CHAPTER FOUR

Strain differences in CP 55,940-induced *c-fos* expression, hypothermia and catalepsy

4.1. Introduction

Lewis rats, unlike other inbred strains of rat, appear to find the main psychoactive constituent of marijuana, Δ^9-THC, rewarding as assessed by the intracranial self-stimulation paradigm (Lepore et al., 1996a). In addition, Lewis rats also show less susceptibility to the anxiogenic effects of the synthetic analogue of Δ^9-THC, CP 55,940, when compared to Wistar rats (see Chapters 2 and 3). These observations have led to the hypothesis that in Wistar rats, the effects of cannabinoids on neural substrates mediating anxiety may outweigh the effects the drug has on neural substrates underlying reward. However, the Lewis strain of rat appears less susceptible to the anxiogenic effects of cannabinoids and this may allow the unmasking of a rewarding effect. Thus, for Lewis rats it is hypothesised that the effects of cannabinoids on neural substrates underlying reward outweigh the effects the drug has on neural substrates mediating anxiety.

The current chapter examines the above hypotheses by quantifying CP 55,940-induced *c-fos* expression in the rat brain in Lewis and Wistar rats. *C-fos*, an immediate early gene (IEG) that produces the Fos protein, is a transcription factor that is rapidly and transiently expressed to various stimuli, including the activation of synaptic receptors. Furthermore, by forming a heterodimer with members of the Jun family of IEGs and by binding to AP-1 DNA binding sites, *c-fos* is thought to regulate the expression of genes, therefore promoting long-term cellular changes (Hughes & Dragunow, 1995). Fos has been used as a marker of neural activation, providing researchers with a unique map of the neural response to a given stimulus (Chaudhuri,

1997; Dragunow & Faull, 1989). *C-fos* expression has also been implicated in the long-term plastic changes occurring in the CNS that are thought to underlie drug addiction and drug craving (Harlan & Garcia, 1998; Hughes & Dragunow, 1995). Some evidence has illustrated the functional importance of *c-fos* in the NAS in the expression of cocaine-induced locomotor hyperactivity (Heilig, Engel, & Soderpalm, 1993). In addition, *c-fos* appears to be functionally critical to the expression of anxiety in rodents. One study has shown that central infusion of a *c-fos* antisense oligonucleotide into the amygdala of rats abolished anxiety as assessed by the Vogel conflict test (Moller, Bing, & Heilig, 1994).

The vulnerability of Lewis rats to the rewarding effects of recreational drugs is not limited to the cannabinoids. Lewis rats are also found to have a higher vulnerability to the rewarding effects of other drugs of abuse (see section 1.6.). These effects may rest on striking differences between Lewis rats and Fischer 344 rats in the biochemistry and electrophysiology of the mesolimbic DA system (see section 1.6.). For instance, Lewis rats have more TH per neuron in the VTA (Beitner-Johnson et al., 1991; Harris & Nestler, 1996), and less TH and inhibitory G proteins in the NAS (Beitner-Johnson et al., 1991; Brodkin et al., 1998). Furthermore, Lewis rats also exhibit less basally active VTA neurons with a higher percentage of burst firing patterns (Minabe et al., 1995) and longer-lasting increases in DA release in the NAS following an injection of cocaine (Strecker et al., 1995). The current chapter will attempt to assess the neural basis of the unique rewarding effects that cannabinoids have on Lewis rats by observing the expression of CP 55,940-induced *c-fos* in this strain and comparing it with that seen in Wistar rats.

The exact reasons for Lewis rats being less susceptible to the anxiogenic effects of CP 55,940 than Wistar rats (see Chapters 2 and 3) are unclear. One obvious hypothesis is that CP 55,940 may have less of an effect on brain structures implicated in anxiety such as the CEA, BNST or PVN in Lewis rats in comparison to Wistar rats (Davis, 1998; Rosen & Schulkin, 1998). The CEA appears to be a critical area involved in cannabinoid-induced anxiety highlighted by a study that showed that

