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Antidepressant-like effects of Δ^9-tetrahydrocannabinol and rimonabant in the olfactory bulbectomised rat model of depression

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ABSTRACT

The endocannabinoid signalling system is widely accepted to play a role in controlling the affective state. Plant cannabinoids are well known to have behavioural effects in animals and humans and the cannabinoid CB_1 receptor antagonist rimonabant has recently been shown to precipitate depression-like symptoms in clinical trial subjects. The aim of the present study was to investigate the behavioural and neurochemical effects of chronic administration of Δ^9-tetrahydrocannabinol (THC) and rimonabant on intact and olfactory bulbectomised (OB) rats used as a model of depression.

As expected, OB rats were hyperactive in the open field. Repeated THC (2 mg/kg, i.p. once every 48 h for 21 days) and rimonabant (5 mg/kg, i.p. once every 48 h for 21 days) reduced this hyperactivity, which is typical of clinically effective antidepressant drugs. In intact animals, chronic THC increased brain derived neurotrophic factor (BDNF) expression levels in the hippocampus and frontal cortex but rimonabant had no effect. Rimonabant increased the levels of phosphorylated extracellular signal regulated kinases (p-$ERKs_{1/2}$) in the hippocampus and prefrontal cortex and THC also increased expression in frontal cortex. OB did not affect BDNF or p-$ERK_{1/2}$ expression in the hippocampus or frontal cortex and in, contrast to the intact animals, neither THC nor rimonabant altered expression in the OB rats.

These findings indicate antidepressant-like behavioural properties of both THC and rimonabant in OB rats although additional studies are required to clarify the relationship between the chronic effects of cannabinoids in other pre-clinical models and in human depression.

1. Introduction

It is estimated by the WHO that depression will be the most important cause of disability in the world by the year 2020 (Murray and Lopez, 1997). The current treatments for depression are only partially effective (Post et al., 2010), necessitating the development of alternative pharmacotherapies. Retrospective studies in cannabis users and small clinical trials have suggested possible therapeutic benefit of cannabinoid use in depression (Gruber et al., 1996). However, other previous studies have suggested that cannabis use may be a contributory cause of depression and suicidal behaviours (Bovasso, 2001). These human findings have their counterpart in animal studies, but the situation is complicated by reports of cannabinoid receptor agonists and antagonists displaying both anxiolytic- and anxiogenic-like effects in rodent models of anxiety and depression (Bambico et al., 2007; Berrendero and Maldonado, 2002; Griebel et al., 2005; Jiang et al., 2005; Viveros et al., 2005). Rimonabant can be classified as a CB1 antagonist but its inverse agonist properties have been well documented by in vitro pharmacological experiments (Howlett et al., 2002). Thus, its biochemical or behavioural effects generally are opposite in direction to effects produced by Δ^9-THC or other CB1 agonists. Rimonabant has been investigated mainly for the treatment of obesity and associated metabolic dysregulation; however, clinical trials showed an increased incidence of psychiatric side effects, mainly anxiety and depression-like states, in obese patients which resulted in rimonabant being withdrawn from the market (Leite et al., 2009).

The involvement of the endocannabinoid system in depression is supported by pre-clinical studies such as that of Hill et al. (2008a) showing increased CB_1 receptor expression and decreased endocannabinoid content in different brain regions in the chronic mild unpredictable stress model; effects that were generally reversed by chronic antidepressant administration. In transgenic animals lacking the cannabinoid CB_1 receptor, there are enhanced behavioural signs of anxiety and depression and an amplified sensitivity to stressful stimuli (Aso et al., 2008). In clinical investigations, Hill et al. (2008b) demonstrated that circulating levels of endocannabinoids were significantly reduced in a population with major clinical depression. Together, these data are consistent with the hypothesis that an endogenous

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endocannabinoid system operates to maintain an appropriate affective state. They are also consistent with the traditional mood-elevating properties of cannabinoids and, perhaps, the anxiety and depression experienced by some patients prescribed the CB_1 receptor antagonist rimonabant as an adjunct for weight reduction (Hill and Gorzalka, 2009).

Animal models of psychiatric disorders represent valuable tools for invasively studying molecular changes in brain tissue which cannot be done in patients (Licinio and Wong, 2004). Thus, olfactory bulbectomy (OB) in rodents has been proposed as a model with high predictive validity for chronic psychomotor agitated depression (Harkin et al., 2003; Kelly et al., 1997). The bilateral removal of the olfactory bulbs creates chronic behavioural, endocrine, neurotransmitter and immunological changes that are qualitatively similar to those occurring in depressed patients (Song et al., 1994a, 1994b; van Riezen and Leonard, 1990). Moreover, in the context of the neurogenesis hypothesis of depression (Duman and Monteggia, 2006), some studies have reported that impaired cell proliferation and/or neuronal degeneration observed following olfactory bulbectomy are reduced by some antidepressants (Jaako-Movits et al., 2006; Jarosik et al., 2007; Keilhoff et al., 2006).

The aim of the present study was to investigate the behavioural and neurochemical effects of chronic administration of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive plant cannabinoid, and the CB1 receptor antagonist rimonabant on intact and OB rats (as a model of depression). Our working hypothesis was that THC would show antidepressant-like activity whilst rimonabant might reflect its clinical side effects and exacerbate the effects of bulbectomy.

2. Methods

2.1. Animals

Male Lister hooded rats (n = 8–10 per group; Charles River UK) weighing 180–300 g were housed four per cage and acclimatised to the laboratory conditions for one week before the experiment. Rats were kept in a temperature-regulated (22 ± 2 °C) room and artificial lighting was provided from 0700 h to 1900 h. Food and water were available ad libitum and each animal was handled daily through the first week. Experimental testing began seven days after the acclimatisation period and was performed during the light cycle.

