic drug treatment began. PPI testing commenced 60 min after injection on days 1 and 18 and 55 min after injection on day 7 (20 min after injection of MK-801). PPI test sessions consisted of 5-min acclimatization to 70-dB background noise followed by 105 trials presented in a pseudo-random order: 5 × 70-dB trials (background); 5 × 80-dB trials; 5 × 100-dB trials; 15 × 120-dB trials (startle) and 15 sets of five trials comprising a prepulse of either 74, 82 or 86 dB presented 32, 64, 128, 256 or 512 ms prior to a startling pulse of 120 dB (PPI response). Inter-trial intervals varied randomly from 10 s to 20 s. Responses to each trial were calculated as average mean amplitudes detected by the accelerometer. Percentage PPI (% PPI) was calculated as

$$\frac{\text{startle response (120 dB)} - \text{PPI response}}{\text{startle response (120 dB)}} \times 100.$$

% PPI was averaged across inter-stimulus (prepulse-startling pulse) intervals to produce a mean % PPI for each prepulse intensity.

#### *Y-maze*

The natural tendency of mice to alternate successive entry choices of arms in a Y-maze (spontaneous alternation) is used as an assessment of memory retention (Hughes, 2004). Mice were placed into the centre of a grey perspex Y-shaped maze (30 cm × 10 cm × 17 cm, arms joined by a triangular centre section) and allowed to explore freely for 8 min. Different patterns on each arm wall provided intra-maze directionality cues. Objects (e.g. a camera tripod) at the end of each arm provided extra-maze directionality cues. Arm entries (defined when all four paws were inside the arm) were recorded manually. The spontaneous alternation score was calculated as the number of novel triplet entries (three consecutive entries into different arms) expressed as a percentage of the maximum possible triplet entries (Hodges, 1996). Mice were tested 30 min after injection on day 16.

### Passive avoidance (PA)

In PA, memory retention is indicated by avoidance of a naturally less aversive dark compartment after it is paired with an electrical footshock (Bovet *et al.* 1969). This hippocampus-dependent learning test is influenced by fear of highly illuminated areas and aversive stimuli (e.g. electrical footshock), and by nociception. Training and retention trials were performed 30 min after injection on days 19 and 20, respectively, using a shuttle box system (TSE Systems, Bad Homburg, Germany). Latency to enter the dark chamber on each trial was measured (cut-off time: 300 s) (previously described; Karl *et al.* 2008). Increased latency in the retention trial indicated memory of the aversive stimulus (0.4 mA footshock; 2 s). Due to apparatus malfunction several mice did not receive a footshock and were excluded from further analysis.

### Statistical analysis

In analyses for all experiments the vehicle group was compared separately to the  $\Delta^9$ -THC (THC 0.3, THC 1, THC 3 and THC 10) and CBD (CBD 1, CBD 5, CBD 10 and CBD 50) groups. Differences between treatment groups were analysed with one-way (factor: treatment) or repeated-measures analyses of variance (ANOVA) [factors: treatment, 5-min block (Dex-induced locomotor activity), prepulse intensity and trial (PA)]. Main and interaction effects were identified when  $p < 0.05$ . Planned contrasts were used to determine which treatment groups were significantly different to vehicle (simple contrasts), to locate differences between levels of within-subjects factors (polynomial contrasts) and to locate interactions between levels of treatment groups and within-subjects factors (special interaction contrasts). Huynh-Feldt corrections for non-sphericity were applied. Degrees of freedom,  $F$  values and  $p$  values from ANOVA are presented in the Results, and  $p$  values from planned contrasts are indicated in the Figures and Tables by asterisks (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). Data are presented as mean  $\pm$  standard error of the mean (S.E.M.). Analysis was performed using SPSS 16.0 for Windows (SPSS Inc., USA).

## Results

### Cannabinoid tetrad A: body temperature, catalepsy and nociception

Acute  $\Delta^9$ -THC (10 mg/kg) produced significant hypothermia [one-way ANOVA:  $F(4, 49) = 9.3$ ,  $p < 0.001$ ] and catalepsy [one-way ANOVA:  $F(4, 43) = 6.0$ ,  $p < 0.001$ ] (Table 2). There was a trend towards an effect of

acute  $\Delta^9$ -THC on nociception (one-way ANOVA:  $p = 0.08$ ).  $\Delta^9$ -THC (10 mg/kg) had a significant antinociceptive effect on day 14 [one-way ANOVA:  $F(4, 44) = 3.9$ ,  $p = 0.01$ ] (Table 2), but not on day 20.

CBD (1 and 10 mg/kg) induced a hyperthermic response on day 7 [one-way ANOVA:  $F(4, 49) = 3.5$ ,  $p < 0.05$ ] but had no effect on catalepsy or nociception (Table 2).

### Cannabinoid tetrad B: spontaneous locomotor activity

$\Delta^9$ -THC decreased overall distance travelled on day 1 [one-way ANOVA:  $F(4, 49) = 3.5$ ,  $p < 0.05$ ] and day 15 [ $F(4, 46) = 3.6$ ,  $p < 0.05$ ] at 10 mg/kg and on day 21 [ $F(4, 44) = 13.3$ ,  $p < 0.001$ ] at 1, 3 and 10 mg/kg (Fig. 1a).  $\Delta^9$ -THC also decreased peripheral distance travelled in the OF test on day 1 [one-way ANOVA:  $F(4, 49) = 3.1$ ,  $p < 0.05$ ] at 10 mg/kg and on day 21 [ $F(4, 44) = 12.8$ ,  $p < 0.001$ ] at 1, 3 and 10 mg/kg (Fig. 1b).  $\Delta^9$ -THC also decreased the total distance travelled in the LD test [one-way ANOVA:  $F(4, 46) = 9.6$ ,  $p < 0.001$ ] at 10 mg/kg (Fig. 1c) and the total number of arm entries in the EPM [one-way ANOVA:  $F(4, 42) = 4.0$ ,  $p < 0.01$ ] at 3 and 10 mg/kg (Fig. 1d), confirming its locomotor suppressant effects.

In contrast, CBD had no effect on any locomotor activity measure in the OF test (one-way ANOVA:  $p > 0.05$ ; Fig. 1e, f). CBD (10 mg/kg) significantly decreased the total distance travelled in the LD test [one-way ANOVA:  $F(4, 44) = 2.6$ ,  $p < 0.05$ ] (Fig. 1g). There was a trend towards an effect of CBD on the total number of EPM arm entries (one-way ANOVA:  $p = 0.06$ ; Fig. 1h).

### Dex-induced locomotor activity

#### Chronic

There were main effects of chronic  $\Delta^9$ -THC (repeated-measures ANOVA:  $F(4, 38) = 4.6$ ,  $p < 0.01$ ) and time (5-min block) [ $F(2.6, 97.8) = 196.2$ ,  $p < 0.001$ ] on the distance travelled in the OF test on day 21, and a moderate trend towards an interaction of  $\Delta^9$ -THC with time ( $p = 0.09$ ) (Fig. 2a). An interaction contrast between the 15-min baseline (BL) and 30-min post-Dex periods showed that Dex increased the distance travelled in all  $\Delta^9$ -THC-treated groups [BL  $\times$  post-Dex:  $F(1, 38) = 284.7$ ,  $p < 0.001$ ]. The magnitude of this increase was dependent on  $\Delta^9$ -THC dose [BL  $\times$  post-Dex  $\times$   $\Delta^9$ -THC interaction:  $F(4, 38) = 2.8$ ,  $p < 0.05$ ]. Doses and time-points at which  $\Delta^9$ -THC attenuated the Dex-induced increase in locomotor activity were determined by special interaction contrasts (BL  $\times$  specific post-Dex time-point  $\times$   $\Delta^9$ -THC) (Fig. 2a).