administration of Δ^9-THC directly into the CEA increased anxiety-like behaviour of mice on the EPM (Onaivi et al., 1996). Further, extrahypothalamic CRH receptors such as those found in the CEA, appear to be critical to cannabinoid-induced anxiety (see section 1.6.). As noted previously, the PVN of Lewis rats has been shown to contain less CRH mRNA than Wistar rats (Oitzl et al., 1995). Moreover, it is possible that the Lewis rats deficiency in CRH gene expression in the PVN may also apply to other brain areas such as the CEA or BNST as these areas also contain CRH receptors. This may explain why the HPA of Lewis rats releases less ACTH and corticosterone to both stress and to the administration of drugs, such as arecoline and methoxamine (see sections 1.6. and 2.1.) in comparison to other rat strains (Calogero et al., 1992; Simar et al., 1996). Moreover, it may help to explain why Lewis rats are subsensitive to the anxiogenic effects of CP 55,940 in comparison to Wistar rats (see Chapters 2 and 3). The current chapter will test this hypothesis by observing the expression of CP 55,940-induced *c-fos* in anxiety-related areas of the brain including the CEA, BNST and PVN.

Previous studies examining the effects of Δ^9-THC and its synthetic analogues on *c-fos* expression have examined only a relatively small number of brain structures (see Table 4.1.). This has made it difficult to compare the map of *c-fos* expression produced by cannabinoid exposure with the maps induced by other drugs of abuse (Harlan & Garcia, 1998). Previous studies have consistently shown *c-fos* expression induced by cannabinoids in the CPU and the NAS (McGregor et al., 1998; Miyamoto et al., 1996; Porcella et al., 1998; Rodriguez de Fonseca et al., 1997). Interestingly, DA D_1 receptors appear to play a permissive role in the expression of *c-fos* in these areas (Miyamoto et al., 1996) which is consistent with Δ^9-THC promoting DA release in the NAS (Chen et al., 1990; Chen et al., 1991; Tanda et al., 1997). Previous studies have demonstrated that Δ^9-THC or synthetic analogues such as HU-210 increased *c-fos* expression in the CEA, PVN (McGregor et al., 1998; Rodriguez de Fonseca et al., 1997), BNST, VTA (Rodriguez de Fonseca et al., 1997), lateral septum (LS) (McGregor et al., 1998) and the fronto-parietal and cingulate cortices (Mailleux, Verslype, Preud'homme, & Vanderhaeghen, 1994; Porcella et al., 1998). In light of

the limited anatomical detail provided by these studies, a more detailed analysis of *c-fos* was provided in the current chapter. Notably, the present chapter assessed CP 55,940-induced *c-fos* expression in the following reward and anxiety-related areas of the brain that have not been previously assessed: 1) the medial prefrontal cortex (MPC), 2) ICjM, 3) PAG and 4) PPTg.

Table 4.1. Previous studies that have assessed the effects of exogenous cannabinoid receptor agonists on *c-fos* expression in the rat brain.

Study	Exogenous CB1 receptor agonist	Brain structures examined	Dose	Strain	Technique
(Glass & Dragunow, 1995)	CP 55,940	CPU patch compartment	2.5 mg/kg	Wistar	Immunohistochemistry
(Mailleux et al., 1994)	Δ^9-THC	Cingulate cortex, frontoparietal cortex, CPU	5 mg/kg	Wistar	*In situ* hybridization
(Miyamoto et al., 1997; Miyamoto et al., 1996)	Δ^9-THC	Dorsomedial striatum, NAS	10 mg/kg	Wistar	Immunohistochemistry
(Rodriguez de Fonseca et al., 1997)	HU-210	Hippocampus, NAS core and shell, CEA, BNST, thalamus, PVN, SCh, SO, VTA, locus coeruleus, central grey, solitary tract, area postrema	100 µg/kg	Wistar	Immunohistochemistry
(McGregor et al., 1998)	Δ^9-THC	LS, CPU, NAS, PVN, CEA	5 mg/kg	Wistar	Immunohistochemistry
(Porcella et al., 1998)	Δ^9-THC	NAS, CPU, cingulate cortex	10 or 15 mg/kg	Sprague-Dawley rats	Western Blot