Animals were assigned to surgical groups with regard to their basal open field activity to ensure a comparable inter-group average activity. After surgery, all animals were allowed two weeks to recover before performing a post-surgical (pre-injection) open field test to verify the effect of the lesion. Body weight was measured daily between 9:00 and 10:00 h.

Efforts were made to minimise animal suffering and to reduce number of animals used. All experiments were carried out in accordance with UK Animals Scientific Procedures Act 1986 and Local Ethical Committee Approval (Project licence 40/2715).

2.2. Bilateral olfactory bulbectomy (OB) surgery

Removal of olfactory bulbs was carried out according to the method of Redmond et al. (1999). Animals were anaesthetised using isofluorane gas (3–4%), mixed with oxygen and nitrous oxide. The top of the skull was shaved and swabbed with an antiseptic. The animals were placed in a stereotaxic instrument (Kopf) using atraumatic-blunt ear bars with local analgesic cream applied to their ends. Topical eye lubricant was used to avoid drying of the cornea during surgery. A midline sagittal incision was made in the skin overlying the skull and the skin was retracted bilaterally. Two burr holes, 2 mm in diameter, were drilled on either side of the skull, 5 mm anterior to bregma, and 2 mm from the midline of the frontal bone overlying the olfactory bulbs. The bulb tissue was removed using a blunt hypodermic needle connected to a water vacuum pump and care was taken not to damage the frontal cortex. The burr holes were packed with haemostatic sponge (Claudius Ash, UK) to prevent blood loss. Animals receiving sham surgery underwent a similar procedure with two burr holes drilled but no removal of olfactory bulbs. All incisions were closed using silk sutures. After surgery, all animals received 1 ml of saline (subcutaneously), and Rimadyl (5 mg/kg, subcutaneously) for post-operative analgesia. Rats were allowed to recover in a standard housing cage (but without sawdust bedding) placed on heated blanket, for 12 h with free access to water and a pot of mash. Rats were then moved to the housing room and were individually housed with twice daily handling (5–10 min each) to reduce the development of aggressive behaviour.

Animals were allowed 14 days to recover from surgery after which they were divided into 4 groups: one sham and three OB-groups (n = 9–10). After recovery, all animals were tested in the open field for lesion verification (hyperactivity) and for equal distribution of the hyperactive rats between treated groups. The OB rats were subdivided into 3 groups according to treatment: OB-vehicle treated, OB-THC treated and OB-SR treated. The sham-operated groups received the same volume of vehicle as OBs.

On the day of killing, rats were quietly taken out of the housing room by the person who handled the animals in the previous weeks, and rats were killed by a blow to the head by sufficient force to cause immediate loss of consciousness followed by decapitation to confirm death (Code of Practice for the Humane Killing of Animals under Schedule 1 to the Animals (Scientific Procedures) Act 1986). Mixed arterio-venous trunk blood was collected in heparinised ice-cold tubes and plasma was prepared by centrifugation (at 1600 *g* for 10 min) and frozen at −80 °C until estimation of corticosterone levels. The brains were rapidly removed from the skull and the anatomical success of the lesions verified visually. One animal was eliminated from the analysis because the surgical damage extended to the frontal cortex. The brains were immediately dissected on ice and brain regions were stored at −80 °C. Finally, both adrenal glands were removed from each rat and weighed.

2.3. Drug administration

Vehicle (Cremophor/ethanol/saline, 1:1:18, 1.0 mg/kg i.p.), THC (Sigma UK, 2.0 mg/kg i.p.) and/or rimonabant (formerly called SR141716-A; 5.0 mg/kg i.p.) were administered to intact, sham or OB animals every 48 h for 21 days (n = 9–10 per group).

The dose and frequency of drug administration was chosen after several preliminary studies by our research group. THC was chosen after several preliminary experiments using different doses (0.5, 1, and 2 mg/kg) on the locomotor activity and protein expression and the 2 mg/kg produced the most consistent effect (data not published). In addition, the used dose of rimonabant (5 mg/kg; every 48 h) was equivalent to human dose of rimonabant that previously used for treatment of obesity (20 mg/kg; daily). Moreover, in our previous study daily i.p. injection of rimonabant (2 mg/kg) did not affect locomotor activity of the same strain of rats (Assareh et al., 2012). The frequency of injection every 48 h was chosen to decrease the stress of daily injection. Rimonabant was generously provided by the NIMH compound synthesis programme. All other drugs and chemicals were purchased from Tocris Bioscience, Bristol, UK or Sigma-Aldrich Company Ltd. Gillingham, UK.

2.4. Locomotor activity

For intact animals, spontaneous locomotor and exploratory behaviour were measured for 65 min following drug or vehicle treatment 10 min after the final injection. The activity box (65 × 45 × 45 cm) was placed in a sound-isolated room with constant illumination of 40 lx and was fitted with two parallel horizontal and vertical infrared

beams 2 cm and 6 cm, respectively, from the floor and activity measured using electronic counters. Cumulative horizontal and vertical counts were recorded for every 5 min, and the data were analysed using one-way ANOVA with Prism 4.0 software followed by a post-hoc Tukey's multiple comparison test where applicable, to compare the total locomotor activity between the groups.

2.5. Open field test

In order to assess neophobia typically produced by OB, the increase in horizontal ambulation and rearing was recorded in a novel open field arena. The open field consisted of a silver circular arena (79 cm in diameter) surrounded by a silver 48 cm high wall. On the experimental day, each animal was removed from the home cage and placed in the centre of the brightly lit (200 lx) novel open field environment. The arena was divided into two zones; the central arena was defined as the area within 10 cm of the outside wall (diameter 59 cm). Locomotor activity was assessed using a computerised video-tracking system (Noldus Etho-Vision Version 3) for 5 min. Total locomotor distance and time spent in both the perimeter and central portion of the arena was measured to determine exploratory behaviour. The arena was cleaned with 20% (v/v) ethanol after removal of each rat. The tests were carried out between 0900 and 1300 h. Open field testing was thus performed three times, once before surgery, a second time before drug injection and finally 24 h after the last injection to minimise the acute behavioural effects of the drugs.