**Table 2.** Body temperature, catalepsy and nociception

Parameter	Day	Vehicle	$\Delta^9$ -THC 0.3	$\Delta^9$ -THC 1	$\Delta^9$ -THC 3	$\Delta^9$ -THC 10	CBD 1	CBD 5	CBD 10	CBD 50
Thermic response: difference in body temperature measured 5 min before and 30 min after injection ( $\Delta$ °C)	1	1.4±0.3	2.3±0.3	2.3±0.4	1.2±0.4	-1.8±1.0*	1.5±0.7	1.4±0.3	2.2±0.5	2.2±0.5
	3	1.4±0.3	1.3±0.3	1.6±0.1	1.5±0.2	0.8±0.4	1.7±0.3	1.0±0.2	1.2±0.3	1.4±0.1
	5	1.2±0.3	1.1±0.5	1.2±0.2	1.4±0.3	1.5±0.2	1.8±0.3	1.2±0.3	1.2±0.3	1.5±0.2
	7	0.9±0.4	1.6±0.3	1.0±0.2	1.7±0.2	1.6±0.3	2.0±0.2**	1.0±0.3	1.8±0.3*	1.1±0.2
	12	1.6±0.2	1.0±0.3	0.8±0.4	1.4±0.3	1.3±0.2	0.8±0.2	1.3±0.2	1.6±0.4	1.1±0.2
	14	1.8±0.4	1.1±0.2	1.4±0.4	1.9±0.4	1.3±0.1	1.6±0.3	1.2±0.4	1.2±0.2	1.4±0.2
Catalepsy: latency to remove both forepaws from bar and place on bench, 20 min after injection (ms)	1	58.7±5.2	71.4±6.8	68.9±6.8	93.9±11.4	390.5±137.9*	50.4±4.5	114.8±40.3	64.0±11.3	55.4±11.3
	3	48.3±6.5	44.7±5.3	70.3±14.3	48.3±6.2	66.1±8.2	46.9±5.9	76.6±17.3	39.6±2.1	59.5±7.8
	5	44.9±3.1	43.7±4.3	85.4±32.4	53.7±4.9	57.2±5.1	37.4±1.9	41.2±2.9	38.9±3.6	47.9±5.6
	7	49.7±8.0	45.8±12.7	49.5±5.9	53.9±10.1	45.1±4.8	41.7±4.5	40.4±4.6	38.4±2.2	42.7±5.7
	12	39.4±1.2	69.0±34.1	56.7±12.2	51.3±5.6	63.9±10.6	42.1±7.0	54.3±4.2	38.3±2.4	79.0±22.2
	14	36.6±1.0	43.2±5.4	56.3±13.2	62.0±6.8	56.1±6.4	38.9±4.7	49.3±6.7	38.8±2.8	49.5±7.1
Nociception: mean latency to flick tail immersed in 52 °C water, 45 min after injection (s), measured over three consecutive trials	1	2.4±0.1	2.6±0.2	2.6±0.2	2.9±0.3	3.5±0.4	2.3±0.1	2.0±0.2	2.4±0.2	2.2±0.1
	14	2.6±0.3	3.1±0.4	3.0±0.2	2.9±0.2	3.8±0.2***	3.0±0.3	2.5±0.2	2.5±0.3	2.4±0.2
	20	2.5±0.3	2.4±0.3	2.5±0.3	2.5±0.3	3.1±0.3	2.5±0.2	2.3±0.3	2.3±0.3	1.8±0.1

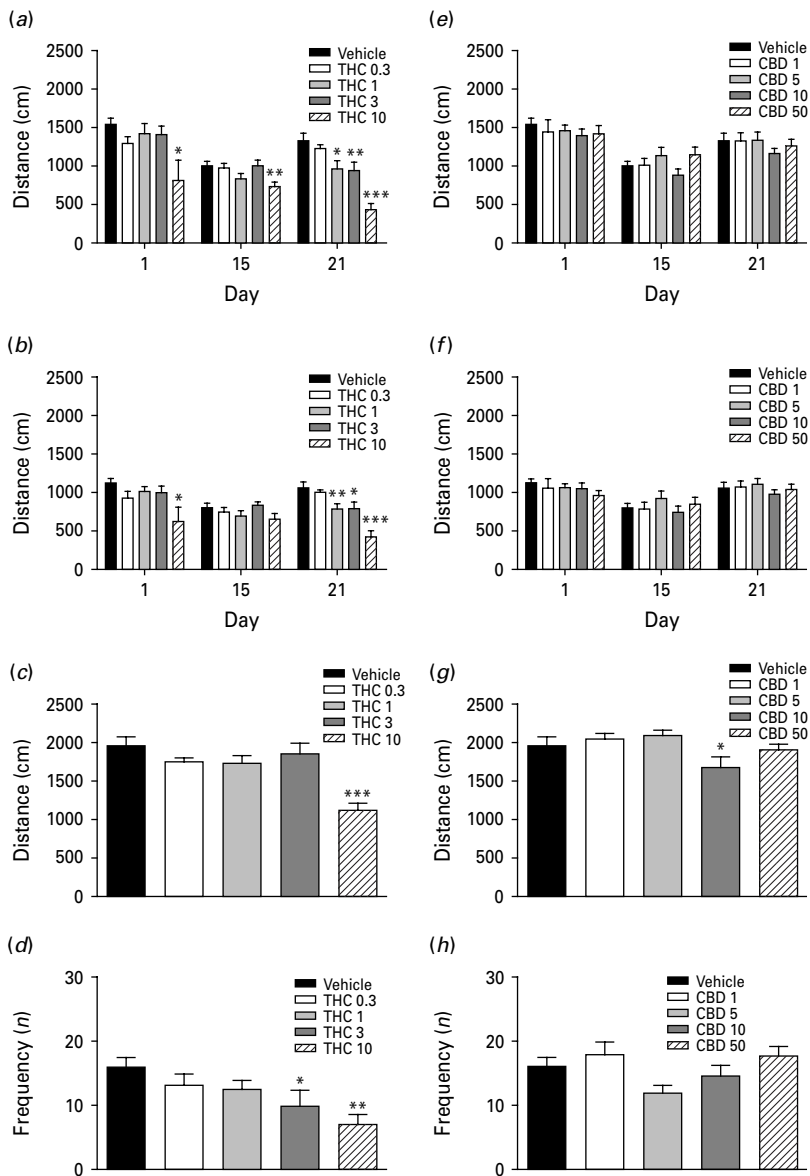
$\Delta^9$ -THC,  $\Delta^9$ -tetrahydrocannabinol; CBD, cannabidiol.

Body temperature, catalepsy and nociception after injection with  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg) on various days of treatment.

Data represent mean ( $\pm$ S.E.M.),  $n=8-10$ .

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. vehicle group (one-way ANOVA and planned contrasts).





