CHAPTER SIX

Effects of CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats

6.1. Introduction

The phenomenon of MFB self-stimulation has provided researchers with a unique insight into the neural and chemical profile of brain reward systems (Wise, 1996). Stimulation of the MFB has been shown to maintain operant responding in a similar fashion to natural rewards, such as food, water and sex. Thus, it is not surprising that the rewarding impact of MFB stimulation may derive from activation of brain circuitry thought to underlie the rewarding effects of food, sex and drugs of abuse. Drugs such as cocaine, opiates and amphetamine, enhance the rewarding potency of MFB stimulation, because they have effects upon common "reward" substrates, of which the mesolimbic DA system appears to play a central role (Wise, 1996).

Early studies indicated that the classical cannabinoid receptor agonist and main psychoactive constituent of cannabis, Δ^9-THC, could reduce the rate of responding for brain stimulation (Bailey & Pradhan, 1972; Bhattacharyya, Aulakh, Pradhan, Ghosh, & Pradhan, 1980; Pradhan, Bailey, & Ghosh, 1972; Pradhan, Bhattacharyya, Aulakh, Pradhan, & Bailey, 1978; Stark & Dews, 1980). However, these studies generally used doses that are beyond those used by humans. Further, these studies could not rule out the possibility that this observed reduction in response rate was just a consequence of Δ^9-THC inhibiting the rats ability to bar press. This is consistent with the findings of Chapter 3, 4 and 5 where CP 55,940 was shown to inhibit motor behaviour based on measures of

catalepsy, locomotor activity and rotarod performance. Thankfully advances in the quantitative assessment of brain stimulation have overcome this problem. The rate frequency curve-shift method was developed to distinguish between the effects of a drug on responding related to reward or the effects of the drug on responding related to motor performance (Edmonds & Gallistel, 1974; Gallistel & Karras, 1984).

Gardner and colleagues first reported that Δ^9-THC (1.5 mg/kg) enhanced the rewarding potency of electrical MFB stimulation using a titrating threshold stimulation paradigm (Gardner et al., 1988). Much later this was repeated using a slightly lower dose of Δ^9-THC (1 mg/kg) using a rate-frequency curve shift method to highlight that this rewarding effect occurred in the absence of performance deficits (Lepore et al., 1996a). Interestingly, the leftward shift observed in the rate-frequency function was small in comparison to the effects of other drugs of abuse such as amphetamine, MDMA or cocaine (Bauco & Wise, 1997; Gallistel & Karras, 1984; Lin, Jackson, Atrens, Christie, & McGregor, 1997).

What appeared to be the most important determinant of the observed rewarding effect of Δ^9-THC on electrical brain stimulation appeared to be the use of Lewis rats (Gardner et al., 1998). In the study by Lepore *et al* (1996a) other strains such as Sprague-Dawley rats only showed a marginal rewarding effect, whereas Fischer 344 rats showed no such effect. This finding is consistent with the notion that Lewis rats are more susceptible to the rewarding effects of cannabinoids because they are less susceptible to the anxiogenic effects of such drugs which is supported by the findings of Chapters 2 and 3. However, the finding of Lepore *et al* (1996a) is inconsistent with the findings of Chapter 4 which showed that Lewis rats were less affected by CP 55,940 than Wistar rats in terms of the amount of *c-fos* expressed in reward-related areas of the brain. To be consistent with the rest of the thesis, the current study tested the effects of CP 55,940 on MFB self-stimulation. This was done to make sure that CP 55,940, which has never been previously tested, has similar rewarding effects to Δ^9-THC on MFB self-stimulation (Lepore et al.,

1996a). As Lewis rats appear more susceptible to the rewarding effects of cannabinoids, the current chapter only utilised this strain of rat.

There is increasing evidence that the endogenous cannabinoid system plays an important role in mediating the rewarding effects of both drugs of abuse and natural reinforcers (see section 1.8.). Recent results show that mice with targeted deletion of the CB_1 receptor gene exhibit reduced self-administration of morphine (Ledent et al., 1999). Further, the selective cannabinoid CB_1 receptor antagonist SR 141716 blocks the acquisition of conditioned place preference to cocaine and morphine in rats (Chaperon et al., 1998). SR 141716 also reduces sucrose, ethanol, and beer consumption in rats suggesting involvement of cannabinoid systems in appetite and alcohol preference (Arnone et al., 1997; Colombo et al., 1998; Gallate & McGregor, 1999).

In the present study it was reasoned that if the central endogenous cannabinoid system plays a critical role in the neural substrate of reward, then SR 141716 should have a powerful inhibitory effect on the rewarding efficacy of electrical stimulation of the MFB. Such an effect is already well documented with DA D_1 receptor antagonists, so for comparison purposes we also assessed the effects of SCH 23390 (Nakajima, 1989). Previous studies have shown that 0.04 mg/kg of SCH 23390 produces a large decrease in the rewarding impact of electrical stimulation of the MFB (Nakajima, 1989; Nakajima, Liu, & Loong Lau, 1993; Nakajima et al., 1991). Furthermore, a dose of 0.08 mg/kg has been shown to completely abolish responding for electrical stimulation of the MFB (Nakajima et al., 1991).