2.6. Protein expression using Western blotting

Expression of BDNF, TrkB, Erk, pErk, CREB, pCREB, transient receptor potential typeV1 (TRPV1) and glucocorticoid receptor GR57 were determined in available brain regions by Western immunoblotting as described previously (Pardon et al., 2005). The following primary antibodies were used: anti-BDNF antibody in blocking solution (1:1000 Santa Cruz Biotechnology), anti-$ERK_{1/2}$ and anti-p-$ERK_{1/2}$ (1:2000 Cell Signalling), anti-CREB (1:2000 Cell Signalling), anti-p-CREB (1:200 Cell Signalling), anti-TRPV1 (1:1000 Tocris), anti-GR57 (1:1000 Affinity Bioreagent) and Anti-β-actin (1:20,000, Sigma). β-actin was used as a loading control. Data were corrected on the basis of β-actin levels to normalise possible differences between each loading volume. The protein levels were presented as percentage changes compared with control treated tissue, designated as 100%, and data were subjected to one-way ANOVA. Post-hoc comparisons were made when appropriate using Tukey's test for multiple comparisons, with $P<0.05$ considered a significant difference.

2.7. Determination of plasma corticosterone levels

Measurement of plasma corticosterone was performed using a commercially available enzyme-immunoassay (EIA) kit (Assay Design, UK) according to the manufacturers' instructions with a lower of detection of approximately 27 pg/ml and intra-assay variation of 6–8% and inter-assay variation 7–13% and less that 1% cross reactivity. All samples were measured in duplicate from the linear (20–80% binding) portion of the curve.

2.8. [^{3}H]-CP55940 binding assay

One half of the frontal cortex was used for determination of cannabinoid receptor density, estimated by measuring, in triplicate, the specific binding of [^{3}H]-CP55940 (specific activity 180.0 Ci/mmol, Perkin Elmer, USA) to a total membrane fraction, as described by Leggett et al. (2004).

2.9. Statistical analysis

Data are presented as means ± standard error of the mean (SEM). Data were analysed by Prism 4 software using one-way ANOVA followed by Bonferroni's post hoc test for analysis of all behavioural measures, protein expression, adrenal gland weight, plasma corticosterone, levels of monoamines and [^{3}H]-CP55940 specific binding to CB_1. Unpaired Student's *t*-test was used to analyse the open field data in the first post-surgical test. The distance moved over the 5-minute open field test and changes in body weight were analysed by two-way repeated measures ANOVA (with lesion/treatment and time as factors) followed by Bonferroni's post hoc test when appropriate. Results were considered statistically significant if $P<0.05$.

3. Results

3.1. Effect of Δ^9-THC on spontaneous locomotor activity

In intact rats, chronic administration of Δ^9-THC (2 mg/kg, i.p.) significantly decreased total locomotor activity (Fig. 1). Chronic treatment with the CB1 receptor antagonist rimonabant (5 mg/kg, i.p.) significantly increased total locomotor activity compared with vehicle-treated rats. Pre-treatment with rimonabant prevented the THC-induced decrease in locomotor activity. Acute administration (30 min) of THC or rimonabant had no significant effect on spontaneous locomotor activity (data not shown).

3.2. Expression of BDNF and $ERK_{1/2}$-like immunoreactivity in the hippocampus and frontal cortex

In intact rats, chronic THC (2 mg/kg) increased BDNF levels in the hippocampus and frontal cortex compared with vehicle-treated rats, but the CB1 receptor antagonist rimonabant alone had no effect on BDNF in either the hippocampus or frontal cortex. The levels of BDNF were still increased when rats were pre-treated with rimonabant before each THC injection (Fig. 2). One of the best characterised neurotrophin-activated signal transduction pathways is the mitogen-activated protein (MAP) kinase cascade, which includes extracellular signal-regulated protein kinase (ERK) as one of the key steps in the pathway (Chang and Karin, 2001; Sweatt, 2001). Treatment with chronic THC, rimonabant and rimonabant plus THC produced no changes in the levels of total extracellular signal regulated kinase ERK_1 and ERK_2 (data not shown). However, all drug-treatments increased the levels of phosphorylated extracellular signal regulated kinase p-ERK_1 in the hippocampus and frontal cortex and p-ERK_2 in frontal cortex. However, only rats pre-treated with rimonabant before each THC injection showed significant increase in p-ERK_2 in the hippocampus (Fig. 3).

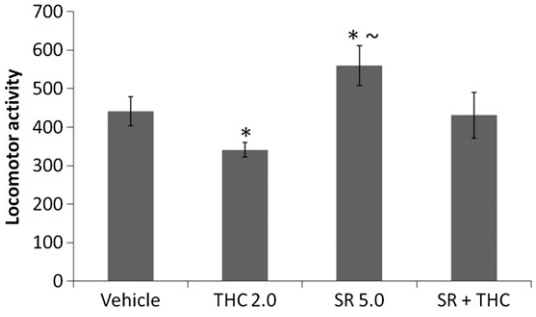

Fig. 1. Effects of chronic administration of THC (2 mg/kg i.p.), rimonabant (SR, 5 mg/kg i.p.) and pre-treatment with rimonabant followed by THC 30 min later, once every 48 h for 21 days. Data are expressed as total number of crossings measured by infrared beams (mean ± SEM). Data were analysed using one-way ANOVA with Prism 4.0 software followed by a post-hoc Tukey's multiple comparison test where applicable to compare the total locomotor activity between the groups (n = 7–8 per group), *$p<0.05$ vs. Vehicle and ~$p<0.05$ vs. THC 2.0.