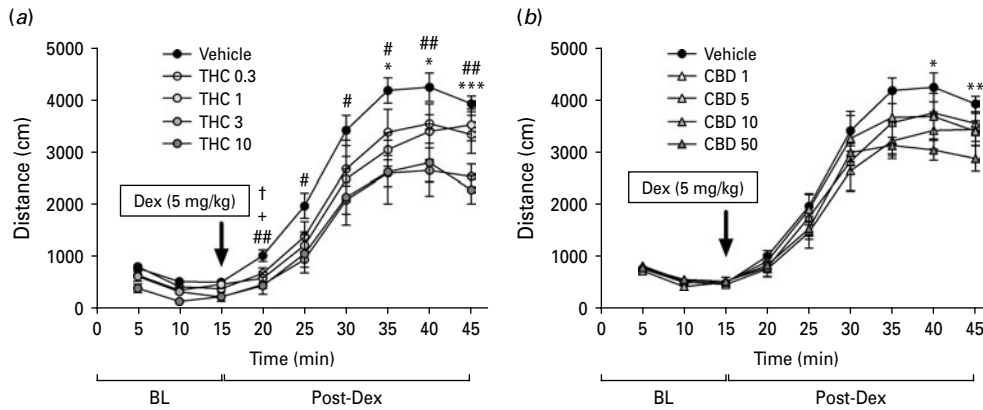
**Fig. 1.** Measures of locomotor activity after injection of  $\Delta^9$ -tetrahydrocannabinol (THC) (0.3, 1, 3 or 10 mg/kg) or cannabidiol (CBD) (1, 5, 10 or 50 mg/kg). (a, e) Total distance travelled (open-field test, 10 min) on days 1, 15 and 21 of treatment. (b, f) Distance travelled in the peripheral area (open-field test). (c, g) Distance travelled (light-dark test, 10 min) on day 17. (d, h) Frequency of entries into open and closed arms (elevated plus maze, 5 min) on day 17. Data represent mean  $\pm$  S.E.M. ( $n=10$ ). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. vehicle (one-way ANOVA and planned contrasts).

There was a main effect of time (5-min block), but not CBD, on distance travelled in the OF on day 21 [repeated-measures ANOVA:  $F(2.2, 92.9) = 225.8$ ,  $p < 0.001$ ] (Fig. 2b). There was no CBD  $\times$  time interaction ( $p > 0.05$ ). An interaction contrast showed that Dex increased the distance travelled in all CBD-treated groups [BL  $\times$  post-Dex:  $F(1, 42) = 330.1$ ,  $p < 0.001$ ]. The magnitude of this increase was independent of CBD dose (BL  $\times$  post-Dex  $\times$  CBD interaction contrast:  $p > 0.05$ ).

Special interaction contrasts (BL  $\times$  specific post-Dex time-point  $\times$  CBD) showed that CBD (50 mg/kg) attenuated the Dex-induced increase in locomotor activity at 35–40 min and 40–45 min (Fig. 2b).

#### Acute CBD

In the follow-up experiment investigating the effect of acute CBD on Dex-induced locomotor activity there



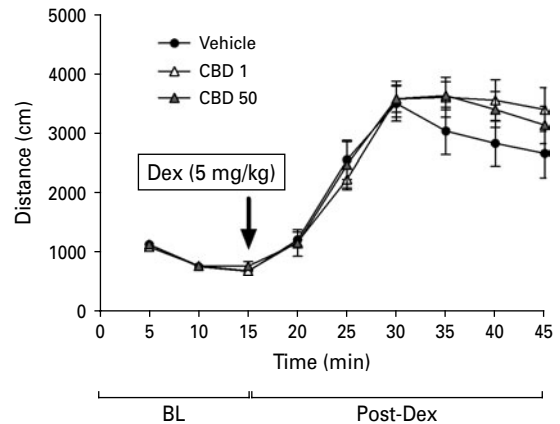
**Fig. 2.** Total distance travelled over time (open-field test, 45 min) after injection of (a)  $\Delta^9$ -tetrahydrocannabinol (THC) (0.3, 1, 3 or 10 mg/kg) or (b) cannabidiol (CBD) (1, 5, 10 or 50 mg/kg) during baseline (BL) and after injection of 5 mg/kg Dex (post-Dex) on day 21 of treatment. Data represent mean  $\pm$  S.E.M. ( $n=7-10$ ).  $\dagger p < 0.05$  ( $\Delta^9$ -THC 0.3 vs. vehicle);  $^+ p < 0.05$  ( $\Delta^9$ -THC 1 vs. vehicle);  $^\# p < 0.05$ ,  $^\#\# p < 0.01$  ( $\Delta^9$ -THC 3 vs. vehicle);  $^* p < 0.05$ ,  $^{**} p < 0.01$ ,  $^{***} p < 0.001$  ( $\Delta^9$ -THC 10 or CBD 50 vs. vehicle) (repeated-measures ANOVA and planned contrasts).

was a main effect of time (5-min block), but not CBD, on distance travelled in the OF test [repeated-measures ANOVA:  $F(2,7,71.1)=112.0$ ,  $p < 0.001$ ] (Fig. 3). There was no CBD  $\times$  time interaction ( $p > 0.05$ ). Within-subjects contrasts showed that Dex increased the distance travelled in all CBD-treated groups [BL vs. post-Dex:  $F(1,26)=211.6$ ,  $p < 0.001$ ]. The magnitude of this increase was independent of CBD dose (BL vs. post-Dex  $\times$  CBD interaction contrast:  $p > 0.05$ ). Special interaction contrasts (BL vs. specific post-Dex time-point  $\times$  CBD vs. vehicle) showed that CBD did not affect the Dex-induced increase in locomotor activity at any time-point ( $p > 0.05$ ).

### Exploratory activity

Vertical activity in the OF test was decreased by  $\Delta^9$ -THC (1, 3 and 10 mg/kg) on day 1 [one-way ANOVA:  $F(4,49)=8.2$ ,  $p < 0.001$ ] and day 15 [ $F(4,46)=7.9$ ,  $p < 0.001$ ] and by  $\Delta^9$ -THC (3 and 10 mg/kg) on day 21 [ $F(4,44)=8.4$ ,  $p < 0.001$ ] (Table 3). This was confirmed by significant inhibition by  $\Delta^9$ -THC (10 mg/kg) of rearing in the LD test [one-way ANOVA:  $F(4,46)=3.5$ ,  $p < 0.05$ ; data not shown] and rearing [one-way ANOVA:  $F(4,42)=4.7$ ,  $p < 0.01$ ] and head dipping [one-way ANOVA:  $F(4,42)=3.1$ ,  $p < 0.05$ ] in the EPM (Table 3).

CBD did not affect vertical activity/rearing in the OF, LD or EPM tests (one-way ANOVA:  $p > 0.05$ ). There was a trend towards an effect of CBD on head dipping in the EPM (one-way ANOVA:  $p = 0.06$ ) (Table 3).



**Fig. 3.** Total distance travelled over time (open-field test, 45 min) after a single injection of cannabidiol (CBD) (1 or 50 mg/kg) during baseline (BL) and after injection of 5 mg/kg Dex (post-Dex). Data represent mean  $\pm$  S.E.M. ( $n=9-10$ ).

