Lack of evidence for appetitive effects of Δ^9-tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents

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Data on the ability of Δ^9-tetrahydrocannabinol (THC) to modify reward processes in experimental animals are inconsistent. This study examined the effects of Δ^9-THC on brain reward function using the rate–frequency curve shift paradigm of intracranial self-stimulation (ICSS) and the conditioned place preference (CPP) paradigm. In ICSS tests, rats were implanted with electrodes into the medial forebrain bundle. After brain stimulation reward thresholds stabilized, rats received intraperitoneal injections of Δ^9-THC (0, 0.5, 1 and 2 mg/kg) or the CB_1 receptor antagonist SR141716A (0, 0.02 mg/kg) and Δ^9-THC (0, 2 mg/kg). The two highest doses of Δ^9-THC significantly increased the threshold ICSS frequency. SR141716A reversed the action of Δ^9-THC (2 mg/kg), without affecting reward thresholds by itself. In the CPP test, mice received intraperitoneal injections of Δ^9-THC (0, 1 or 3 mg/kg). Δ^9-THC showed neither statistically significant preference nor aversion in either of the doses tested. These findings indicate that Δ^9-THC, in contrast to other drugs of abuse, does not facilitate ICSS or support CPP under the present experimental conditions, but rather has a dose-dependent inhibitory influence on ICSS. *Behavioural Pharmacology* 18:311–319 © 2007 Lippincott Williams & Wilkins.

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Introduction

Most drugs abused by humans are thought to produce their effects by acting on brain reward pathways. In most cases these drugs also yield reinforcing and rewarding effects in experimental animals as assessed by the intracranial self-stimulation (ICSS), self-administration and conditioned place preference (CPP) paradigms. Although marijuana is considered to be one of the oldest and most widely used recreational drugs, our knowledge and understanding of the way marijuana and its main psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), act in the central nervous system to exert their reinforcing/rewarding effects is far from complete.

Animal studies indicate that Δ^9-THC and other synthetic cannabinoid agonists can induce both appetitive and aversive effects, using several paradigms and under various experimental conditions (Gardner and Vorel, 1998; Gardner, 2002; Maldonado, 2002; Maldonado and Rodriguez de Fonseca, 2002; Tanda and Goldberg, 2003; Justinova *et al.*, 2005). In general, the results of animal studies suggest that Δ^9-THC and other synthetic cannabinoids do not produce effects typical of other drugs of abuse in preclinical models of dependence and addiction.

Drugs that have a high abuse liability generally enhance the rewarding effects of electrical brain stimulation, whereas drugs that have a low abuse liability usually fail to enhance brain stimulation reward (Wise, 1996). Thus, the ICSS paradigm has been extensively used to measure the reward-related properties of addictive drugs. Only a few studies, however, have been conducted on the effects of Δ^9-THC and other cannabinoids in the ICSS paradigm (see Table 1). Importantly, different effects have been observed with different strains of animals after the administration of Δ^9-THC or other CB_1 receptor agonists and cannabinoid modulators. According to Gardner and colleagues, low doses of Δ^9-THC decrease the ICSS threshold in Lewis, but not in Sprague–Dawley and Fisher rats (Gardner *et al.*, 1988, 1989; Gardner and Vorel, 1998). In contrast, Stark and Dews (1980) and Kucharski *et al.* (1983) failed to see an enhancement of brain stimulation reward with Δ^9-THC and other cannabinoid drugs. Similarly, Arnold and colleagues (2001) have reported that the cannabinoid 1 (CB_1) receptor agonist CP 55,940 does not affect the rewarding efficacy of self-stimulation. In an analogous manner, we have recently shown that the CB_1 receptor agonists WIN 55,212-2, CP 55,940 and HU-210, as well as the indirect cannabinoid agonists PMSF, AM-404, OMDM-2 and URB-597, either

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Table 1 Evaluation of the reinforcing/rewarding properties of cannabinoids in experimental animals