Experiment 6A assesses the effects of CP 55,940 on responding for electrical stimulation of the MFB using a rate-frequency curve-shift procedure in Lewis rats using doses equivalent to those employed by Lepore *et al* (1996a). Experiment 6A also tests whether withdrawal from the acute exposure to CP 55,940 causes a reduction in the rewarding value of MFB stimulation 24 hours after a single injection of CP 55,940. This reduction in the rewarding action of brain stimulation has been reported to occur 24 hours

after acute exposure to Δ^9-THC (Gardner, 1999). Experiment 6B tests whether the synthetic cannabinoid receptor antagonist SR 141716 reduces responding for electrical stimulation of the MFB. For purposes of comparison Experiment 6B also assesses the effects of the DA D_1 receptor antagonist SCH 23390 on MFB self-stimulation.

6.2. Experiment 6A. The effects of CP 55,940 on MFB self-stimulation in Lewis rats

6.2.1. Method

6.2.1.1. Subjects. The subjects were seven Lewis rats weighing between 330-380 g at the time of surgery. Rats were singly housed in a temperature controlled room (20 ± 2 ˚C) with food and water provided *ad libitum*. All testing occurred during the light hours of a 12 h:12 h light/dark cycle.

6.2.1.2. Apparatus. Two identical operant boxes [25 cm (L) x 25 cm (W) x 30 cm (H)] (Coulbourn Instruments) were used. Each perspex box contained a standard response lever that was positioned on one side of the box elevated 3 cm above its grid floor. These individual boxes were located within their own sound attenuating chambers [75 cm (L) x 50 cm (W) x 60 cm (H)]. Lever presses delivered a 500 msec stimulation train of 0.1 msec wide cathodal rectangular pulses. Stimulation was supplied via a programmable stimulator and constant current generator through mercury filled commutators via connecting leads. A PC compatible computer controlled the delivery of stimulation and recorded all behavioural data while a Macintosh™ computer controlled the programmable stimulator to allow a descending series of stimulation frequencies to be automatically presented.

6.2.1.3. Drug. CP 55,940 (Pfizer) (0, 10, 25 and 50 µg/kg) was prepared as was done throughout the thesis (see section 2.2.1.3.). Identical solutions minus the drugs were used as vehicle solutions. All injections were given i.p. in a volume of 1 ml/kg.

6.2.1.4. Surgery. Rats were anaesthetised with an i.p. injection of ketamine (60 mg/kg) and xylazine (9 mg/kg) and stereotaxically implanted with a monopolar electrode in the right hemisphere of the brain and an indifferent electrode, as described previously (Hunt & McGregor, 1998). The electrode tip was aimed at the lateral

hypothalamic level of the MFB using the following stereotaxic coordinates: 2.5 mm posterior to Bregma, 1.6 mm lateral to the midline and 9.7 mm ventral from the superior surface of the skull (Paxinos and Watson, 1997). Rats were allowed to recover from surgery for at least 3 weeks before testing.

6.2.1.5. Procedure. Rats were trained to lever press under a fixed-interval (FI) 1 sec schedule of reinforcement with the stimulation frequency and current set at 100 Hz and 150 μA respectively. Once the rats started self-stimulating, currents were manipulated to yield high rates of responding (70 - 130 responses per min). Rats were then trained using a rate-frequency paradigm (Coulombe & Miliaressis, 1987) involving a descending series of 10 different frequencies, beginning at 200 Hz and finishing at 25 Hz. At the beginning of each series of frequencies rats received 5 priming stimulations over 20 sec. Individual frequencies were available for 2 min before the frequency was decreased by 0.1 $\log_{10}$ units. Once all 10 frequencies had been presented, the procedure was repeated. If the rat did not respond for a period of 90 sec, one forced stimulation was supplied.

Responding was deemed stable when the rats M_{50} (see section 6.2.1.7.) did not vary beyond 0.1 $\log_{10}$ units over three consecutive days. This was a less stringent criterion of stability in responding than in Experiment 6B but is consistent with what others classify as stable responding in the literature (Johnson & Stellar, 1994; Lin et al., 1997). Once a rat's responding had become stable it was given a dose of CP 55,940 30 min before the start of testing. Testing proceeded such that a rat received no more than one dose per week. All seven rats were administered doses of CP 55,940 (0, 10, 25 and 50 μg/kg) in a randomised order. These doses of CP 55,940 were selected based on previous doses used in the thesis (see Chapters 2, 3, 4 and 5). The rats were then tested drug-free 24 h after the administration of CP 55,940 to assess whether prior cannabinoid administration decreases the rewarding impact of MFB stimulation.

6.2.1.6. Histology. Upon completion of drug testing rats were killed with a lethal injection of pentobarbitone (120 mg/kg, i.p.). They were then decapitated and their brains were removed. Brains were blocked and placed on a microtome stage before being frozen to – 14 °C and sliced into 40 µm sections. Frozen slices were examined under a light microscope to confirm that the electrodes were positioned at the lateral hypothalamic level of the MFB according to the brain atlas of Paxinos and Watson (1997). The tissue was then mounted on slides and left overnight to dry. These were stained with toluidine blue and coverslipped.