For comparison purposes, the *c-fos* expression induced by cocaine was also examined in the present chapter. The distribution of cocaine-induced *c-fos* expression has some commonalities with that produced by acute exposure to a cannabinoid receptor agonist. Cocaine-induced increases in *c-fos* expression have been observed in the cortex, CPU, NAS, septum, ICjM, paraventricular nucleus of the thalamus (PV), amygdala, lateral habenula, suprachiasmatic nucleus of the hypothalamus (SCh), lateral hypothalamic area (LH) and the cerebellum (Harlan & Garcia, 1998). In the current study, the effects of cocaine on *c-fos* expression were also tested in Lewis and Wistar rats to provide a comparison to the effects of CP 55,940. In terms of behaviour, Lewis

rats are more susceptible to the rewarding effects of cocaine as assessed by self-administration and conditioned place preference models when compared to Fischer 344 rats (Kosten et al., 1994; Kosten et al., 1997). In addition, Lewis rats are more sensitive to acutely administered cocaine where they show greater locomotor activity than Fischer 344 rats (Camp et al., 1994). Furthermore, Lewis rats are more susceptible to the effects of the chronic administration of cocaine where they exhibit enhanced sensitization of locomotor activity compared to other rat strains (Kosten et al., 1994 and also see Chapter 5). Thus, assessing the effects of cocaine on *c-fos* expression in Lewis and Wistar rats provides a useful contrast to the effects of CP 55,940. In addition, this study assesses the possibility that Lewis rats are not generally subsensitive to the effects of drugs of abuse on *c-fos* expression.

4.2. Experiment 4A. CP 55,940-induced *c-fos* expression, hypothermia, inhibition of locomotor activity and catalepsy in Lewis and Wistar rats

4.2.1. Methods

4.2.1.1. Subjects. The subjects were 15 Lewis rats and 15 Wistar rats matched for age (75-90 days old). Wistar rats weighed between 370 and 550 g and Lewis rats weighed between 350 and 430 g at the time of testing. Rats were housed in large plastic tubs in groups of eight and maintained under a 12h:12h reversed light:dark cycle (lights off at 9 a.m.) with food and water available *ad libitum.* All experimentation occurred during the dark cycle.

4.2.1.2. Drug. CP 55,940 was prepared and administered i.p. 30 min prior to behavioural testing as stated in section 2.2.1.3. Two doses were used in this study, 50 µg/kg and 250 µg/kg. It was of interest to observe whether Lewis and Wistar rats would diverge in their response to a relatively high dose of CP 55,940 (250 µg/kg). The 250 µg/kg dose resembles doses that have been used in previous studies assessing the effects of cannabinoids on *c-fos* expression in the rat brain. Previous studies have reported that *c-fos* expression in the rat brain is magnified by Δ^9-THC exposure (3 - 10 mg/kg) (Mailleux et al., 1994; McGregor et al., 1998; Miyamoto et al., 1997; Miyamoto et al., 1996; Porcella et al., 1998), HU-210 (100 µg/kg) (Rodriguez de Fonseca et al., 1997) and anandamide (20 mg/kg) (McGregor et al., 1998). However, the problem with administering such large doses is that they are much higher than what most humans use recreationally. Furthermore, doses in this range cause profound behavioural deficits such as catalepsy in rodents. Thus, the current study also employs 50 µg/kg of CP 55,940 which is a smaller and more ecologically relevant dose. In addition, as shown in Chapters 2 and 3, this dose had divergent effects on anxiety-like behaviour in Lewis and Wistar rats.

4.2.1.3. Procedure.

4.2.1.3.1. ***Behavioural testing.*** Rats from each strain were extensively handled for a week prior to the start of the experiment and were randomly assigned to one of three groups (n = 5 per group) to receive either 0, 50 or 250 µg/kg of CP 55,940.

Two habituation days were given immediately prior to the test day in order to minimise any *c-fos* expression due to novelty of testing, handling or injection procedures. On these habituation days the rats were subjected to exactly the same procedures to those used on the test day except that they were given only saline injections.

On the test day individual rats were removed from their home cage and brought through to the test room in a small enclosed rectangular tub filled with wood shavings. All rats were given a single i.p. injection of either 0, 50 or 250 µg/kg CP 55,940. 30 min following this injection, rats were subjected to a series of tests that consisted of measuring catalepsy, axillary temperature and locomotor activity.

First rats were tested for catalepsy using a bar test (Wickens & Pertwee, 1993). The forepaws of the rat were placed on a horizontal metal bar (25 cm x 0.5 cm in diameter) that was held 10 cm above the bench using a retort stand. The experimenter used a stopwatch to measure the time that it took the rat to move both paws onto the bench. Each rat was given a maximum of 2 min to do this. This procedure was repeated twice and the average of these measures formed the basis for statistical comparison.