Behavioral Model	Cannabinoid drug	Dose	Effect	Animal	References
CPP	Δ^9-THC	1 mg/kg	CPA	Long–Evans rats	Lepore *et al.* (1995)
		2 and 4 mg/kg	CPP		
		2 and 4 mg/kg (wash-out period)	CPA		
		1 mg/kg	CPP		
	Δ^9-THC	1 mg/ml	CPA	Lewis and Sprague–Dawley rats	Parker and Gillies (1995)
	CP 55,940	100 μg/kg	CPA	Wistar rats	McGregor *et al.* (1996)
	Δ^9-THC	1.5 mg/kg	–	Sprague–Dawley rats	Sañudo-Peña *et al.* (1997)
		15 mg/kg	CPA		
	WIN 55,212-2	0.3–1 mg/kg	CPA	Wistar rats	Chaperon *et al.* (1998)
	SR141716A	Up to 10 mg/kg	– (reversing effect on WIN 55,212-2)		
	Δ^9-THC	20 mg/kg	CPA	CD1 mice	Hutcheson *et al.* (1998)
	Δ^9-THC	1 and 1.5 mg/kg	CPA	Wistar rats	Mallet and Benninger (1998)
	AEA	Up to 16 mg/kg	–		
	HU-210	20, 60, 100 μg/kg	CPA	Lister Hooded rats	Cheer *et al.* (2000)
	Δ^9-THC	1.5 mg/kg	CPA		
	Δ^9-THC	5 mg/kg	CPA	CD1 mice	Valjent and Maldonado (2000)
		1 mg/kg	–		
		5 mg/kg (not standard protocol-pretreatment)	–		
		1 mg/kg	CPP		
	CP 55,940	20 μg/kg	CPP	Wistar rats	Braida *et al.* (2001)
	SR141716A	0.5 mg/kg	– (reversing effect on CP 55,940)		
	Δ^9-THC	5 mg/kg	–	Dynorphin-deficient mice	Zimmer *et al.* (2001)
	Δ^9-THC	0.075–0.75 mg/kg	CPP	Wistar rats	Braida *et al.* (2004)
	SR141716A	0.25–1 mg/kg	(Reversing effect on Δ^9-THC)		
	Δ^9-THC	1 mg/kg	CPP	A_{2A}KO and wild-type mice	Soria *et al.* (2004)
		5 mg/kg	CPA		
	URB-597	0.03–0.3 mg/kg	–	Wistar, Sprague–Dawley rats	Gobbi *et al.* (2005)
	AM-404	1.25–10 mg/kg	CPP	Rats (anxiety models)	Bortolato *et al.* (2006)
			–		
	Δ^9-THC	0.1 mg/kg	CPP	Sprague–Dawley rats	Le Foll *et al.* (2006)
ICSS	Δ^9-THC, nabilone, canbisol	0.12–10 mg/kg	↑ threshold	Long–Evans rats	Stark and Dews (1980)
	Levonantradol	0.2, 0.3 mg/kg	↑ threshold	Albino CDF rats	Kucharski *et al.* (1983)
	Δ^9-THC	1.5 mg/kg	↓ threshold	Lewis rats	Gardner *et al.* (1988)
	Δ^9-THC	1 and 1.5 mg/kg	↓ threshold	Lewis rats	Gardner *et al.* (1989)
	Δ^9-THC	1 mg/kg	–	Sprague–Dawley rats	Lepore *et al.* (1996)
			–	Fischer 344 rats	
			↓ threshold	Lewis rats	
	CP 55,940	10, 25, 50 μg/kg	–	Lewis rats	Arnold *et al.* (2001)
	SR141716A	1, 3, 10 mg/kg	↑ threshold	Sprague-Dawley rats	Deroche-Gamonet *et al.* (2001)
	WIN 55,212-2	0.1, 0.3 and 1 mg/kg	↑ threshold	Sprague–Dawley rats	Vlachou *et al.* (2003)
	WIN 55,212-2	0.1, 0.3, 1, 3 mg/kg	↑ threshold	Sprague–Dawley rats	Vlachou *et al.* (2005)
	CP 55,940	10, 30, 56, 100 μg/kg	(Reversing effect on agonists)		
	HU-210	10, 30, 100 μg/kg			
	SR141716A	0.02 mg/kg			
	AMG-3	1, 2, 4, 8 mg/kg	↑ threshold	Sprague–Dawley rats	Antoniou *et al.* (2005)
	PMSF	15, 30, 60 mg/kg	↑ threshold	Sprague–Dawley rats	Vlachou *et al.* (2006)
	OMDM-2	3, 10, 30 mg/kg	(Reversing effect on modulators)		
	URB-597	0.3, 1, 3 mg/kg			
	SR141716A	0.02 mg/kg			

AEA, anandamide; CPA conditioned place aversion (avoidance), CPP, conditioned place preference; ICSS, intracranial self-stimulation; Δ^9-THC, Δ^9-tetrahydrocannabinol; –, no effect; ↑, increase; ↓, decrease.

do not affect or increase ICSS threshold, depending on the dose used (Vlachou *et al.*, 2003, 2005, 2006).