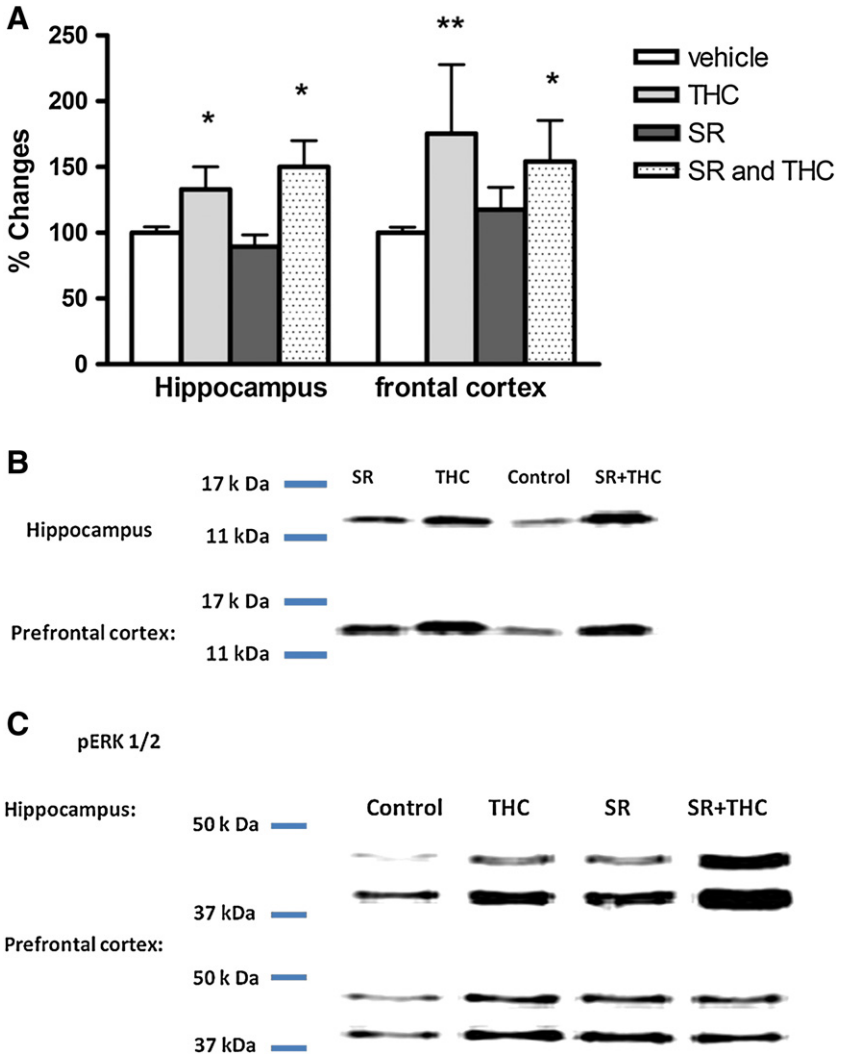

Fig. 2. A) Effects of chronic treatment with 2 mg/kg THC, 5 mg/kg rimonabant (SR) and SR + THC (5 mg/kg rimonabant + 2 mg/kg THC) compared to vehicle-treated controls on changes in BDNF protein levels in the hippocampus and prefrontal cortex of intact rats. B) Illustration of BDNF protein bands in the hippocampus and prefrontal cortex. C) Illustration of p-ERK$_{1/2}$ protein bands in the hippocampus and prefrontal cortex. Data are expressed as percentage changes compared with control group and presented as mean ± SEM. One-way ANOVA followed by a post-hoc Tukey's multiple comparison test where applicable for inter-group comparisons (n = 7–8 per group; $^{*}p<0.05$, $^{**}p<0.01$).

3.3. Effect of chronic administration of THC and rimonabant on olfactory bulbectomised (OB) rats

3.3.1. Body weight

All rats gained weight over the course of the experiment (Two-way RM ANOVA, $F_{(2, 68)} = 680.2$; $P<0.0001$, data not shown). All OB groups weighed significantly less than sham controls on both 15 days and 37 days after surgery, irrespective of drug treatment such that there was a main effect of OB (Two-way RM ANOVA $F_{(3, 68)} = 9.4$; $P = 0.0001$). Bonferroni post hoc testing confirmed that all OB rats weighed the same on equivalent days whether treated with THC, rimonabant or saline.

3.3.2. Open field test

In the open field test, post-surgery but prior to drug injection, the OB rats showed the expected hyperactivity in an open field 14 days after surgery. A two way ANOVA of the distance moved over the 5-minute open field test revealed a significant main effect of time [$F_{(4, 144)} = 5.2$, $P = 0.0006$] and a significant effect of lesion [$F_{(1, 144)} = 17.57$, $P = 0.0002$] but no significant interaction [$F_{(1, 144)} = 0.44$, $P = 0.78$] (Fig. 4A). In addition, there was a significant increase in the total distance moved (unpaired Student's *t*-test, $P = 0.0002$) (Fig. 4B). The time spent in the more aversive central zone was calculated as a percentage of the total time in the arena. OB rats spent less time in the central zone compared to sham treated rats (unpaired Student's *t*-test, $P = 0.002$) (Fig. 4C).

The number of rears was also significantly increased in the OB group (unpaired Student's *t*-test, $P = 0.02$) (Fig. 4D).