### Anxiety measures

#### Chronic

$\Delta^9$ -THC (10 mg/kg) significantly decreased the time spent in the central zone of the OF test on day 15 [one-way ANOVA:  $F(4,46)=2.8$ ,  $p < 0.05$ ] and day 21 [ $F(4,44)=3.4$ ,  $p < 0.05$ ] (Fig. 4a) and decreased the distance ratio on day 1 [ $F(4,49)=3.9$ ,  $p < 0.01$ ], day 15 [ $F(4,46)=3.1$ ,  $p < 0.05$ ] and day 21 [ $F(4,44)=8.6$ ,  $p < 0.001$ ] (Fig. 4b). Similarly,  $\Delta^9$ -THC (10 mg/kg) decreased the time spent in the light compartment [one-way ANOVA:  $F(4,46)=3.5$ ,  $p < 0.05$ ] and the distance

Table 3. Exploratory activity

Parameter	Day	Vehicle	$\Delta^9$ -THC 0.3	$\Delta^9$ -THC 1	$\Delta^9$ -THC 3	$\Delta^9$ -THC 10	CBD 1	CBD 5	CBD 10	CBD 50
Vertical activity (OF) (n)	1	107.9 ± 6.0	75.8 ± 7.4	67.8 ± 9.9*	29.9 ± 13.2***	32.4 ± 16.9***	104.7 ± 17.0	111.9 ± 11.5	88.0 ± 10.5	93.2 ± 7.3
	15	62.9 ± 7.6	53.9 ± 5.8	45.4 ± 5.9*	38.5 ± 5.1**	19.1 ± 4.3***	67.9 ± 9.6	75.9 ± 8.8	53.3 ± 6.3	82.3 ± 10.6
	21	68.3 ± 7.6	61.6 ± 7.0	55.3 ± 15.5	35.3 ± 6.7**	4.3 ± 1.7***	79.6 ± 9.3	63.8 ± 7.9	63.0 ± 6.2	69.9 ± 10.7
Rearing (EPM) (n)	17	11.2 ± 2.0	12.0 ± 1.7	12.1 ± 2.1	7.4 ± 2.4	2.8 ± 1.1**	12.3 ± 2.2	12.3 ± 1.8	13.4 ± 1.4	15.5 ± 1.2
Head dipping (EPM) (n)	17	13.1 ± 1.5	12.2 ± 1.5	9.9 ± 1.9	9.7 ± 2.7	4.3 ± 1.5**	12.4 ± 2.8	10.5 ± 0.9	12.0 ± 1.3	17.6 ± 2.0

$\Delta^9$ -THC,  $\Delta^9$ -tetrahydrocannabinol; CBD, cannabidiol; OF, open-field; EPM, elevated plus maze.

Frequency of vertical activity (OF test, 10 min) and rearing and head dipping (EPM, 5 min) after injection of  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg) on various days of treatment.

Data represent mean ( $\pm$  SEM),  $n = 7-10$ .

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. vehicle group (one-way ANOVA and planned contrasts).

ratio [one-way ANOVA:  $F(4, 46) = 2.8$ ,  $p < 0.05$ ] in the LD test (Fig. 4c,d). There was a trend towards an effect of  $\Delta^9$ -THC on time spent in the inner open arm in the EPM (one-way ANOVA:  $p = 0.08$ ), but no effect on the open-arm entry ratio (data not shown).

In contrast, CBD (50 mg/kg) increased the time spent in the central zone of the OF test on day 15 [one-way ANOVA:  $F(4, 48) = 3.5$ ,  $p < 0.05$ ] (Fig. 4e). There was a trend towards a significant effect of CBD on the distance ratio on day 15 (one-way ANOVA:  $p = 0.07$ ) (Fig. 4f). In the LD test, CBD (1 mg/kg) significantly increased the time spent in the light compartment [one-way ANOVA:  $F(4, 44) = 3.0$ ,  $p < 0.05$ ] and the distance ratio [ $F(4, 44) = 2.8$ ,  $p < 0.05$ ] (Fig. 4g,h). There was a moderate trend towards an effect of CBD on time spent in the inner open arm in the EPM (one-way ANOVA:  $p = 0.09$ ), but no effect on the open-arm entry ratio or percentage of time spent on open arms (data not shown).

#### Acute CBD

In the follow-up experiment investigating the effect of CBD in the EPM there was no effect of CBD on total number of EPM arm entries, percentage time in the open arms, ratio of open-arm entries to total arm entries, time in the centre zone, head dipping, or rearing (one-way ANOVA:  $p > 0.05$ ; data not shown).

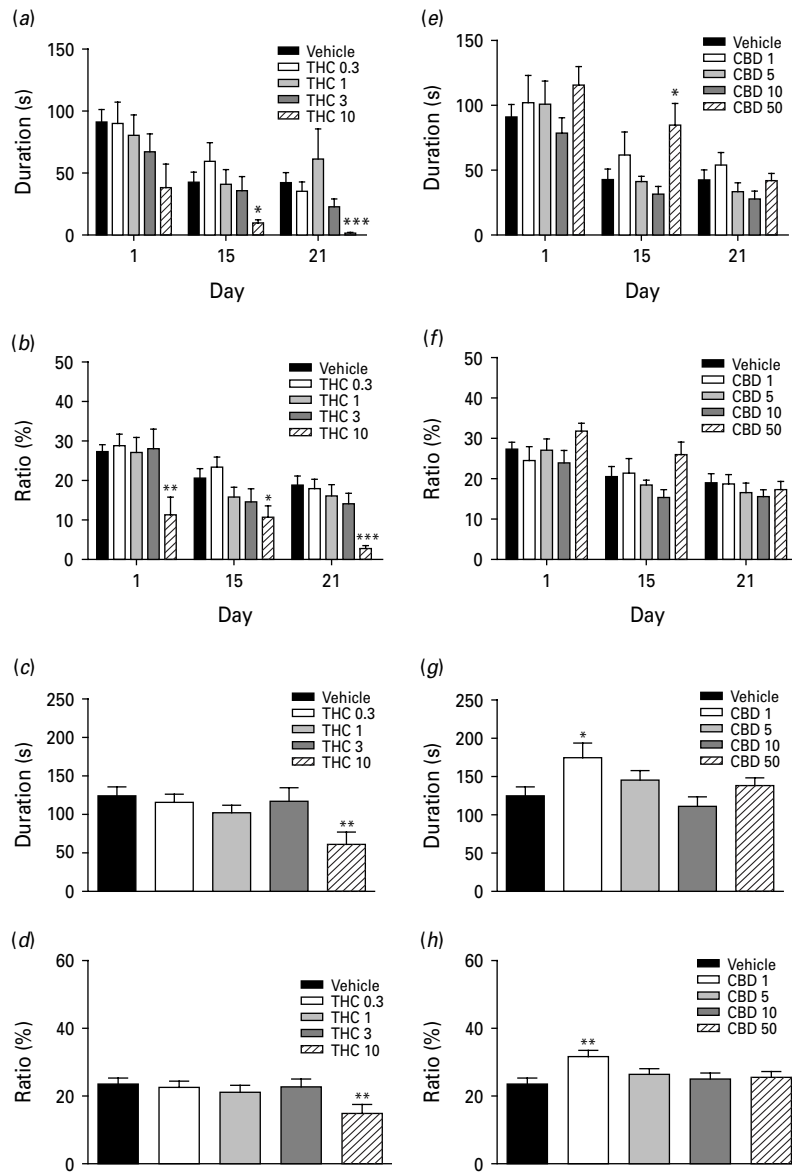
#### Startle response and PPI

All mice displayed identical startle response and PPI at baseline testing 1 d prior to the first injection (data not shown). There was a main effect of prepulse intensity on PPI ( $p < 0.001$ ) and a significant linear contrast between levels of prepulse intensity ( $p < 0.001$ ) on all test days, indicating that PPI increased with increasing prepulse intensity.

$\Delta^9$ -THC (10 mg/kg) decreased the startle response on day 1 [one-way ANOVA:  $F(4, 48) = 3.1$ ,  $p < 0.05$ ] and day 18 [ $F(4, 44) = 3.1$ ,  $p < 0.05$ ] (Table 4). There was no effect of  $\Delta^9$ -THC on the startle response when mice were treated with MK-801 (data not shown).