In the CPP procedure, drug administration is associated with characteristics of a specific environment in a manner that allows the drug to produce appetitive or aversive properties (for reviews, see Hoffman, 1989; Tzschentke, 1998). A number of studies with Δ^9-THC and other cannabinoid agonists have shown that they elicit either CPP (Lepore *et al.*, 1995; Valjent and Maldonado, 2000; Braida *et al.*, 2001; Braida *et al.*, 2004; Soria *et al.*, 2004; Bortolato *et al.*, 2006; Le Foll *et al.*, 2006) or conditioned place aversion (Parker and Gillies, 1995; McGregor *et al.*, 1996; Sañudo-Peña *et al.*, 1997; Chaperon *et al.*, 1998; Hutcheson *et al.*, 1998; Mallet and Benninger, 1998; Cheer *et al.*, 2000) or no effect (Zimmer *et al.*, 2001; Gobbi

et al., 2005), under different methodological conditions and manipulations (see Table 1). Valjent and Maldonado (2000) have shown that reducing the possible dysphoric effects of the first exposure of the animals to Δ^9-THC, by a priming injection 24 h before the first conditioning session, allows the subsequent administration of Δ^9-THC to support CPP in mice.

Taking into consideration the disparate results of the above studies, and as the interest in therapeutic applications of cannabinoids and, more specifically, Δ^9-THC has been restrained by the fear of a potentially harmful abuse liability, the aim of this study was to further examine the possible reinforcing/rewarding properties of Δ^9-THC in the ICSS and CPP paradigms in rats and mice, respectively. Finally, the involvement of CB_1 receptors in tentative Δ^9-THC-induced effects was investigated by pretreating the animals with the CB_1 receptor antagonist SR 141716A.

Methods

Intracranial self-stimulation

Subjects and surgery

Male Sprague–Dawley rats ($n = 36$) weighing 300–350 g at the time of surgery were used. Sprague–Dawley rats were bred at the Laboratory of Behavioral Neuroscience, Department of Psychology, University of Crete. Before surgery, they were housed in groups of three under a 12 : 12-h light–dark cycle with free access to food and water. The animals were anesthetized with an intramuscular injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Atropine sulphate (0.6 mg/kg, intramuscular) was injected to reduce bronchial secretion. Moveable monopolar stimulating electrodes (Model SME-01, Kinetrods, Ottawa, Ontario, Canada) were lowered into the medial forebrain bundle at the level of the lateral hypothalamus (coordinates Anteroposterior (AP): −2.5 mm from bregma, Laterae (L): −1.7 mm from the midline, Ventrodorsal (VD): −8.0 from a flat skull), according to Paxinos and Watson (1998).

The electrodes consisted of a plastic guiding base and a 0.25-mm diameter moveable stainless-steel wire, which were insulated with Epoxylite, except for the conically shaped tip. The anode was an amphenol pin connected to five miniature skull screws. After implantation and for the entire duration of the experiments, the animals were housed individually.

Animal care and the procedures used were in accordance with National Institutes of Health public document 85–23 (1985).

Apparatus and procedures

One week after surgery, the animals were tested for self-stimulation in an operant chamber that was made of transparent Plexiglas (25 cm wide, 25 cm deep, 30 cm high). A stainless-steel rodent lever protruded 2 cm from the left wall at a height of 4 cm from the floor. Each bar-press triggered a constant current generator that delivered a 0.4-s train of rectangular cathodal pulses of constant duration (0.1 ms) and intensity (250 μA) and variable frequency (25–125 Hz, i.e. 10–50 number of pulses/0.4 s). The pulse frequency, that is, the number of pulses within a train, was progressively increased up to 40 per stimulation train until the animal showed vigorous self-stimulation. If the implantation site failed to support self-stimulation, the electrode was lowered by steps of 0.16 mm (one step every 24 h), until a self-stimulation site was found. The electrode position was held unchanged in all subsequent testing. During the acquisition phase, the animals were trained to self-stimulate for at least three consecutive days (1 h daily), using stimulation parameters that maintained near maximal bar-pressing rates. After self-stimulation had been acquired and stabilized for a given pulse frequency, animals were trained under a protocol in which frequency was systematically manipulated to generate rate–frequency response curves. On this protocol, the animals were tested at several stimulation frequencies, beginning with frequencies that sustained responding at maximal rates and descending to frequencies that did not sustain responding. The pulse frequency was varied by steps of approximately 0.1 log units. Fourteen rate–frequency trials were conducted during each session. At the beginning of each trial, the animals received three trains of priming stimulation, at the frequency of the stimulation that was available for that trial. Each frequency was tested within trials of 60-s duration, followed by an extinction period of 30 s (intertrial interval). A rate–frequency determination (i.e. the entire session) lasted about 45 min. One rate–frequency curve was established daily, for 10–12 days, depending on the period when the self-stimulation indices (i.e. shifts in the lateral position of the curve and threshold measure) were stable.