In the second open field test, post-surgery after drug treatment, two way ANOVA of the distance moved during each consecutive 5 min epoch during the open field test revealed a significant main effect of time [$F_{(4, 100)} = 10.38$, $P<0.0001$] and a significant effect of treatment [$F_{(2, 100)} = 12.28$, $P = 0.0002$] but the interaction was not significant [$F_{(8, 100)} = 0.91$, $P = 0.51$]. Post hoc analysis showed a significant difference between the OB THC-treated group and the OB vehicle-treated group at the first minute (Fig. 5A).

Analysis of the total distance moved by OB rats by one-way ANOVA indicated significant changes [$F_{(2, 27)} = 12.28$, $P = 0.0002$], such that the Bonferroni post-hoc test showed that both THC and rimonabant reduced the distance moved by OB rats ($P<0.001$, $P<0.01$ respectively) compared to vehicle-treated OB rats (Fig. 5B).

There were no significant differences in the percentage of the total time spent in the central zone [$F_{(2, 27)} = 0.49$, $P = 0.62$] or in the total

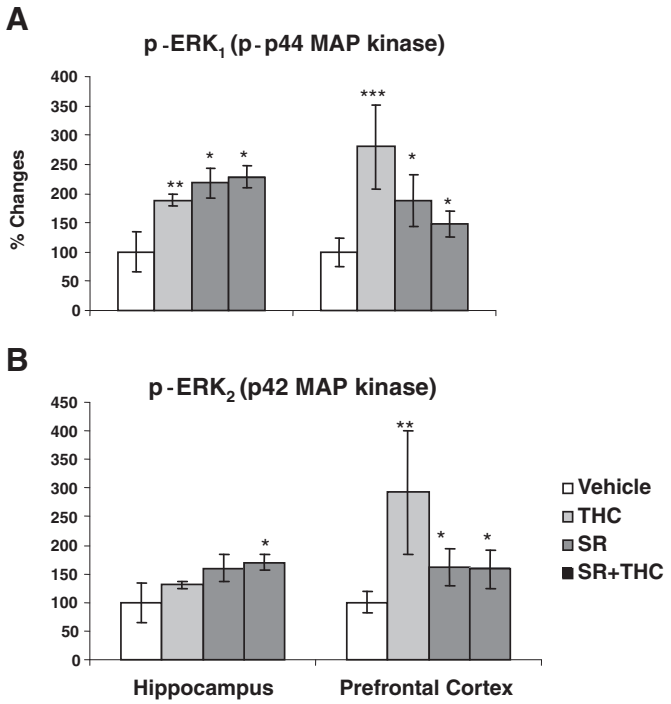

Fig. 3. Effects of chronic treatment with 2 mg/kg THC, 5 mg/kg rimonabant (SR) and SR + THC compared to vehicle-treated controls on changes in phosphorylated or activated extracellular signal regulated kinase (A) p-ERK$_1$ and (B) p-ERK$_2$ protein levels in the hippocampus and prefrontal cortex of rats. Data are expressed as percentage changes compared with control group and presented as mean ± SEM. One-way ANOVA was performed with Prism 4.0 software followed by a post-hoc Tukey's multiple comparison test where applicable for inter-group comparisons (n = 7–8 per group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

number of rears [$F_{(2, 27)} = 1.46$, $P = 0.25$] after treatment with either THC or rimonabant (Fig. 5C and D).

3.3.3. Protein expression

OB did not affect BDNF expression significantly in the hippocampus or frontal cortex and in, contrast to the intact animals, neither THC nor rimonabant affected expression in the OB rats (one way ANOVA, $F_{(3, 37)} = 0.54$, $P = 0.66$; $F_{(3, 37)} = 0.27$, $P = 0.85$ respectively). While there was a trend towards a reduction in the expression of the BDNF receptor TrkB in the hippocampus (76.6 ± 12.6% sham) and frontal cortex (57.1 ± 9.0% sham) of OB rats which tended to be reversed by THC and rimonabant, this failed to reach significance (one way ANOVA, $F_{(3, 34)} = 0.55$, $P = 0.65$; $F_{(3, 36)} = 0.87$, $P = 0.47$ respectively).

There were no changes due to olfactory bulbectomy or THC/rimonabant treatment in total ERK$_{1/2}$ expression in the hippocampus or frontal cortex or expression of the activated forms (pERK$_{1/2}$) in response to any treatment.

In the hippocampus, there were no significant changes in total CREB, pCREB or the pCREB/CREB ratio (one way ANOVA, $P = 0.87$, $P = 0.74$, $P = 0.35$ respectively). Likewise, there were no significant changes in total CREB, pCREB or pCREB/CREB ratio (one way ANOVA, $P = 0.70$, $P = 0.60$, $P = 0.92$ respectively) in the frontal cortex.

One-way analysis of variance showed no significant changes in TRPV1 expression in the hippocampus (one way ANOVA, $F_{(3, 37)} = 0.02$, $P = 0.995$). While there was a strong trend towards reduction in TRPV1 expression in the frontal cortex of OB rats (64.2 ± 12.4% sham) which tended to be reversed by rimonabant (115.1 ± 25.0% sham), once again this failed to reach significance (one way ANOVA, $F_{(3, 34)} = 1.8$, $P = 0.160$).

One-way analysis of variance showed no significant changes in expression of the corticosteroid receptor GR57 in the hippocampus or frontal cortex (one way ANOVA, $F_{(3, 31)} = 1.66$, $P = 0.20$, and $F_{(3, 35)} = 0.85$, P 0.48 respectively) due to bulbectomy or to drug treatment in the OB rats.

3.3.4. Adrenal weight and plasma corticosterone

OB significantly reduced adrenal gland weight (to 51.17 ± 3.80 g in the OB-vehicle-treated group versus 65.53 ± 3.42 g in the sham-vehicle-treated group) but neither THC (53.57 ± 3.37 g) nor rimonabant (49.84 ± 2.51 g) reversed this effect, such that there was a main effect of OB (one way ANOVA, $F_{(3, 37)} = 4.76$, $P = 0.007$.