There was no effect of CBD on startle response on any day (one-way ANOVA:  $p > 0.05$ ). There was no main effect of  $\Delta^9$ -THC on PPI on days 1, 7 or 18 (repeated-measures ANOVA:  $p > 0.05$ , data not shown for day 7) (Fig. 5a,b). There was a significant interaction between  $\Delta^9$ -THC and prepulse intensity on day 1 [ $F(6.5, 71.4) = 5.0$ ,  $p < 0.001$ ] and day 18 [ $F(7.0, 70.2) = 5.6$ ,  $p < 0.001$ ]. Planned contrasts showed that acute  $\Delta^9$ -THC (0.3, 3 and 10 mg/kg) significantly increased PPI (Fig. 5a).

There was a main effect of CBD on PPI on day 1 [repeated-measures ANOVA:  $F(4, 44) = 4.9$ ,  $p < 0.01$ ]



**Fig. 4.** Anxiety-related measures after injection of  $\Delta^9$ -tetrahydrocannabinol (THC) (0.3, 1, 3 or 10 mg/kg) or cannabidiol (CBD) (1, 5, 10 or 50 mg/kg). (a, e) Time spent in the central area (open-field test, 10 min) on days 1, 5 and 21 of treatment. (b, f) Distance ratio (open-field test). (c, g) Time spent in the light compartment (light-dark test, 10 min) on day 17. (d, h) Distance ratio (light-dark test). Data represent mean  $\pm$  S.E.M. ( $n=7-10$ ). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. vehicle group (one-way ANOVA and planned contrasts).

(Fig. 5c), but not on day 7 (data not shown) or day 18 ( $p > 0.05$ ) (Fig. 5d). There was no CBD  $\times$  prepulse intensity interaction ( $p > 0.05$ ). Planned contrasts showed that acute (1, 5 and 50 mg/kg) (Fig. 5c) and chronic (1 mg/kg) (Fig. 5d) CBD increased PPI.

#### Social interaction

$\Delta^9$ -THC did not alter total SI time (one-way ANOVA:  $p > 0.05$ ), but decreased the combined frequency of the

social behaviours general sniffing, anogenital sniffing, allogrooming, following and climbing over/under [one-way ANOVA:  $F(4, 45) = 13.2$ ,  $p < 0.001$ ] (Table 5).  $\Delta^9$ -THC reduced general sniffing frequency [one-way ANOVA:  $F(4, 45) = 18.6$ ,  $p < 0.001$ ] at 3 and 10 mg/kg and its duration [one-way ANOVA:  $F(4, 45) = 3.9$ ,  $p < 0.01$ ] at 10 mg/kg (Table 5).  $\Delta^9$ -THC (10 mg/kg) also decreased anogenital sniffing frequency [one-way ANOVA:  $F(4, 45) = 4.5$ ,  $p < 0.01$ ].

**Table 4.** Startle response

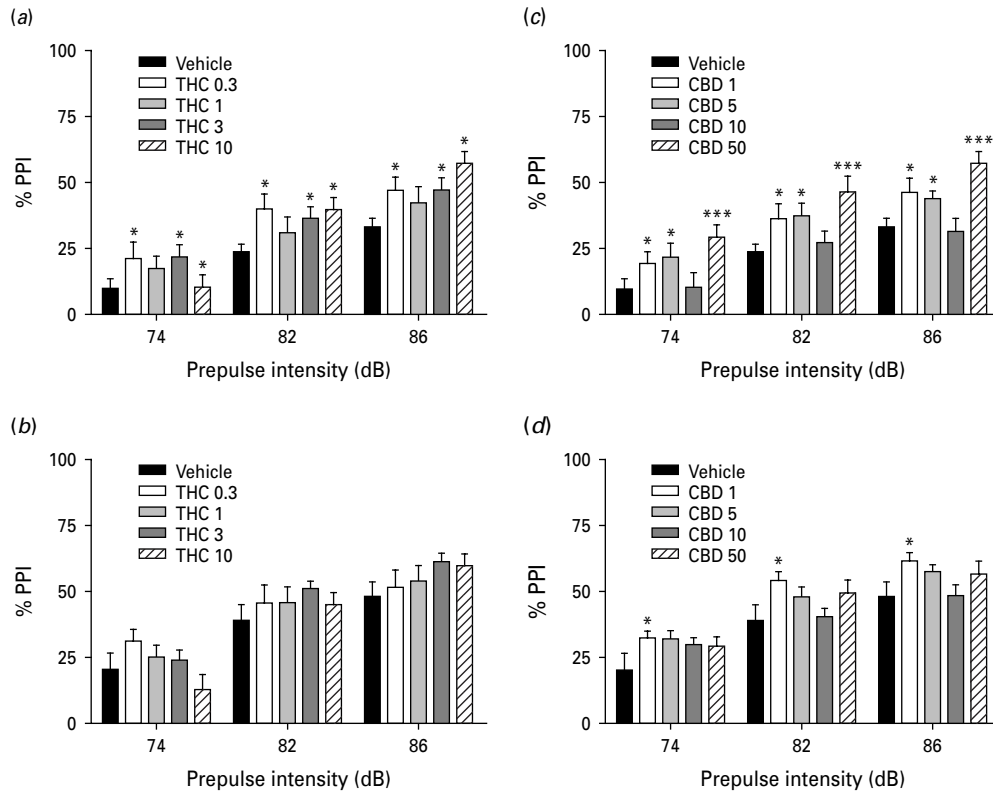
Day	Vehicle	$\Delta^9$ -THC 0.3	$\Delta^9$ -THC 1	$\Delta^9$ -THC 3	$\Delta^9$ -THC 10	CBD 1	CBD 5	CBD 10	CBD 50
1	58.1±4.0	49.8±3.4	50.4±6.2	48.8±4.3	35.7±4.6**	44.3±6.6	57.9±4.7	52.9±3.5	52.5±5.8
18	66.8±4.9	56.0±9.0	65.3±7.9	50.1±6.0	39.0±5.2*	54.3±10.5	71.0±8.1	64.3±4.9	65.6±6.1

$\Delta^9$ -THC,  $\Delta^9$ -tetrahydrocannabinol; CBD, cannabidiol.

Startle response (arbitrary units) following 120 dB acoustic stimuli [prepulse inhibition (PPI), 30 min] after injection with  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg) on various days of treatment.

Data represent mean ( $\pm$ S.E.M.),  $n=8-10$ .

\* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle group (one-way ANOVA and planned contrasts).



**Fig. 5.** % Prepulse inhibition (PPI) after injection of  $\Delta^9$ -tetrahydrocannabinol (THC) (0.3, 1, 3 or 10 mg/kg) or cannabidiol (CBD) (1, 5, 10 or 50 mg/kg) on (a, c) day 1 and (b, d) day 18 of treatment. Data represent mean  $\pm$  S.E.M. ( $n=8-10$ ).

\* $p < 0.05$ , \*\*\* $p < 0.001$  vs. vehicle group (repeated-measures ANOVA and planned contrasts).

There was no effect of CBD on SI (one-way ANOVA:  $p > 0.05$ ) (Table 5).

#### Y-maze

There was no effect of treatment on the percentage of novel entry triplets, indicating equal spontaneous alternation. Neither  $\Delta^9$ -THC nor CBD affected the number of total arm entries (data not shown).