6.2.1.7. Data Analysis. Data collected consisted of the number of reinforced responses for each frequency presented. Non-linear regression analyses were performed on these data and the Gompertz equation was used to provide three estimates of rewarding efficacy to enter into statistical analysis: 1) M_{50}, which is the frequency supporting half maximal rates of responding, and 2) Y_{max} which is the maximal or asymptotic response rate (Coulombe & Miliaressis, 1987). The M_{50} provides an estimate of the rewarding efficacy of MFB stimulation, while the Y_{max} provides an indication of any drug effects on performance (Coulombe & Miliaressis, 1987; Wise, 1996).

M_{50} and Y_{max} data for all drug, vehicle and saline tests were expressed as a percentage change from the drug-free baseline on the previous day. The resultant percentages for each of the two sessions within each test were averaged to give a single percentage change score. Average percentage change scores for M_{50} and Y_{max} were then separately analysed using one-factor ANOVA with drug administration treated as a repeated measure. Individual comparisons between each dose of CP 55,940 (either 10, 25, or 50 µg/kg) and vehicle were constructed using Bonferroni corrections to control the type 1 error rate (Keppel, 1991). These same comparisons were conducted on data retrieved 24 h following CP 55,940 administration to assess whether prior cannabinoid exposure reduces the rewarding impact of MFB stimulation.

6.2.2. Results

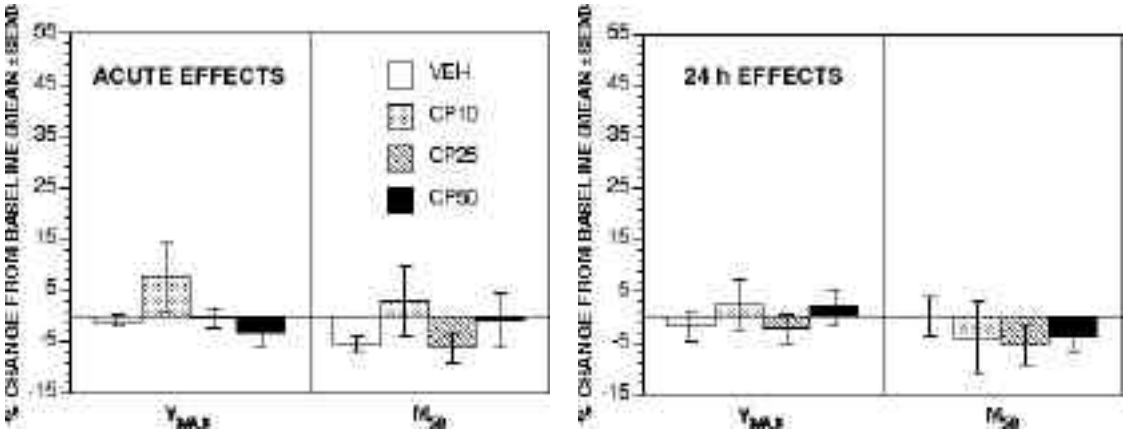

Figure 6.1. (left) Effects of CP 55,940 [0 (VEH), 10 (CP10), 25 (CP25) and (CP50) 50 µg/kg] on MFB self-stimulation as assessed by Y_{max} and M_{50} (n=7 per group). (right) Acute CP 55,940 administration (0, 10, 25, 50 µg/kg) on MFB self-stimulation as measured 24 hours after this administration when tested drug-free as assessed by Y_{max} and M_{50}.

CP 55,940 was not effective in modulating either the rewarding efficacy of MFB stimulation or the ability of rats to perform the bar-press response. This was reflected in no significant changes in M_{50} or Y_{max} (see Figure 6.1.). Individual contrasts comparing each CP 55,940-treatment group and vehicle revealed CP 55,940 had no effect upon the M_{50} at any dose tested (F*s* < 1.7). In addition, CP 55,940 had no effect upon Y_{max} at any dose tested (F*s* < 2.6).

Twenty-four hours after the administration of CP 55,940 there was no significant shifts in either M_{50} or Y_{max} (see Figure 6.1.). Individual contrasts comparing each CP 55,940-treatment group and vehicle revealed CP 55,940 given 24 hours earlier had no effect upon the M_{50} at any dose tested (F*s* < 1). In addition, prior CP 55,940 administration had no effect upon Y_{max} at any dose tested (F*s* < 1).

Electrode tips were confirmed to be within the boundaries of the MFB at the level of the LH. Some representative photomicrographs of the electrode tip locations can be seen in Figure 6.2.