There was no significant effect of removal of the olfactory bulbs on plasma corticosterone levels (118.72 ± 34.14 ng/ml in OB-vehicle-treated group versus 114.03 ± 26.68 ng/ml in the sham-vehicle-treated group). Also, chronic treatment with neither THC (108.20 ± 19.99 ng/ml) nor rimonabant (107.85 ± 26.08 ng/ml) had any effect on corticosterone plasma levels of olfactory bulbectomised rats.

3.3.5. Cannabinoid CB$_1$ receptor density

This was estimated in the frontal cortex by measuring [^{3}H]-CP55940 binding to a total particulate membrane preparation. Specific binding was unaffected by either OB or chronic drug treatment of OB rats with THC or rimonabant (data not shown).

4. Discussion

In the present study, acute administration of THC (2.0 mg/kg) failed to affect total locomotor activity in intact animals. However, chronic THC treatment (2.0 mg/kg) in intact animals inhibited spontaneous locomotor activity and this effect was completely reversed by the cannabinoid CB$_1$ antagonist rimonabant, perhaps indicating a role for CB$_1$ receptors in modulating motor behaviours as previously reported (Barg et al., 1995), although a CB receptor-independent physiological antagonism due to the opposing effects of the two drugs is possible. Several previous studies, however, have shown that administration of cannabinoids can stimulate locomotion at lower doses but inhibit at higher doses (Rodríguez de Fonseca et al., 1998; Sanudo-Pena et al., 2000; Sulcova et al., 1998).

The present data show that treatment of intact rats with chronic THC produced significant increases in expression of BDNF and its upstream regulator/downstream effector p-ERK$_{1/2}$ in the hippocampus and frontal cortex. These data are in agreement with previous immunostaining data (Butovsky et al., 2005) which reported high levels of BDNF in the hippocampus following chronic treatment with THC. In this instance, pre-treatment with the CB$_1$ antagonist rimonabant failed to reverse the changes in the BDNF levels, suggesting that THC's effect on neurotrophin expression is not CB$_1$ receptor-dependent.

In the present study, the olfactory bulbectomy model of depression was used to study the potential antidepressant activities of the cannabinoid receptor agonist and antagonist.

The OB rats gained less weight than sham-operated rats which may be related to a loss of olfactory sensation or to lesion-induced changes in hypothalamic function. The reduction in body weight in OB rats was consistent with previous studies (Hellweg et al., 2007; van Riezen and Leonard, 1990) as well as human studies in depressed patients (Hopkinson, 1981). The change in body weight of OB rats was not reversed by THC or rimonabant administration, which suggests that it may occur by a mechanism independent of the changes in behaviour which were sensitive to drug treatment.

In the open field test, OB rats showed the expected hyperactivity both in the form of increased total distance moved 14 days after surgery and in less time being spent in the more aversive central zone and more time in the periphery; this corresponds to the reported general increase of exploration and neophobic pattern of behaviour of OB rats (Leonard and Tuite, 1981). Unexpectedly, chronic administration of either THC

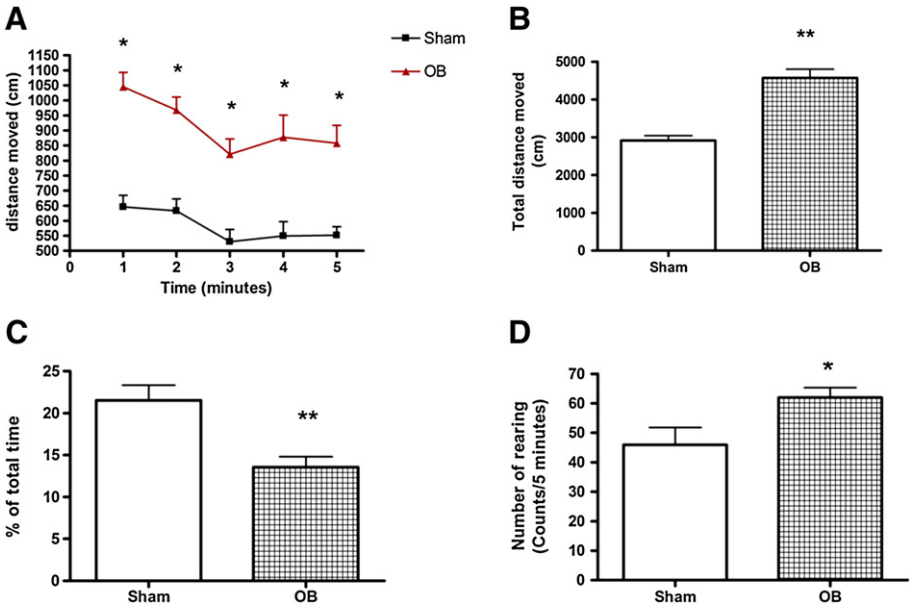

Fig. 4. Behavioural performance in the open field test performed 14 days after olfactory bulbectomy (OB).(A) Distance moved over the 5-minute duration in 1-minute time bins. (B) Total distance moved. (C) Percentage of time spent by rats in the central zone. (D) Rearing Frequency. Results show that the OB rats were hyperactive in the open field compared to sham controls. Data are presented as mean ± SEM and analysed by unpaired Student's *t*-test except in (A) A two way ANOVA of the distance moved over 5 min was done using GraphPad Prism 4 software. *P<0.05, **P<0.001 versus sham operated rats. N = 9–10, OB: olfactory bulbectomy.

or rimonabant reversed the increased activity in the final open field test, which could be interpreted as an antidepressant-like action.