Experimental procedure

Drug testing began for each animal when the function relating bar-pressing rate to pulse frequency (the rate–frequency function) was stable for at least three consecutive days. The criterion for stability was met when the frequency thresholds did not vary by more than 0.1 log units. Each drug or vehicle self-stimulation test consisted of a baseline and a drug rate–frequency function determination (for 45 min each). After the baseline period, each animal was injected with the drug or its vehicle. The animals were tested 10 min after the last injection. This time interval has also been used in self-stimulation studies with other drugs of abuse and appears to be critical for the observation of other behavioral and physiological effects of cannabinoids.

In this study, we used a mixed design, that is, some animals received only one treatment, whereas other

animals received all doses for only one drug treatment tested (see below for numbers of animals in each group). All animals took part in only one experiment, either by receiving only one drug treatment or by receiving all drug treatments of the experiment. An initial analysis, not presented in the paper, showed no difference in the reward and performance measurements of the animals used in both designs (within and between-subjects design). We used animals that received all drug treatments and animals that received only one drug treatment in each experiment because, as is already known, cannabinoids have 'carry-over' effects, owing to their lipophilicity and sustained exposure in the body. We tried to control these effects by allowing a 3-day period between injections – this period is considered sufficient for the behavior of the animals to return to stable, pretreatment levels, without being affected by prior cannabinoid administration, and by using animals that would receive only one drug treatment in one experiment. In fact, the use of animals that received only one treatment gave us confidence that the results obtained were not confounded by such a 'carry-over' effect, and as there was no statistical difference in the responses between the groups of animals with different treatment history (see below), the data were pooled and presented together. In the case of animals receiving more than one drug injection, the sequence of injections for the different drug doses was counterbalanced with respect to order and a 3-day period was allowed between injections, so that no carry-over effects of the cannabinoids would be detected.

Experiment 1. Effects of systemically administered Δ^9-tetrahydrocannabinol on brain stimulation reward Twenty-one rats were used. Five of them received all doses of Δ^9-THC (0.5, 1 and 2 mg/kg, intraperitoneal) or its vehicle in a randomized order, whereas 16 received only one drug treatment.

Experiment 2. Effects of SR141716A on Δ^9-tetrahydrocannabinol-induced changes in brain stimulation reward Fifteen rats were used. Three of them received SR141716A (0.02 mg/kg, intraperitoneally) or its vehicle, followed 5 min later by Δ^9-THC (0.02 mg/kg, intraperitoneally) or its vehicle in a randomized order, whereas 12 received only one combination of SR141716A and Δ^9-THC.

Histology

At the end of the experiments, the animals were given a lethal dose of sodium pentothal. The location of the terminal stimulation site was then marked according to the following procedure: a direct anodal current of 0.1 mA and 15 s duration was passed through the electrode tip. The animals were perfused intracardially with 0.9% NaCl, which was followed by a 50 ml solution of potassium ferrocyanide (3%) and trichloroacetic acid (0.5%) in 10% formalin. The brains were then removed and stored in 10% formalin for 3 days, and for 2 days in a 30% sucrose solution. Finally, the brains were sliced in a cryostat microtome and the sections containing the electrode tract were mounted on slides and stained with cresyl violet. All rats included in this study were found to have electrode tracks verified to be located in the medial forebrain bundle. Electrode tips were examined in all animals tested.

Drugs

Δ^9-THC (Sigma-Aldrich, St Louis, Missouri, USA) and SR141716A (synthesized by Lilly Research Laboratories, Indianapolis, Indiana, USA) were dissolved in a vehicle solution that consisted of 5% dimethylsulfoxide, 5% cremophor EL and 90% of 0.9% NaCl and injected intraperitoneally at a volume of 3 ml/kg of body weight. Control animals received, intraperitoneally, the corresponding vehicle solutions in the same injection volume.