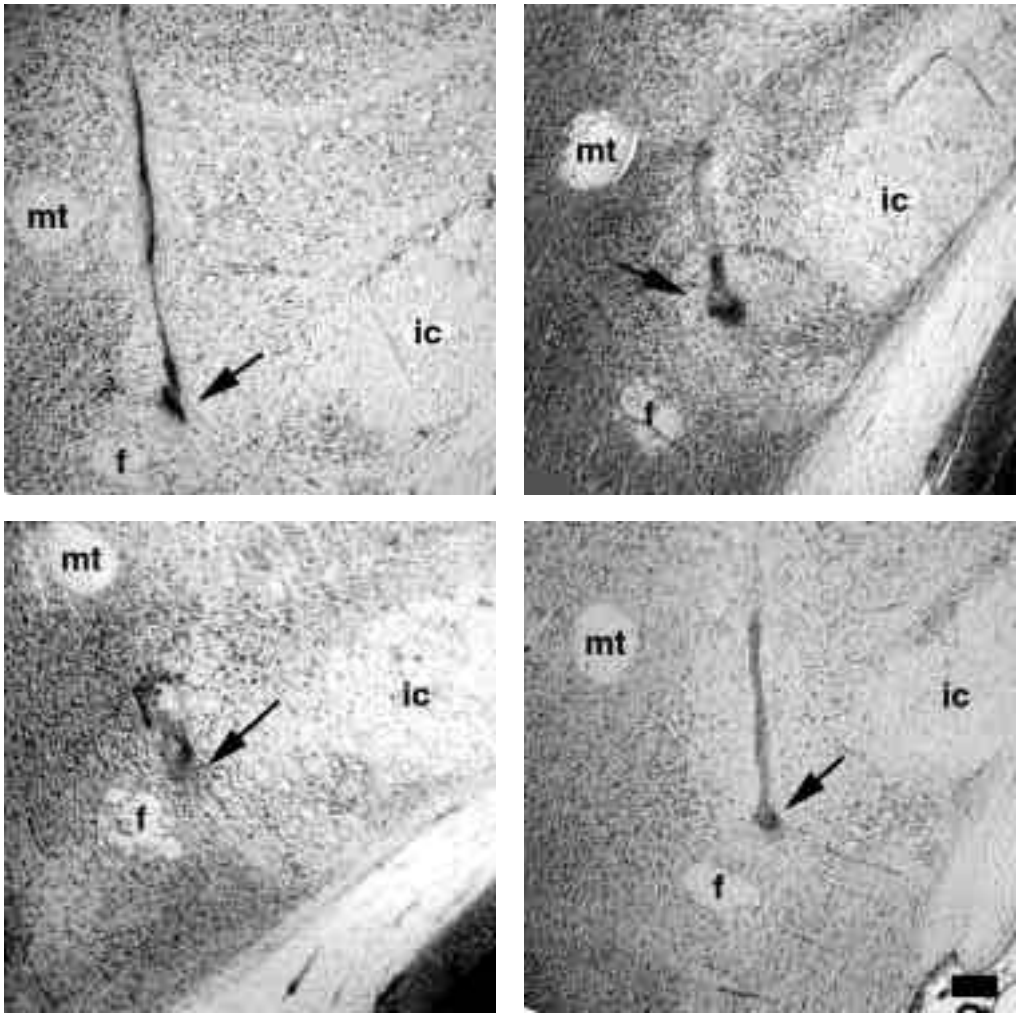

Figure 6.2. Photomicrographs of representative electrode placements. The electrode placements were confirmed to be within the boundaries of the MFB at the level of the LH. The arrow indicates the placement of electrode tips. **f** refers to the fornix, **ic** to the internal capsule and **mt** to the mammillothalamic tract. Scale bar = 250 µm.

6.3. Experiment 6B. The effects of SR 141716 and SCH 23390 on MFB self-stimulation in Lewis rats

6.3.1. Method

6.3.1.1. Subjects. The subjects were six Lewis rats weighing between 330-380 g at the time of surgery. Rats were maintained in exactly the same way as in Experiment 6A (see section 6.2.1.1.).

6.3.1.2. Apparatus. The same apparatus was used as in Experiment 6A (see section 6.2.1.2.).

6.3.1.3. Drugs. SR 141716 {*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-IH-pyrazole-3-carboxamide} (Sanofi Recherche) is the best characterised competitive CB_1 receptor antagonist (Pertwee, 1997). The cannabinoid receptor antagonist is provided as a salt (SR 141716A) or as a free base (SR 141716). SR 141716A reversibly displaces CP 55,940 from specific CB_1 receptor binding sites and antagonizes the inhibitory effects that cannabinoids have on adenylate cyclase activity in the rat brain (Rinaldi-Carmona et al., 1994). SR 141716 also reverses the behavioural effects of a wide range of cannabinoid receptor agonists (Carriero et al., 1998; Compton et al., 1996c; Gallate, Saharov, Mallet, & McGregor, 1999; Lichtman & Martin, 1996, 1997; Lichtman et al., 1998; Mallet & Beninger, 1998; Rinaldi-Carmona et al., 1994; Vivian et al., 1998). Further, SR 141716A is selective for the CB_1 receptor by showing that it has no affinity for many other known receptors, such as CB_2, histamine, DA, adrenergic, adenosine, 5-HT, excitatory amino acid or GABA receptors (Rinaldi-Carmona et al., 1994).