The OB rats showed increased numbers of rears in the open field 14 days after surgery, also consistent with their enhanced tendency to escape the arena, and this was significantly reduced after chronic treatment with rimonabant and also trended to be reduced after THC treatment. These changes in rearing are consistent with those reported by Song and Leonard (1994) who demonstrated that rearing scores were increased in OB rats and were decreased by chronic treatment with two serotonin selective reuptake inhibitor antidepressants (sertraline and fluvoxamine). All the OB rats spent more time in the central zone in the final open field test although they spent less time in the same zone in the open field test performed 14 days after surgery. Habituation might explain this behaviour as the rats were exposed to the open field three times in total. Reversal of the OB-induced hyperactivity in the open field could be suggested to be an artefact due to non-specific motor effects of THC, but there was no reduction in rearing, which would be expected if this was the case, and there is no evidence for a hypolocomotor effect of rimonabant at the dose used (Przegalinski et al., 2005). In fact, in the present study, in intact animals, rimonabant increased locomotor activity (Fig. 1), suggesting that the reversal of OB-induced hyperactivity is not a non-specific motor effect.

These behavioural data parallel those generated in studies of chronic antidepressant treatment (Jancsar and Leonard, 1981; Kelly et al., 1997) and the antidepressant-like effect of THC is consistent with previous reports using THC and cannabinoid agonists in other models of depression and anxiety (Bambico et al., 2007; Fokos and Panagis, 2010). It was, particularly unexpected that rimonabant

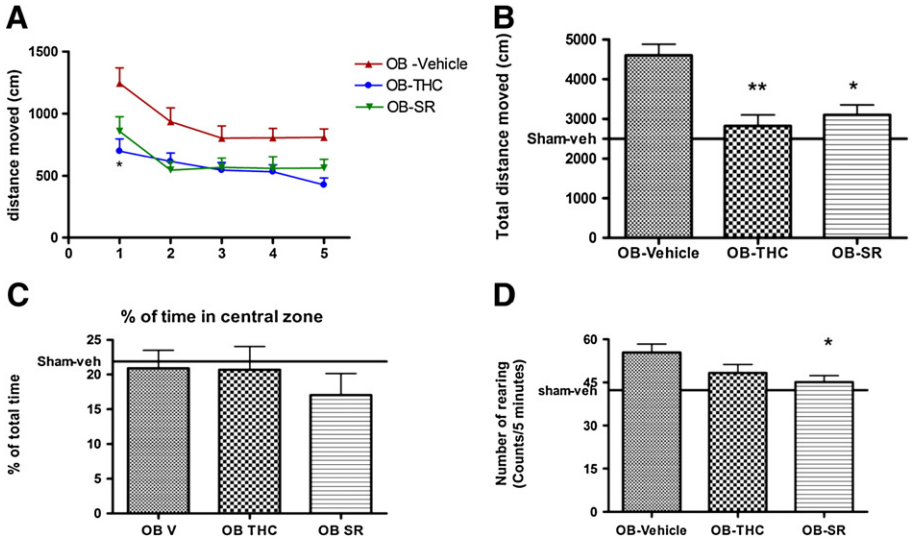

Fig. 5. Performance in the open field after chronic treatment of OB rats by THC or rimonabant.(A) Distance moved over the 5-minute duration in 1-minute time bins. (B) Total distance moved. (C) Percentage of time spent by rats in the central zone. (D) Rearing Frequency. Treatment with THC or rimonabant reduced the effects of OB in the open field. Data are presented as mean ± SEM and analysed by one way ANOVA except in (A) A two way ANOVA of the distance moved over 5 min was done using GraphPad Prism 4 software. *P<0.05, **P<0.001 versus sham operated rats. OB: olfactory bulbectomy, SR: rimonabant.

treatment should produce antidepressant-like behavioural effects since it has been withdrawn from the market as an appetite suppressant, due to the development of anxiety and depression-like symptoms in some clinical trial participants (Hill and Gorzalka, 2009). However, evidence from other animal studies is consistent with the current finding that both cannabinoid receptor antagonists and activators of the endocannabinoid system can have antidepressant-like properties (Griebel et al., 2005; Takahashi et al., 2008; Tzavara et al., 2003a; Witkin et al., 2005). The similar effects of the cannabinoid agonist and antagonist in the OB model could be explained in various ways. For instance, the drugs could target a common site unrelated to the CB_1 receptor, such as PPARs (O'Sullivan, 2007) or GPR55 (Ross, 2009). In addition, rimonabant has been reported to increase serotonin efflux and produce elevations in dopamine and norepinephrine levels in the prefrontal cortex (Tzavara et al., 2003a). Indeed the neurochemical effects of CB_1 receptor agonists and antagonists are not necessarily in opposition, as rimonabant and a low dose of a CB_1 receptor agonist both have been shown to increase acetylcholine release in the hippocampus (Tzavara et al., 2003b). Although our own experiments in OB rats have produced some evidence for changes in striatal dopamine and hippocampal 5HT turnover following rimonabant treatment, there were no parallel THC-induced changes (ElBatsh, unpublished), arguing against a common monoamine-dependent mechanism.

According to the neurotrophic theory of depression (Nestler et al., 2002), BDNF and CREB levels might be expected to be reduced after OB and increased after chronic treatment with THC and rimonabant if they are, indeed, antidepressant. Although, in intact animals, there was evidence for THC-induced increases in BDNF and both THC and rimonabant-induced enhancements in MAP kinase expression, this was not the case in the OB animals, There were no changes in BDNF in any group in either brain region, although there was a trend towards a reduction in expression of the neurotrophin receptor TrkB in the hippocampus and frontal cortex of OB rats which appeared to be reversed by THC and rimonabant treatment. This apparent lack of changes in BDNF in OB rats is consistent with reports of Van Hoomissen et al. (2003) and, indeed, Hellweg et al. (2007) reported significant increases in hippocampal and frontal cortical BDNF protein in OB mice.