Data analysis and statistics

Data gathered from pre- and postinjection portions of each session were curve-fitted and threshold and asymptote estimates were obtained using the Gompertz sigmoid model (Coulombe and Miliaressis, 1987):

$$f(X) = \alpha e^{-e^{b(X_i - X)}}$$

In this equation, α represents the maximum rate (asymptote), whereas X_i (X at inflection) represents the threshold frequency. The latter is the pulse number producing 36.7% of the asymptotic rate, that is, the rate lying on the fastest-accelerating region of the curve. Parameter b represents an index of the slope whereas e is the base of natural logarithms.

The posttreatment threshold and asymptote values were expressed as percentage of predrug values. The results were statistically evaluated using one-way (effects of Δ^9-THC alone) or two-way (effects of combined administration) analyses of variance (ANOVA) followed, whenever appropriate, by the lysergic acid diethylamide (LSD) test for multiple contrasts. It should be noted that in our design, some animals received all treatments and others received only one (see above). To combine these two data sets, for all rats we expressed drug effects as a percentage of preinjection baseline, which was established independently for each drug or vehicle treatment. Each drug test was thus treated as an independent measure for statistical analysis.

Conditioned place preference

Subjects

Male mice ($n = 30$) of a C57B1/6LX129Sv-derived strain, bred at the University of Sussex, were used in the experiment. Animals, weighing 25–35 g at the beginning of the experiment, were housed in groups of 2 to 3, under a 12 : 12-h light/dark cycle (lights on at 07.00 h) and had free access to rodent chow (Bekay Feeds, Hull, UK) and

water. The temperature was held at $20 \pm 3^{\circ}C$ and humidity was $50 \pm 10\%$. The experiment was carried out under the UK Animal Experimental Procedures Act (1986).

Conditioned place preference apparatus

The apparatus comprised eight CPP boxes, each of which consisted of two distinct compartments ($20 \times 20 \times 20$ cm) separated by a corridor ($20 \times 4.5 \times 20$ cm) that prevented the animals from having direct access to the alternative compartment. One compartment had white walls and a mesh floor, whereas the other compartment had black and white diagonal walls and a clear smooth Perspex floor. Infrared beams across the entrances to the compartments, and within the corridor, allowed the location of the mouse to be determined, and the time spent in each compartment to be calculated, using in-house software.

Experimental procedure

An unbiased place conditioning procedure was used to evaluate the effects of Δ^9-THC. During the preconditioning phase, the animals were placed in the middle of the apparatus, in the corridor, without the guillotine doors and were allowed to explore it for 30 min, being in a drug-free state. To reduce any potential dysphoric effects induced by the first drug exposure, all animals next received a priming injection of Δ^9-THC (1 mg/kg, intraperitoneally) in their home cage, 24 h before starting the first place preference conditioning session. In the next (conditioning) phase, animals were subjected to eight 30-min conditioning sessions (four pairings with the drug and four with vehicle), one each day. The compartment paired with drug, the order of drug administration (odd or even days), and the drug dose were randomly chosen for each animal and counterbalanced. Each animal was injected with Δ^9-THC (0, 1 or 3 mg/kg, intraperitoneally), returned to its home cage, and placed in one of the compartments (drug-paired) with the guillotine doors closed, 15 min after the injection ($n = 10$ for each group). On the alternate days, the mice were administered vehicle and placed in the alternative compartment. The test phase was conducted in the same way as the preconditioning phase. The time in each compartment (drug-paired or vehicle-paired) was recorded in the test-phase (after conditioning) for each animal.

Drug preparation and doses

Δ^9-THC (Sigma-Aldrich) was dissolved in a solution that consisted of 5% dimethylsulfoxide, 5% cremophor EL in 0.9% NaCl and injected intraperitoneally at a volume of 10 ml/kg of body weight.

Statistical analysis

The time spent in each compartment during the post-conditioning phase was calculated. The effect of Δ^9-THC on the time (expressed as mean ± SEM) spent in each compartment after conditioning was analyzed using a two-way ANOVA, with compartment (drug-paired or vehicle-paired) as a within-subjects factor and doses of Δ^9-THC (0, 1, and 3 mg/kg) as a between-subjects factor.

Results

Intracranial self-stimulation

Experiment 1. Effects of systemically administered Δ^9-THC on brain stimulation reward

The changes of self-stimulation threshold and asymptotic rate of responding after systemic injection of Δ^9-THC are presented in Fig. 1a and b, respectively. Δ^9-THC (0.5, 1 and 2 mg/kg, intraperitoneally) significantly increased self-stimulation thresholds [$F(3,32) = 10.92$, $P < 0.001$], whereas it had no effect on the asymptotic rate of responding [$F(3,32) = 0.27$, NS]. Post-hoc analysis with the LSD test showed that these effects on the self-stimulation thresholds were significant at the two highest doses tested ($P < 0.02$ and $P < 0.001$, respectively), compared with the control group. The LSD test also showed that the 2 mg dose of Δ^9-THC also differed significantly from the 0.5 and 1 mg doses ($P < 0.001$ and $P < 0.01$, respectively).