Some studies have shown that SR 141716 when given alone produces effects opposite to that of classical cannabinoid receptor agonists. For instance, SR 141716A increased locomotor activity in mice (Compton et al., 1996c) and improved social memory in rats and mice (Terranova et al., 1996). These results can be interpreted in two possible

ways. First, it is possible that endogenous cannabinoid tone is blocked by SR 141716. Thus, the tonically inhibitory effects that endogenous cannabinoids have on locomotor activity or on memory may be diminished. Alternatively, some recent studies indicate that SR 141716A sometimes acts as an inverse agonist (Bouaboula et al., 1997; MacLennan et al., 1998). Thus, SR 141716 may have a direct intrinsic effect on the CB_1 receptor that is opposite to the usual inhibitory effects that CB_1 receptor activation has on locomotor activity or memory.

SR 141716 was prepared by initially dissolving its powder formulation in 100% ethanol. Tween 80 was added to this solution and the ethanol was completely evaporated under a stream of nitrogen gas. The resulting solution was diluted with 0.9% saline to give a final solution containing 1:19 (Tween 80:saline).

SCH 23390 (Research Biochemicals Inc.) is a competitive D_1 receptor antagonist. It was prepared by dissolving it in 0.9% saline to create a solution with a concentration of 0.06 mg/ml.

6.3.1.4. Surgery. The surgery was performed in the same fashion as in Experiment 6A (see section 6.2.1.4.).

6.3.1.5. Procedure. Drug testing commenced once individual responding on the rate-frequency paradigm had stabilised according to a criterion of 3 consecutive days where the M_{50} (see section 6.2.1.7.) varied within only 0.03 $\log_{10}$ units. To maintain stability, rats were tested daily except on weekends. Once a rat's responding had become stable it was administered a dose of SR 141716 20 min before the start of testing. Testing proceeded such that a rat received no more than one dose per week. All six rats were administered doses of SR 141716 (0, 1, 3, 10 and 20 mg/kg) in a randomised order. These doses of SR 141716 were selected according to the effective dose range reported in previous studies (Arnone et al., 1997; Chaperon et al., 1998; Gallate & McGregor, 1999).

Once testing with SR 141716 was complete, the same rats were tested with SCH 23390. Testing with SCH 23390 occurred over three consecutive days, where the first day served as a baseline, the second as a control day where saline was administered, and the final day as a test where 0.06 mg/kg of SCH 23390 was administered. This dose was selected on the basis of a previous study showing that a slightly higher dose (0.08 mg/kg i.p.) completely blocks MFB self-stimulation (Nakajima, 1989).

6.3.1.6. Histology. The same histological procedures were carried out as outlined in section 6.2.1.6.

6.3.1.7. Data analysis. Average percentage change scores for M_{50} and Y_{max} were derived as described in section 6.2.1.7. These scores were separately analysed using a one-factor ANOVA with drug administration (either SR 141716 or SCH 23390) treated as a repeated measure. Individual comparisons between each SR 141716 dose (1, 3, 10, or 20 mg/kg) and vehicle were constructed using Bonferroni corrections to control the type 1 error rate (Keppel, 1991).

6.3.2. Results

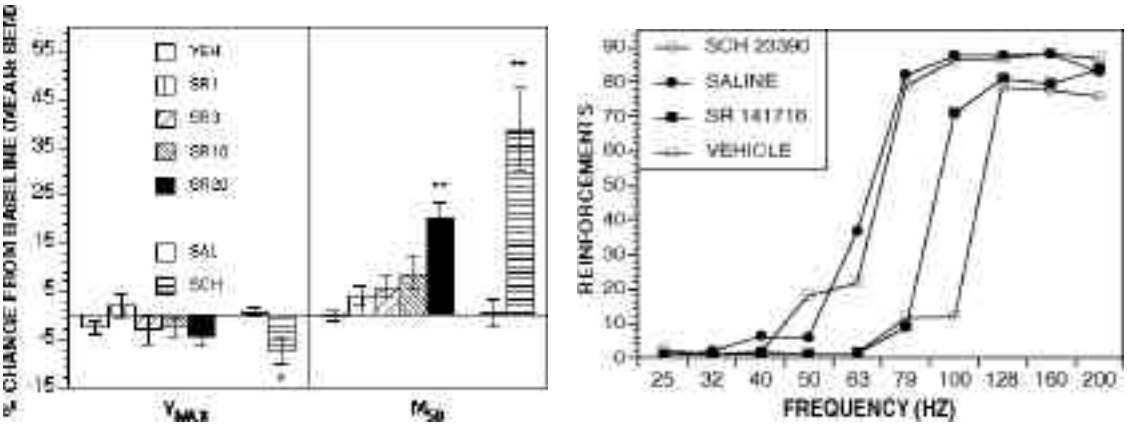

Figure 6.3. (left) Effects of SR 141716 (0, 1, 3, 10 and 20 mg/kg) and SCH 23390 (0 and 0.06 mg/kg) on MFB self-stimulation as assessed by Y_{max} and M_{50} (n=6 per group). SR 141716 (20 mg/kg) and SCH 23390 (0.06 mg/kg) significantly attenuated the rewarding efficacy of the electrical stimulation as shown by decreased M_{50}, while only SCH 23390 significantly reduced Y_{max}: $^{*}p < 0.05$, $^{**}p < 0.01$. (right) Effects of SR 141716 (20 mg/kg) and SCH 23390 (0.06 mg/kg) and their vehicles on a typical rat responding for MFB self-stimulation. Data are expressed as the number of reinforcements obtained per frequency of stimulation.