Although Nibuya et al. (1995) reported that BDNF upregulation is a common feature of antidepressant treatments, more recent data do not support this notion (Hanson et al., 2011). In accordance with these findings, BDNF may be a target for antidepressants but certainly not the sole mediator of the clinical condition. This then implies that BDNF downregulation is not a necessary condition for the development of a depression like phenotype or its reversal a prerequisite of antidepressant activity.

CREB expression and function are reported to be reduced in models of depression and to be reversed by antidepressants (Li et al., 2009; Qi et al., 2008; Xu et al., 2006), although, Romeas et al. (2009) reported that OB rats showed increased phosphorylation of striatal CREB. In the present study, there was no evidence for any effect of OB surgery or of the cannabinoid receptor ligands on CREB/phospho-CREB expression, thereby producing no support for the possibility of the drugs having an antidepressant action via changes in CREB-controlled gene expression.

Human studies in depressed suicide victims have shown reductions in phospho-ERKs in the frontal cortex and hippocampus (Dwivedi et al., 2001; Feng et al., 2003; Qi et al., 2006) although phosphorylation of $ERK_{1/2}$ has been reported to be significantly enhanced in the prefrontal cortex of chronically stressed animals (Trentani et al., 2002). In the present study, despite chronic treatment with THC increasing phospho-$ERK_{1/2}$ expression in the hippocampus and frontal cortex of intact rats, there were no changes either in the expression of total $ERK_{1/2}$, or p-$ERK_{1/2}$ in the hippocampus and frontal cortex of OB rats, or due to treatment with THC or rimonabant. Although the mechanism underlying the differences in drug effects induced by bulbectomy is unclear, the lack of effect of THC and rimonabant on ERK and CREB phosphorylation in the OB rats provides a clear disconnection between the drugs' antidepressant-like behavioural effects and changes in these intracellular signalling systems.

Gourley et al. (2008) reported that modelling depression, using corticosterone administration, revealed reductions in ERK phosphorylation in the dentate gyrus which were reversed by antidepressant treatment but in a brain region-specific fashion with other areas showing no, or even opposite responses. Such data emphasise the fact that drug responses can vary dramatically between different parts of the brain and depend strongly on the state of the animals, whether naive or modified to model the depressed state.

TRPV1 receptors act as endocannabinoid receptor ion channels and Di Marzo et al. (2008) have reviewed the evidence which suggests that hippocampal TRPV1 is implicated in a number of neuropsychiatric disorders. In the present study, hippocampal TRPV1 expression was not statistically altered in any experimental group although, in the frontal cortex, there was a trend for reduced TRPV1 in the OB vehicle-treated and OB THC-treated groups and this reduction appeared to be reversed in OB rats treated with rimonabant. This clearly warrants further investigation.

Some expected endocrine changes, illustrated by reduced adrenal gland weight, were evident in the OB rats but this was not attenuated by chronic treatment with either rimonabant or THC. In addition, there was no difference in plasma corticosterone or hippocampal glucocorticoid receptor (GR) expression. This lack of change in plasma corticosterone in OB rats confirms the findings of Broekkamp et al. (1986) but is at variance with the report of Cairncross et al. (1979). Even when basal levels of corticosterone have been reported to be increased in OB animals it is notable that antidepressant drugs have not always been found to produce a reversal (Bissette, 2001; Marcilhac et al., 1999; Xu et al., 2010).

CB_1 receptor protein expression, binding density and signal transduction are reported to be increased in the prefrontal cortex of depressed suicide victims (Hungund et al., 2004). In the present study, however, there was no change in CB_1 receptor density in the OB rats treated with vehicle, THC or rimonabant, compared with sham controls, although it was expected that CB_1 receptor densities might decrease and increase respectively after chronic THC and rimonabant administration. Recently, Rodriguez-Gaztelumendi et al. (2009) reported an increase in CB_1 receptor density and CB_1 receptor-mediated [^{35}S] GTPγS binding in prefrontal cortex of OB rats which was decreased by chronic fluoxetine treatment. These inconsistencies may result from differences in rat strain used or difference in methodology. Moreover, the changes in CB_1 receptor density may be brain region-specific and the tissue sample of whole frontal cortex which was used for membrane preparations in this study could mask the changes localised in certain sub-regions. It remains unclear whether the effects of rimonabant are attributable to the blockade of endogenous cannabinoid tone, or to inverse agonist properties of rimonabant. Moreover, there is evidence suggesting that this inverse agonism is not mediated by the CB1 receptor (Pertwee, 2005). The lack of changes in CB1 receptors may suggest inverse agonist action of rimonabant. In addition, rimonabant could target other sites unrelated to the CB1 receptor, such as PPARs (O'Sullivan, 2007) or GPR55 (Ross, 2009).

In conclusion, our studies indicate antidepressant-like properties of both THC and rimonabant, as demonstrated by their common ability to reverse the enhanced aversion to an open field arena following OB. However, although the drugs modified the expression of BDNF and its signalling-related ERKs in intact rats, such changes were no longer apparent when the animals were bulbectomised, suggesting that the behavioural modifications were not related to neurotrophin expression and signalling which is often assumed to be a common property of clinically effective antidepressants. The reported precipitation of anxiety and depression in some obese patients treated with rimonabant, suggests that rimonabant could have a different

effect in non-depressed obese and depressed patients. This emphasises the need for additional long-term multi-dose studies of THC and rimonabant in obese rats and/or models of affective disease.

Disclosure/Conflict of interest

The authors have no conflicts of interest to disclose.

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