Experiment 2. Effects of SR141716A on Δ^9-THC-induced changes in brain stimulation reward

Figure 1c and d presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of SR141716A or its vehicle and Δ^9-THC or its vehicle. Two-way ANOVA showed that Δ^9-THC (2 mg/kg, intraperitoneal) produced an increase in self-stimulation threshold [$F(1,20) = 11.78$, $P < 0.005$]. Administration of SR141716A (0.02 mg/kg, intraperitoneally) blocked this effect [$F(1,20) = 9.21$, $P < 0.01$]. Two-way ANOVA also showed that neither Δ^9-THC (2 mg/kg) [$F(1,20) = 1.95$, NS], nor SR141716A (0.02 mg/kg) [$F(1,20) = 0.31$, NS], or their combination [$F(1,20) = 0.44$, NS] affected the asymptotic rate of responding (Fig. 2).

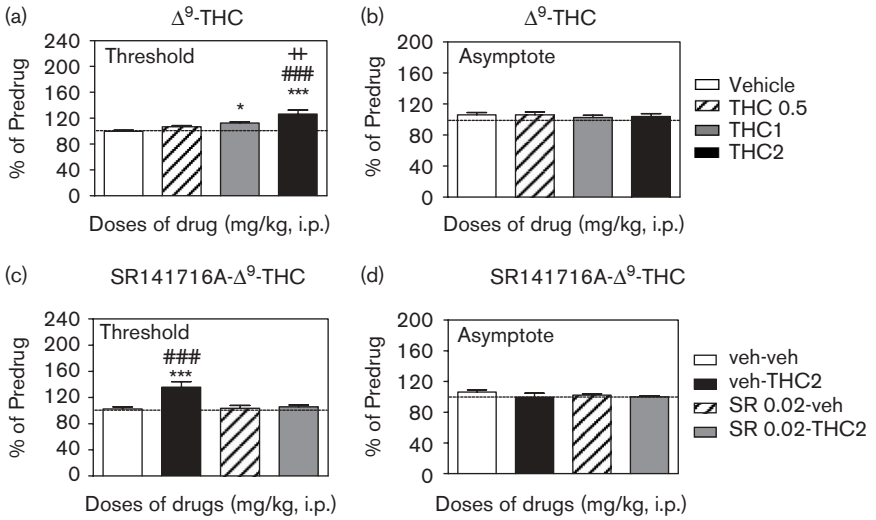
Conditioned place preference

Δ^9-THC (0, 1 and 3 mg/kg, intraperitoneal) produced neither preference nor aversion at any of the doses tested [$F(2,27) = 0.23$, NS] (Fig. 3).

Discussion

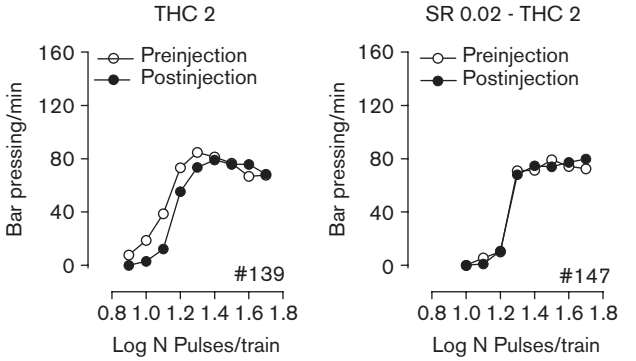
This study provides clear evidence that Δ^9-THC lacks appetitive properties in the ICSS and CPP paradigms. It is important to notice that, under these experimental conditions, typical drugs of abuse, such as cocaine, amphetamine, morphine and nicotine, have also exhibited either appetitive or aversive properties in the ICSS and CPP paradigms (Panagis and Spyraki, 1996; Panagis *et al.*, 1998, 2000; Mead and Stephens, 1999; Mead *et al.*, 2005).