SR 141716 caused a dose-dependent decrease in the rewarding efficacy of MFB self-stimulation without causing any significant performance deficit (see Figure. 6.3.). Rate-frequency data for a typical rat are shown in Figure 6.3. Contrasts comparing each SR 141716 dose to vehicle revealed that only the 20 mg/kg dose significantly increased the M_{50} [$F(1,20) = 26.54$, $p < 0.001$]. However, remaining contrasts comparing 1, 3 and 10 mg/kg of SR 141716 separately to vehicle exhibited only a tendency toward significance (F*s* = 1.20, 2.37, 5.01 respectively).

When first placed in the self-stimulation apparatus it was noticed that rats treated with 20 mg/kg of SR 141716 exhibited clear deficits in coordinated movement and a marked loss of muscle tone. However, these clear behavioural deficits did not impede bar pressing. Thus, no effect of SR 141716 on asymptotic responding was evident, with no significant decreases in Y_{max} for any SR 141716 dose tested (F*s* < 1).

SCH 23390 (0.06 mg/kg i.p.) also caused a decrease in the rewarding efficacy of MFB self-stimulation as assessed by M_{50} [$F(1,5) = 23.29$, $p < 0.01$]. A one-factor ANOVA directly comparing the inhibitory effects of 20 mg/kg of SR 141716 and 0.06 mg/kg of SCH 23390 on M_{50} indicated a significantly larger effect of SCH 23390 [$F(1,5) = 12.61$, $p < 0.05$]. A slight decrease in asymptotic responding was evident in SCH 23390-treated rats, as shown by a significant decrease in Y_{max} [$F(1,5) = 10.99$, $p < 0.05$].

Electrode tips were found to be within the boundaries of the MFB at the level of the LH in similar regions to the representative photomicrographs shown in Figure 6.2.

6.4. Discussion

The current chapter reports for the first time the effects of the synthetic cannabinoid receptor agonist CP 55,940 and the selective cannabinoid receptor antagonist SR 141716 on MFB self-stimulation in Lewis rats. CP 55,940 was shown to have no effect upon MFB self-stimulation behaviour. This is consistent with a previous study that showed that the cannabinoid, levonantradol, had no effect on electrical brain stimulation thresholds (Kucharski et al., 1983). Furthermore withdrawal from acute administration of CP 55,940 did not elevate electrical stimulation reward thresholds when measured 24 hours later under drug-free conditions.

Previous studies by Gardner and colleagues have shown that Δ^9-THC (1.5 and 1 mg/kg respectively) enhances the rewarding potency of electrical brain stimulation using two different procedures, namely, a titrating threshold stimulation paradigm and a rate-frequency curve shift paradigm (Gardner et al., 1988; Lepore et al., 1996a). In general, brain stimulation reward psychophysicists agree that the rate-frequency curve shift paradigm provides the best method of assessing the effects of drugs on electrical brain stimulation behaviour (Bauco & Wise, 1997; Gallistel & Freyd, 1987; Miliaressis, Rompre, Laviolette, Philippe, & Coulombe, 1986; Wise & Rompre, 1989). Both previous findings by Gardner and colleagues might be seen as complimentary to the finding reported here showing that CB_1 receptor antagonism caused only a modest inhibitory effect on the rewarding efficacy of self-stimulation in Lewis rats (Gardner et al., 1988; Lepore et al., 1996a). However, the current investigation failed to demonstrate a facilitatory effect of CP 55,940 (10, 25 and 50 µg/kg), a synthetic analogue of Δ^9-THC (see section 2.2.1.3.). Thus, this appears to contradict the findings of Lepore *et al.* (1996a) which also used a rate-frequency curve shift paradigm and a comparable dose of Δ^9-THC.

This disagreement could be due to subtle differences between Δ^9-THC and CP 55,940 in their chemical structures. For instance, CP 55,940 lacks the central pyran ring of

the Δ^9-THC molecule (see Figure 1.1.). Thus, differences in the chemical structure between these cannabinoids might be responsible for differences found in the effects of CP 55,940 and Δ^9-THC on signal transduction processes (Bonhaus et al., 1998). Furthermore, it may also contribute to CP 55,940 having less of an effect on the reward-relevant NAS in comparison to Δ^9-THC. Thus, it is possible that slight differences in the chemical structures of Δ^9-THC and CP 55,940 may be responsible for CP 55,940 having less of an effect on the reward-relevant NAS than Δ^9-THC. This in turn may help to explain why CP 55,940 did not affect brain stimulation behaviour in the current chapter while Δ^9-THC did in the study by Lepore *et al* (1996a).