#### Passive avoidance

A main effect of trial on the latency to enter the dark chamber in vehicle- and  $\Delta^9$ -THC-treated mice [repeated-measures ANOVA:  $F(1, 26) = 31.0$ ,  $p < 0.001$ ] indicated that all mice learned to avoid the dark chamber after training (Fig. 6a). There was no main effect of  $\Delta^9$ -THC ( $p > 0.05$ ) but there was a significant  $\Delta^9$ -THC treatment  $\times$  trial interaction [ $F(4, 26) = 3.4$ ,

Table 5. Social interaction

Parameter	Vehicle	$\Delta^9$ -THC 0.3	$\Delta^9$ -THC 1	$\Delta^9$ -THC 3	$\Delta^9$ -THC 10	CBD 1	CBD 5	CBD 10	CBD 50
General sniffing duration (s)	30.6 ± 2.9	23.5 ± 1.2	28.2 ± 2.3	24.7 ± 1.7	18.7 ± 2.7**	24.5 ± 1.8	29.1 ± 3.7	30.0 ± 2.1	27.1 ± 2.5
General sniffing (n)	55.5 ± 3.6	48.1 ± 2.0	49.6 ± 4.1	43.1 ± 2.5*	21.4 ± 2.2***	47.7 ± 3.6	51.3 ± 3.9	52.3 ± 3.4	51.0 ± 3.2
Anogenital sniffing (n)	16.1 ± 2.0	16.3 ± 2.1	16.6 ± 2.9	13.6 ± 2.1	5.7 ± 1.2*	16.8 ± 2.8	15.8 ± 2.8	11.6 ± 1.6	19.7 ± 1.4
Total social interaction duration (s)	50.2 ± 4.5	43.7 ± 2.9	48.3 ± 3.6	52.2 ± 9.5	50.9 ± 13.1	42.4 ± 4.6	48.5 ± 6.5	48.6 ± 6.0	49.6 ± 3.8
Total social interaction (n)	84.3 ± 5.9	79.3 ± 5.4	83.4 ± 6.4	71.5 ± 6.0	34.6 ± 3.2***	77.9 ± 8.3	80.1 ± 6.9	80.3 ± 7.6	86.6 ± 4.7

$\Delta^9$ -THC,  $\Delta^9$ -tetrahydrocannabinol; CBD, cannabidiol.

Duration and frequency of general sniffing, anogenital sniffing and total social interaction (SI) (SI test, 10 min) with a standard opponent A/JArc mouse after injection with  $\Delta^9$ -THC (0.3, 1, 3 or 10 mg/kg) or CBD (1, 5, 10 or 50 mg/kg) on day 18 of treatment.

Data represent mean ( $\pm$  S.E.M.),  $n = 8-10$ .

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. vehicle group (one-way ANOVA and planned contrasts).

$p < 0.05$ ]. Planned interaction contrasts showed that  $\Delta^9$ -THC (10 mg/kg) increased the latency to enter the dark chamber in the retention trial.

CBD-treated mice also learned to avoid the dark chamber after training, indicated by a main effect of trial [repeated-measures ANOVA:  $F(1, 31) = 37.6$ ,  $p < 0.001$ ] (Fig. 6b). There was no main effect of CBD or a CBD  $\times$  trial interaction.

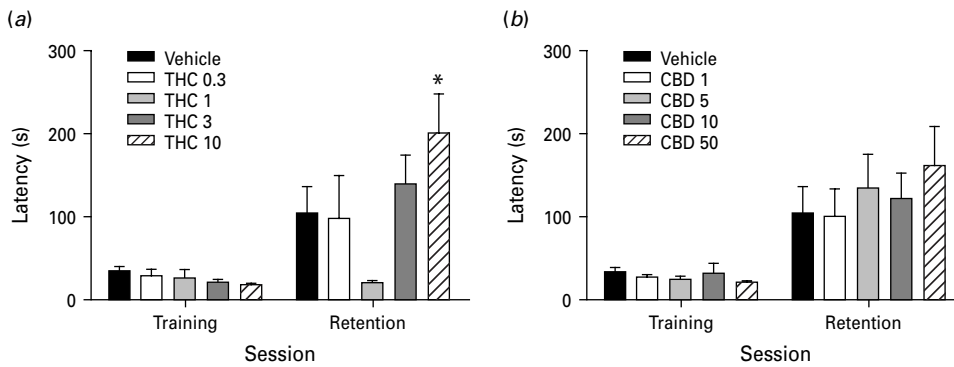
## Discussion

This study is the first to compare acute and chronic effects of two cannabinoids, the prototypical psychoactive constituent  $\Delta^9$ -THC and the non-psychoactive CBD, in a battery of schizophrenia and anxiety-relevant behavioural tests in male adult C57BL/6JArc mice. The major findings were: (i) acute  $\Delta^9$ -THC and CBD enhanced PPI; (ii) chronic CBD, but not  $\Delta^9$ -THC, enhanced PPI; (iii) chronic  $\Delta^9$ -THC and CBD, but not acute CBD, attenuated Dex-induced hyperlocomotion and (iv) chronic, but not acute, CBD produced moderate anxiolytic-like effects.

$\Delta^9$ -THC produced the cannabinoid 'tetrad' of acute effects (Compton *et al.* 1993), consistent with its accepted profile as a partial CB<sub>1</sub> agonist (Pertwee, 2008). In contrast, CBD had no effect on tetrad measures of catalepsy, nociception or locomotion and produced only modest hyperthermia, suggesting that acute CBD does not produce the sedative or hypolocomotor effects of  $\Delta^9$ -THC in C57BL/6JArc mice (Compton *et al.* 1992; Onaivi *et al.* 1990; Varvel *et al.* 2006).

Our novel finding that  $\Delta^9$ -THC (acute) enhanced PPI in C57BL/6JArc mice adds to an inconclusive database on the effects of  $\Delta^9$ -THC on PPI and represents the first such report of CBD effects. The results may be species- and strain-dependent since cannabinoid agonists have previously disrupted PPI in ddY mice (Nagai *et al.* 2006) and rats (Schneider & Koch, 2002), whereas in other animal models,  $\Delta^9$ -THC may disrupt (e.g. in socially isolated rats; Malone & Taylor, 2006) or enhance PPI (e.g. in neuregulin 1 mutant mice; Boucher *et al.* 2007). Importantly, we observed PPI effects in the absence of changes to the startle response except with 10 mg/kg  $\Delta^9$ -THC, which concomitantly enhanced PPI and decreased the startle response.

In contrast, few studies exist on the effects of CBD on PPI, and our findings that acute (1, 5 and 50 mg/kg) and chronic (1 mg/kg) CBD enhanced PPI represent the first such report of CBD's effects. While these findings do not in themselves represent antipsychotic-like effects, it is interesting to speculate on whether such improved baseline sensorimotor gating as that



**Fig. 6.** Latency to enter dark chamber (passive avoidance test, 5 min) 30 min after injection of (a)  $\Delta^9$ -tetrahydrocannabinol (THC) (0.3, 1, 3 or 10 mg/kg) or (b) cannabidiol (CBD) (1, 5, 10 or 50 mg/kg) on days 19 (training) and 20 (retention) of treatment. Data represent mean  $\pm$  S.E.M. ( $n=5-8$ ). \*  $p < 0.05$  vs. vehicle group (repeated-measures ANOVA and planned contrasts).

observed with acute and chronic CBD here might interact with the effect of genetic or pharmacological challenges on PPI. Previously, CBD alone had no effect on PPI in Swiss mice but dose-dependently increased the startle response and reversed an MK-801-induced PPI deficit at 5 mg/kg (Long *et al.* 2006). We aimed to assess whether repeated treatment with CBD could reverse the effects of MK-801, which dose-dependently disrupts PPI in rodents (Mansbach & Geyer, 1989; Varty *et al.* 2001; Yee *et al.* 2004) and mimics the glutamatergic hypofunction thought to occur in schizophrenia. However, MK-801 did not disrupt PPI in our vehicle group. Since mice experienced two PPI sessions prior to testing under MK-801, PPI enhancement may have masked its disruptive effect (Plappert *et al.* 2006). Future studies will confirm whether repeated CBD treatment can reverse the psychotomimetic effect of MK-801 on PPI.