Fig. 1



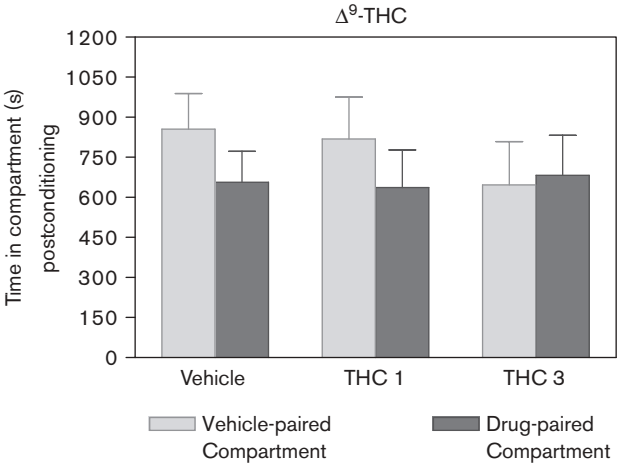
Changes in self-stimulation threshold [(a), (c)] and asymptote [(b), (d)] (expressed as percentage of predrug values) following acute Δ^9-THC (0, 0.5, 1 and 2 mg/kg, intraperitoneal) administration and SR141716A (0, 0.02 mg/kg) and Δ^9-THC (0, 2 mg/kg, intraperitoneal) administration. Vertical bars represent the SEMs. Asterisks (*) signify an ICSS threshold significantly different from the control group. ICSS, intracranial self-stimulation; Δ^9-THC, Δ^9tetrahydrocannabinol. $^{*}P<0.05$, $^{***}P<0.001$, compared with the control group; $^{\#\#\#}P<0.001$ compared with the 0.5 mg/kg dose (a) or the SR0.02 + veh and SR0.02 + THC2 groups (c); $^{++}P<0.01$ compared with the 1 mg/kg dose.

Fig. 2



Rate–frequency functions (rate of lever pressing as a function of stimulation frequency) taken from representative animals for each drug treatment. Each plot represents data from a single animal under predrug and drug conditions. Rate–frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.

Fig. 3



Effects of Δ^9-THC (0, 1 and 3 mg/kg, intraperitoneally) on place conditioning. The histogram represents the time (mean ± SEM) spent in the control and drug compartment after conditioning. Δ^9-THC, Δ^9tetrahydrocannabinol.

The results from the first experiment show that acute administration of Δ^9-THC in Sprague–Dawley rats did not reveal any appetitive properties in the ICSS paradigm. On the contrary, it exhibited anhedonic effects at the two highest doses tested (1 and 2 mg/kg). Δ^9-THC did not affect maximal rates of responding at any of the doses tested. Strong evidence exists that the presently used ICSS paradigm provides reward threshold estimates that are unaffected by performance effects of drug treatments or other experimental manipulations (Edmonds and Gallistel, 1974; Miliaressis and Rompré, 1987; Markou and Koob, 1992). This is also evident in this study. Thus, the increases in self-stimulation thresholds produced by Δ^9-THC were not accompanied by significant changes in asymptotic rates of responding.

The anhedonic effects of Δ^9-THC are probably mediated via CB_1 receptor stimulation, as they were blocked by pretreatment with SR141716A (0.02 mg/kg), which by itself was ineffective in altering brain stimulation reward thresholds. The reason we chose to administer only one dose of SR141716A (0.02 mg/kg) is because we have previously shown that this dose is effective in reducing the increase in self-stimulation threshold caused by CB_1 receptor agonists and endocannabinoid modulators, whereas it does not affect the rewarding efficacy of self-stimulation *per se* (Vlachou *et al.*, 2003, 2005, 2006; Antoniou *et al.*, 2005). Moreover, the fact that SR141716A reversed the decrease in the efficacy of self-stimulation rewarding caused by Δ^9-THC is in agreement with previous studies that utilized different behavioral paradigms (Tanda *et al.*, 2000; Braida *et al.*, 2004). Additionally, SR141716A has been shown to change the self-stimulation threshold at doses much higher than the one used in our study (Arnold *et al.*, 2001; Deroche-Gamonet *et al.*, 2001).