An alternative explanation of why CP 55,940 did not modulate reward is based on close scrutiny of the methodology employed by Lepore *et al* (1996a). First, it is important to note that the magnitude of effect of Δ^9-THC found in their study was very small compared to the effects of other drugs of abuse such as amphetamine, MDMA and cocaine (Bauco & Wise, 1997; Gallistel & Karras, 1984; Lin et al., 1997). These drugs can reliably shift the rate-frequency function to the left by between 0.2 and 0.5 $\log_{10}$ units (Bauco & Wise, 1997; Gallistel & Karras, 1984; Lin et al., 1997). However, the Lepore *et al* (1996a) study showed that Δ^9-THC could only shift the rate-frequency function to the left by approximately 0.05 $\log_{10}$ units. Second, it is possible that the effect of CP 55,940 was unable to be observed within the accepted amount of baseline variation in responding over days in the current study because this was larger than that accepted in the Lepore *et al* (1996a) study. Lepore *et al* (1996a) used a very strict criterion of stable responding (within 0.01 $\log_{10}$ units over days) possibly to make sure that the subtle effect of Δ^9-THC could be distinguished from the variation over days in baseline responding. Interestingly, the magnitude of effect of Δ^9-THC in this study was smaller than what most investigators judge as being stable baseline responding (within 0.1 $\log_{10}$ units over consecutive days) which indicates the measurements are sensitive and reliable for animals to be tested for the effects

of drugs. Thus, any subtle effects of CP 55,940 in the current study may have been lost within baseline variation in responding over days.

Acute administration of CP 55,940 (10, 25 and 50 µg/kg) produced no elevations in electrical brain stimulation thresholds (using the M_{50} estimate of threshold) when measured 24 hours after this administration under drug-free conditions. This is inconsistent with a previous report by Gardner and colleagues which showed that *acute* administration of 1 mg/kg of Δ^9-THC caused an elevation in electrical brain stimulation thresholds 24 hours later under drug-free conditions (Gardner et al., 1998, 1999). Such elevations in reward threshold have been promoted as a model of the depression or dysphoria experienced by human users who are dependent on a drug when they attempt to abstain from using that drug. In the animal model such elevations in threshold have been reported to occur upon withdrawal from *chronic* administration of a number of different drugs of abuse, including nicotine (Epping-Jordan, Watkins, Koob, & Markou, 1998; Watkins, Stinus, Koob, & Markou, 2000), ethanol (Schulteis, Markou, Cole, & Koob, 1995), amphetamine (Kokkinidis & Zacharko, 1980; Lin, Koob, & Markou, 2000; Wise & Munn, 1995), cocaine (Baldo, Koob, & Markou, 1999; Frank, Manderscheid, Panicker, Williams, & Kokoris, 1992; Kokkinidis & McCarter, 1990; Markou & Koob, 1991) and morphine (Schulteis, Markou, Gold, Stinus, & Koob, 1994). Close scrutiny of these findings shows that such elevations in reward threshold can not be discerned after acute administration of these drugs. Thus, the finding of Gardner *et al* (1998, 1999) appears to be doubtful which underscores the importance of future studies that: 1) attempt to replicate the finding of Gardner *et al* (1998, 1999) and; 2) seek to determine whether withdrawal from chronic administration of a cannabinoid receptor agonist, such as Δ^9-THC, can elevate reward thresholds as assessed by the intracranial self-stimulation paradigm.

SR 141716 significantly attenuated the rewarding potency of MFB stimulation, but only at the highest dose tested (20 mg/kg). Further this effect was small in comparison to the effects of the D_1 receptor antagonist SCH 23390. It is notable that in previous studies,

much lower doses of SR 141716 (0.1 - 3 mg/kg) have been effective in reducing the rewarding effects of morphine and cocaine and inhibiting appetite for palatable foods and alcoholic beverages (Arnone et al., 1997; Chaperon et al., 1998; Gallate & McGregor, 1999). Thus 3 mg/kg SR 141716 almost completely blocked operant responding for beer in an animal model of alcohol craving (Gallate & McGregor, 1999) while 0.1 - 3 mg/kg prevented the acquisition of a conditioned place preference to cocaine (Chaperon et al., 1998). The need to use a much higher dose of SR 141716 to affect self-stimulation may be explained by electrical MFB stimulation being a much more potent reinforcer than food, alcohol or drugs. Alternatively, it might be argued that the Lewis rats used here may be less sensitive to the effects of SR 141716 than other rat strains, since to our knowledge this is the first study to administer SR 141716 to the Lewis strain of rat. This hypothesis is consistent with the results of Chapter 4 which showed that Lewis rats were generally less sensitive to the effects of CP 55,940 on *c-fos* expression in reward-relevant areas of the brain compared to Wistar rats.

The fact that such a high dose of SR 141716 was needed to affect self-stimulation behaviour also raises the possibility that such a dose acts on non-cannabinoid systems. An *in vitro* study showed that SR 141716A, at 1µM concentrations, did not bind to any other known receptors that have been tested including DA, opioid, glutamate, 5-HT and GABA (Rinaldi-Carmona et al., 1994). However, it is likely that 20 mg/kg of SR 141716 exceeds the concentrations that were used in this *in vitro* study. Therefore, it can not be ruled out that SR 141716 also affected non-cannabinoid receptors critical to reward. To settle this dispute future studies could attempt to reverse the effect of SR 141716 on electrical brain stimulation behaviour using a cannabinoid receptor agonist to show that the effect was selectively mediated by CB_1 receptors.