We also investigated the effect of chronic  $\Delta^9$ -THC or CBD on the locomotor response to the psychostimulant Dex. Dex produced a typical increase in spontaneous locomotor activity that was attenuated in mice pre-treated with  $\Delta^9$ -THC (3 or 10 mg/kg) or CBD (50 mg/kg).  $\Delta^9$ -THC-treated mice showed decreased baseline activity, suggesting a functional antagonism by  $\Delta^9$ -THC of Dex-induced hyperlocomotion. In contrast, CBD attenuated Dex-induced hyperlocomotion without altering baseline locomotor activity. Importantly, acute CBD at low (1 mg/kg) or high (50 mg/kg) doses did not alter Dex-induced hyperlocomotion. Although previous studies report that at similar doses acute CBD reduced Dex- and ketamine-induced hyperlocomotion (Moreira & Guimaraes, 2005), our divergent results may be due to genetic differences between mouse strains used. Further studies may elucidate potential cumulative effects of CBD on

brain regions associated with the response to the psychostimulant. Indeed, changes in hypothalamic *c-fos* protein expression and catecholamine release in the nucleus accumbens core following intracerebroventricular CBD administration in rats (Murillo-Rodriguez *et al.* 2006) raise intriguing possibilities for mechanisms that may underlie the effects we observed. Our study is the first to report the effects of chronic CBD on behaviours related to schizophrenia and suggests that it might modulate such measures as PPI and Dex-induced hyperlocomotion.

Another area of therapeutic potential for CBD is in treating anxiety. We observed that low- and high-dose chronic CBD produced anxiolytic-like effects, in contrast to the anxiogenic effects observed with acute and chronic  $\Delta^9$ -THC. The significant effects of  $\Delta^9$ -THC and CBD on distance ratios in the OF and LD tests provide measures of anxiety-related behaviour that are relatively independent of locomotor activity. Interestingly, acute doses of CBD (1 and 50 mg/kg) that produced anxiolytic-like effects in the OF and LD tests after chronic administration had no effect in the EPM. This contrasts with previous reports of anxiolytic-like effects of acute CBD (Campos & Guimaraes, 2008; Guimaraes *et al.* 1994; Moreira *et al.* 2006; Onaivi *et al.* 1990) and may again be due to species and strain effects (Belzung, 2001). In the SI test, which measures anxiety and social withdrawal,  $\Delta^9$ -THC (10 mg/kg) reduced general sniffing and anogenital sniffing frequency, consistent with reports after high dose (van Ree *et al.* 1984) or chronic  $\Delta^9$ -THC treatment (Quinn *et al.* 2008). However, sedation might have reduced the overall activity of our  $\Delta^9$ -THC (10 mg/kg)-treated mice. In contrast, CBD did not alter baseline SI, consistent with previous reports in rats (Malone *et al.* 2009; van Ree *et al.* 1984). Overall, we provide novel

evidence that chronic treatment with CBD produces anxiolytic-like effects in the LD test in C57BL/6JArc mice.

Learning and working memory are behavioural domains of therapeutic interest for schizophrenia, which is marked by cognitive deficits (Elvevag & Goldberg, 2000). In the present study  $\Delta^9$ -THC had no effect on spontaneous alternation or total arm entries in the Y-maze. Interestingly,  $\Delta^9$ -THC did not significantly reduce the number of arm entries, perhaps due to the less aversive nature of the Y-maze compared with the anxiogenic components of the LD or OF apparatus. In the PA test,  $\Delta^9$ -THC dose-dependently increased the latency to enter the aversive stimulus-paired dark chamber, an unexpected effect since higher doses of  $\Delta^9$ -THC generally disrupt short-term memory (Fadda et al. 2004; Quinn et al. 2008; Silva de Melo et al. 2005). Nevertheless,  $\Delta^9$ -THC no longer induced analgesia at this stage of treatment, excluding nociceptive interference with the aversive nature of the electric footshock, and any locomotor effects were controlled for in the statistical analysis. CBD did not affect spontaneous alternation in the Y-maze nor latency to enter the dark chamber in the PA test. These results, combined with previous observations that acute CBD had no effect in the radial arm maze (Lichtman et al. 1995) and that CBD-rich cannabis extracts had no effect in the Morris water maze (Fadda et al. 2006), suggest that CBD does not disrupt memory.

In conclusion, using a comprehensive behavioural phenotyping strategy in order to capture a number of features associated with schizophrenia and anxiety we provide the first report that chronic CBD facilitates sensorimotor gating, exerts anxiolytic-like effects and attenuates Dex-induced hyperlocomotion in C57BL/6JArc mice. Meanwhile, acute, but not chronic,  $\Delta^9$ -THC facilitates PPI. The chronic onset of the CBD effects and their complex dose-dependency are novel findings that raise intriguing avenues for further studies of its therapeutic potential. In particular, investigations using specific behavioural models of schizophrenia, particularly in genetic mouse models, will be useful in the future.

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#### Statement of Interest

None.

#### References

- Belzung C** (2001). The genetic basis of the pharmacological effects of anxiolytics: a review based on rodent models. *Behavioural Pharmacology* **12**, 451–460.
- Boucher AA, Arnold JC, Duffy L, Schofield PR, et al.** (2007). Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of Delta(9)-tetrahydrocannabinol. *Psychopharmacology* **192**, 325–336.
- Bovet D, Renzi P, Oliverio A** (1969). Transfer of responding between different stimuli in rats trained in two avoidance tasks. *Life Sciences* **8**, 575–582.
- Braff DL, Geyer MA, Swerdlow NR** (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* **156**, 234–258.
- 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* **199**, 223–230.
- Compton DR, Johnson MR, Melvin LS, Martin BR** (1992). Pharmacological profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents. *Journal of Pharmacology and Experimental Therapeutics* **260**, 201–209.
- Compton DR, Rice KC, De Costa BR, Razdan RK, et al.** (1993). Cannabinoid structure–activity relationships: correlation of receptor binding and in vivo activities. *Journal of Pharmacology and Experimental Therapeutics* **265**, 218–226.
- Crippa JA, Derenusson G, Zuardi AW, Wichert-Ana L, et al.** (2009). The effect of cannabidiol (CBD), a *Cannabis sativa* constituent, on neural correlates of anxiety: a regional cerebral blood flow study. *Proceedings of the 12th International Congress on Schizophrenia Research* (p. 197). San Diego, CA, USA.
- Crippa JA, Zuardi AW, Garrido GE, Wichert-Ana L, et al.** (2004). Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology* **29**, 417–426.
- D'Souza DC, Abi-Saab WM, Madonick S, Forselius-Bielen K, et al.** (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry* **57**, 594–608.
- Denenberg VH** (1969). Open-field behavior in the rat: what does it mean? *Annals of the New York Academy of Sciences* **159**, 852–859.