Our results differ from those of previous studies by Gardner and colleagues (Gardner *et al.*, 1988, 1989; Lepore *et al.*, 1996; Gardner and Vorel, 1998), which reported that Δ^9-THC increases the rewarding efficacy of brain stimulation. This could be due to differences in the experimental design or the strains of the animals used. It should, however, be noted that the rewarding effects of Δ^9-THC in the above-mentioned studies are much less than the rewarding actions shown with typical drugs of abuse, such as morphine, cocaine, amphetamine, 3,4-methylene-dioxymethamphetamine or nicotine, in the ICSS paradigm (Kornetsky and Esposito, 1979; Bauco and Wise, 1994, 1997; Lin *et al.*, 1997). These drugs can reliably shift the rate–frequency function to the left by between 0.2 and 0.5 log units. In the study by Lepore *et al.*, Δ^9-THC, however, could only shift the rate–frequency function to the left by approximately 0.05 log units. Additionally, Lepore *et al.*, adopt a very strict criterion of stable responding (i.e. 0.01 log units for three consecutive days), which indicates that the observed effect of Δ^9-THC in their study could reflect normal baseline variation over days. In our study, the criterion of stable responding was defined within 0.1 log units over three consecutive days, in agreement with most other studies utilizing the ICSS paradigm (see, e.g. Arnold *et al.*, 2001). Interestingly, if we examine carefully the data presented in the study by Lepore and colleagues we can draw different inferences, depending on the criterion used to analyze the effect of Δ^9-THC in the ICSS paradigm. In Sprague–Dawley rats, Δ^9-THC exhibits reinforcing properties only with the Θ_0 criterion, and not the M_{50} criterion. It is worth noting that in our study, the criterion used resembles the M_{50} criterion of Lepore's study. Evaluated in this manner, our data are in agreement with the data of Lepore *et al.* (1996) in Sprague–Dawley rats. Δ^9-THC produced neither CPP nor aversion in any of the doses tested. All animals received a priming injection of Δ^9-THC 24 h before the preconditioning session to reduce any potential dysphoric effects induced by the first injection. Even after this experimental manipulation, no CPP, however, was observed with the 1 mg dose of Δ^9-THC, in contrast to the observations of Valjent and Maldonado (2000) (for review see, Maldonado, 2002). Other studies have shown CPP after administration of Δ^9-THC (Lepore *et al.*, 1995; Braida *et al.*, 2004; Celerier *et al.*, 2006; see also, Introduction; Table 1). This inconsistency in results could be attributable to the different strain of animals used, the number of pairings or the periods of conditioning and administration of the drugs. On the other hand, our results are consistent with previous studies, showing that Δ^9-THC or CB_1 receptor agonists, like WIN 55,212-2 and HU-210, have no appetitive actions in the CPP paradigm or even induce conditioned place avoidance (Lepore *et al.*, 1995; Sañudo-Peña *et al.*, 1997; Chaperon *et al.*, 1998; Mallet and Beninger, 1998; Cheer *et al.*, 2000). Similarly, Gobbi and colleagues (2005) have also shown that the endocannabinoid modulator URB-597 does not exhibit reinforcing properties in the CPP paradigm.

It is worth noting that other behavioral models, such as the self-administration paradigm, have also provided inconsistent results with both Δ^9-THC and other cannabinoid agonists (Tanda *et al.*, 2000; Braida *et al.*, 2001; Fattore *et al.*, 2001; Navarro *et al.*, 2001; Justinova *et al.*, 2003, 2004, 2005; Zangen *et al.*, 2006), adding to the complexity of drawing conclusions on the rewarding actions of Δ^9-THC. A clear example of the above statement is the study of Martellotta and colleagues (1998), in which it was shown that WIN 55,212-2, a CB_1 receptor agonist, was able to elicit both rewarding and aversive effects, depending on the concentration used. It should be noted that most of the self-administration studies showing self-administration of Δ^9-THC or CB_1 agonists have been conducted with animals that have been food deprived (Fattore *et al.*, 2001) or that have previously learnt to self-administer other addictive drugs (Tanda *et al.*, 2000). Some more recent studies, however, have shown that Δ^9-THC has maintained the self-administration behavior in drug-naive animals (Justinova *et al.*, 2003, 2004, 2005). All these studies support the notion that cannabinoids differ from other addictive drugs, when considering their rewarding properties.

The results of this study do not seem to be consistent with the known pleasurable effects of cannabis preparations in humans. Several reasons might explain this difference. First of all, cannabis is more than simply Δ^9-THC. Indeed, cannabis preparations contain several cannabinoid and noncannabinoid compounds, which may contribute to the behavioral effects of cannabis. Second, there may be significant species differences in functions

of the cannabinoid system in rodents and humans. Perhaps the recreational use of cannabis by humans reflects more complex sensory and cognitive effects that cannot be experienced by lower animals. This may, also, explain why cannabinoids do not induce the typical pattern of obsessive drug-seeking and compulsive drug-taking behavior observed in humans addicted to typical drugs of abuse, such as cocaine, heroin, alcohol and nicotine.

Taken together, our findings indicate that Δ^9-THC, in contrast to other drugs of abuse, does not exhibit facilitating properties in the ICSS nor support CPP, under the present experimental conditions.

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