If it is assumed that the effect of 20 mg/kg of SR 141716 on self-stimulation was selectively mediated by CB_1 receptors, then cannabinoid systems have a relatively minor influence on this phenomenon. Both the need to use such a high dose, and the lack of

effects observed with lower doses (1, 3, 10 mg/kg) emphasise this view. One explanation for the effect of SR 141716 in the present study is that it blocks a tonic activation of reward-relevant neural substrates provided by endogenous cannabinoid ligands such as 2-AG and anandamide. Consonant with this, Δ^9-THC is known to activate the mesolimbic DA pathway, thought to be a critical brain reward substrate (Gardner et al., 1998, 1999; Tanda et al., 1997). If a tonic level of endogenous cannabinoid activity in the mesolimbic DA system contributes to the rewarding effects of MFB self-stimulation, then antagonising this tone with SR 141716 will presumably reduce the rewarding efficacy of MFB stimulation. One problem with this hypothesis is that SR 141716 has been found to have no effect on DA release in the shell of the NAS at doses up to 10 mg/kg (Alonso et al., 1999; Tanda et al., 1997). However it still remains possible that the inhibitory effects of 20 mg/kg of SR 141716 on MFB self-stimulation are mediated by decreased DA efflux in the NAS.

Another possible explanation of the effects of SR 141716 seen here relies on recent evidence that SR 141716 acts as an inverse agonist at the CB_1 receptor (Bouaboula et al., 1997; MacLennan et al., 1998) (see section 6.2.1.3.). Based on biochemical evidence, SR 141716A appears to have direct effects on the receptor-mediated intracellular transduction signaling that are opposite to those seen with cannabinoid receptor agonists (Bouaboula et al., 1997). This raises the issue of whether the effects of SR 141716 on self-stimulation reflect a silent inhibition of endogenous cannabinoid tone or whether they rely on an intrinsic effect of SR 141716 on the CB_1 receptor and signal transduction processes. The issue of whether SR 141716 is a competitive antagonist or inverse agonist could be reconciled by future investigations.

Apart from drugs of abuse affecting a common neural substrate of reward, it is also possible that they affect common post-receptor, signal transduction pathways. It has been hypothesised that the reinforcing effects of psychostimulants and opiates may be derived from DA and opioid receptor coupling to a common G protein-second messenger pathway (Self & Stein, 1992; Self, Terwilliger, Nestler, & Stein, 1994). Interestingly, inactivation

of $G_{i/o}$ proteins in the NAS attenuates the reinforcing efficacy of cocaine and heroin (Self et al., 1994). Further, it is also possible that the rewarding effects of cannabis be derived from modulating this same common signal transduction pathway because CB_1 receptors, like D_2 receptors and μ and δ opioid receptors, are linked to G_i proteins. Therefore, an alternative interpretation of the effects of SR 141716 in the current study is based on the hypothesis that a common signal transduction mechanism underlies the rewarding effects of psychostimulants, opiates and cannabis.

It has been recently reported that SR 141716A, apart from blocking CB_1 receptors, may also block G_i proteins and the usual intracellular responses they mediate (Bouaboula et al., 1997). A recent study has shown that SR 141716A sequesters $G_{i/o}$ proteins from a common pool, an action that prevents α_{2} adrenergic and somatostatin receptors from transducing their biological signals (Vasquez & Lewis, 1999). In the present results, it is possible that SR 141716 inactivated $G_{i/o}$ proteins from a common G protein pool used by DA and endogenous opioid receptors. Therefore, SR 141716 by reducing the function of DA and endogenous opioid receptors at the level of signal transduction, may explain why SR 141716 reduced the rewarding efficacy of MFB stimulation.

In conclusion, the present results suggest that endogenous cannabinoid systems may only have a small role in the neural substrate of brain stimulation reward if they have any role at all. This is consistent with research showing that endogenous cannabinoids have a subtle neuromodulatory role in brain function (Di Marzo et al., 1998). In the current chapter only 20 mg/kg of SR 141716 was able to reduce electrical MFB stimulation thresholds in Lewis rats. Lower doses (1 - 10 mg/kg) of SR 141716 had no such effect. Future studies may attempt to test whether the effect of 20 mg/kg of SR 141716 is selectively mediated by CB_1 receptors. If the effect is mediated only by CB_1 receptors then the role of the endogenous cannabinoid system in the substrate of MFB stimulation reward is relatively modest at best. This is highlighted by the magnitude of effect of SR 141716 being relatively small in comparison to the effects of the D_1 receptor antagonist SCH 23390.

The current chapter also reported that acutely administered CP 55,940 (10 - 50 µg/kg) had no effect on MFB self-stimulation thresholds. This is consistent with the results of Chapter 4 which showed that CP 55,940 had a relatively minor effect on *c-fos* expression in reward-related areas of the brain, such as the shell of the NAS. In addition, withdrawal from acute administration of CP 55,940 did not elevate electrical brain stimulation reward thresholds when measured 24 hours later under drug-free conditions.