- Ellenbroek BA, Cools AR** (2000). Animal models for the negative symptoms of schizophrenia. *Behavioural Pharmacology* **11**, 223–233.
- Elvevag B, Goldberg TE** (2000). Cognitive impairment in schizophrenia is the core of the disorder. *Critical Reviews in Neurobiology* **14**, 1–21.
- Fadda P, Robinson L, Fratta W, Pertwee RG, Riedel G** (2004). Differential effects of THC- or CBD-rich cannabis extracts on working memory in rats. *Neuropharmacology* **47**, 1170–1179.
- Fadda P, Robinson L, Fratta W, Pertwee RG, Riedel G** (2006). Scopolamine and MK801-induced working memory deficits in rats are not reversed by CBD-rich cannabis extracts. *Behavioural Brain Research* **168**, 307–311.
- File SE, Seth P** (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology* **463**, 35–53.
- Griebel G, Rodgers RJ, Perrault G, Sanger DJ** (1999). Behavioural profiles in the mouse defence test battery suggest anxiolytic potential of 5-HT<sub>1A</sub> receptor antagonists. *Psychopharmacology* **144**, 121–130.
- Guimaraes FS, de Aguiar JC, Mechoulam R, Breuer A** (1994). Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. *General Pharmacology* **25**, 161–164.
- Guimaraes VM, Zuardi AW, Del Bel EA, Guimaraes FS** (2004). Cannabidiol increases Fos expression in the nucleus accumbens but not in the dorsal striatum. *Life Sciences* **75**, 633–638.
- Henquet C, Krabbendam L, Spauwen J, Kaplan C, et al.** (2005). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *British Medical Journal* **330**, 11.
- Hodges H** (1996). Maze procedures: the radial-arm and water maze compared. *Brain Research. Cognitive Brain Research* **3**, 167–181.
- Hogg S** (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology, Biochemistry and Behavior* **54**, 21–30.
- Hughes RN** (2004). The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience and Biobehavioral Reviews* **28**, 497–505.
- Karl T, Duffy L, Herzog H** (2008). Behavioural profile of a new mouse model for NPY deficiency. *European Journal of Neuroscience* **28**, 173–180.
- Karl T, Duffy L, Scimone A, Harvey RP, Schofield PR** (2007). Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes, Brain and Behavior* **6**, 677–687.
- Kask A, Nguyen HP, Pabst R, von Horsten S** (2001). Factors influencing behavior of group-housed male rats in the social interaction test: focus on cohort removal. *Physiology and Behavior* **74**, 277–282.
- Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM** (2000). Different effects of nabilone and cannabidiol on binocular depth inversion in man. *Pharmacology, Biochemistry and Behavior* **66**, 175–181.
- Lichtman AH, Dimen KR, Martin BR** (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology* **119**, 282–290.
- Long LE, Malone DT, Taylor DA** (2006). Cannabidiol reverses MK-801-induced disruption of prepulse inhibition in mice. *Neuropsychopharmacology* **31**, 795–803.
- Ludewig K, Geyer MA, Vollenweider FX** (2003). Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. *Biological Psychiatry* **54**, 121–128.
- Malone DT, Jongegan D, Taylor DA** (2009). Cannabidiol reverses the reduction in social interaction produced by low dose Delta(9)-tetrahydrocannabinol in rats. *Pharmacology Biochemistry and Behavior* **93**, 91–96.
- Malone DT, Taylor DA** (2006). The effect of Delta(9)-tetrahydrocannabinol on sensorimotor gating in socially isolated rats. *Behavioural Brain Research* **166**, 101–109.
- Mansbach RS, Geyer MA** (1989). Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. *Neuropsychopharmacology* **2**, 299–308.
- Metz GA, Schwab ME** (2004). Behavioral characterization in a comprehensive mouse test battery reveals motor and sensory impairments in growth-associated protein-43 null mutant mice. *Neuroscience* **129**, 563–574.
- Moore THM, Zammit S, Lingford-Hughes A, Barnes TRE, et al.** (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet* **370**, 319–328.
- Moreira FA, Aguiar DC, Guimaraes FS** (2006). Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. *Progress in Neuro-psychopharmacology and Biological Psychiatry* **30**, 1466–1471.
- Moreira FA, Guimaraes FS** (2005). Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. *European Journal of Pharmacology* **512**, 199–205.
- Morgan CJ, Curran HV** (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry* **192**, 306–307.
- Murillo-Rodriguez E, Millan-Aldaco D, Palomero-Rivero M, Mechoulam R, Drucker-Colin R** (2006). Cannabidiol, a constituent of Cannabis sativa, modulates sleep in rats. *FEBS Letters* **580**, 4337–4345.
- Nagai H, Egashira N, Sano K, Ogata A, et al.** (2006). Antipsychotics improve [Delta]9-tetrahydrocannabinol-induced impairment of the prepulse inhibition of the startle reflex in mice. *Pharmacology Biochemistry and Behavior* **84**, 330–336.
- Onaivi ES, Green MR, Martin BR** (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *Journal of Pharmacology and Experimental Therapeutics* **253**, 1002–1009.
- Pellow S, Chopin P, File SE, Briley M** (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods* **14**, 149–167.

- Pertwee RG** (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: [Delta]9-tetrahydrocannabinol, cannabidiol and [Delta]9-tetrahydrocannabivarin. *British Journal of Pharmacology* **153**, 199–215.
- Plappert CF, Kuhn S, Schnitzler H-U, Pilz PKD** (2006). Experience increases the prepulse inhibition of the acoustic startle response in mice. *Behavioral Neuroscience* **120**, 16–23.
- Powell CM, Miyakawa T** (2006). Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? *Biological Psychiatry* **59**, 1198–1207.
- Quinn HR, Matsumoto I, Callaghan PD, Long LE, et al.** (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.
- Resstel LBM, Joca SRL, Moreira FA, Correa FMA, Guimaraes FS** (2006). Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. *Behavioural Brain Research* **172**, 294–298.
- Rottanburg D, Robins AH, Ben-Arie O, Teggin A, Elk R** (1982). Cannabis-associated psychosis with hypomanic features. *Lancet* **2**, 1364–1366.
- Rung JP, Carlsson A, Ryden Markinhuhta K, Carlsson ML** (2005). (+)-MK-801 induced social withdrawal in rats; a model for negative symptoms of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **29**, 827–832.
- Schneider M, Koch M** (2002). The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behavioural Pharmacology* **13**, 29–37.
- Silva de Melo LC, Cruz AP, Rios Valentim Jr. SJ, Marinho AR, et al.** (2005). Delta(9)-THC administered into the medial prefrontal cortex disrupts the spatial working memory. *Psychopharmacology* **183**, 54–64.
- van Ree JM, Niesink RJ, Nir I** (1984). Delta 1-Tetrahydrocannabinol but not cannabidiol reduces contact and aggressive behavior of rats tested in dyadic encounters. *Psychopharmacology* **84**, 561–565.
- Varty GB, Walters N, Cohen-Williams M, Carey GJ** (2001). Comparison of apomorphine, amphetamine and dizocilpine disruptions of prepulse inhibition in inbred and outbred mice strains. *European Journal of Pharmacology* **424**, 27–36.
- Varvel S, Wiley J, Yang R, Bridgen D, et al.** (2006). Interactions between THC and cannabidiol in mouse models of cannabinoid activity. *Psychopharmacology* **186**, 226–234.
- Yee BK, Chang DT, Feldon J** (2004). The effects of dizocilpine and phencyclidine on prepulse inhibition of the acoustic startle reflex and on prepulse-elicited reactivity in C57BL6 mice. *Neuropsychopharmacology* **29**, 1865–1877.
- Zuardi A, Crippa J, Hallak J, Pinto J, et al.** (2008). Cannabidiol for the treatment of psychosis in Parkinson's disease. *Journal of Psychopharmacology*. Published online: 18 September 2008. doi:10.1177/0269881108096519.
- Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS** (2006a). Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Brazilian Journal of Medical and Biological Research* **39**, 421–429.
- Zuardi AW, Hallak JE, Dursun SM, Morais SL, et al.** (2006b). Cannabidiol monotherapy for treatment-resistant schizophrenia. *Journal of Psychopharmacology* **20**, 683–686.
- Zuardi AW, Rodrigues JA, Cunha JM** (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology* **104**, 260–264.