Rats were then tested for their axillary (underarm) temperature using a digital thermometer and thermocouple probe (Yellow Spring Instruments, BAT-12). Typically the probe remained under the rats arm for 30 sec before the temperature stabilized and a reading was taken. Measuring axillary temperature is thought to be considerably less stressful than taking rectal temperature yet gives a highly reliable index of body temperature (McGregor et al., 1996b).

Figure 4.1. The locomotor activity testing cage with sound attenuating chamber.

The rats were then immediately placed in one of eight identical chambers [30 cm (L) x 25.5 cm (W) x 50cm (H)] for 30 min of locomotor activity testing. The side walls and roof of the chambers were aluminum, while the front wall and rear wall were made of clear perspex. The cage floors consisted of 16 metal bars (0.5 cm diameter, spaced 1 cm apart) connected to a high impedance amplifier. When the rat moved on the grid so that contact or breaking of contact between any four bars and the other twelve occurred, an activity count was recorded by a Macintosh™ computer running WorkbenchMac™ data acquisition software (McGregor, 1996a,c). Each cage was encased in a wooden sound attenuation chamber that was equipped with a fan that provided masking noise during testing.

Immediately after the locomotor activity test, axillary temperature was taken again. Rats were then placed in individual plastic tubs in an adjacent darkened holding room for a further 60 min. On the two habituation days the rats were returned to their group housing in the colony room. On the test day, the rats were removed to a laboratory where they were given an overdose of pentobarbitone (120 mg/kg, i.p.) in preparation for immunohistochemical analysis (see section 4.2.1.3.2.).

4.2.1.3.2. ***Immunohistochemistry.*** The immunohistochemical technique was performed as described previously (Hunt & McGregor, 1998; Stephenson, Hunt, Topple, & McGregor, 1999). Rats were perfused transcardially with 100 ml of 0.1 M phosphate-buffered saline (PBS) followed by 250 ml of 4% paraformaldehyde in PBS (pH 7.3). The brains were removed and placed in paraformaldehyde overnight at 4°C and stored in cold 30% sucrose for 72 h. The brains were then placed on microtome stages, frozen to -17°C and sliced at 40 µm with slices collected in PBS.

Free floating sections were incubated for 30 min in 1% hydrogen peroxide in PBS and then for 30 min in 3% normal horse serum in PBS. They were incubated in the primary *c-fos* antibody (rabbit polyclonal; Santa Cruz Biotechnology, specific for the amino terminus of *c-fos* p62, non cross-reactive with FosB, Fra-1 or Fra-2) diluted 1:2000 in phosphate buffered horse serum (0.1% bovine serum albumin, 0.2% Triton X-100, 2% normal horse serum in PBS) for 72 h at 4°C. Sections were washed for 30 min in PBS at room temperature and then incubated for 1 h in biotinylated anti-rabbit IgG (Vector Laboratories; diluted 1:500) in phosphate buffered horse serum. They were then washed in PBS for a further 30 min and then incubated for 2 h in ExtrAvidin-horseradish peroxidase (Sigma; diluted 1:1000 in phosphate buffered horse serum). After three 30 min washes in PBS, horseradish peroxidase activity was visualised with the nickel diaminobenzidine and glucose oxidase reaction. This reaction was terminated after approximately 10 min by washing in PBS. The sections were then mounted onto slides, dehydrated, xylene cleared and coverslipped.

4.2.1.3.3. ***Counting of labeled cells.*** The amount of *c-fos* expression to CP 55,940 (0, 50 and 250 µg/kg) was quantified in 33 different brain regions or subregions with reference to the rat brain atlas of Paxinos and Watson (Paxinos & Watson, 1997). As described previously (Hunt & McGregor, 1998) quantification was performed manually by an observer who was blind to group assignment. Counts, for the most part, were made within a graticule which equated to 0.5 mm^2 at a magnification of 20 times. The location of where Fos-labelled cells were counted is represented in Figure 4.2. Regions not square in shape in Figure 4.2 represent areas in

which the graticle was not used to count cells. A neuron was classified as Fos-positive when the nucleus appeared round or oval, completely filled, and dark brown or black in colour.

4.2.1.4. Data analysis. Data for *c-fos* expression, locomotor activity, catalepsy and axillary temperature were analysed by two-factor ANOVA. The two factors were strain (Lewis versus Wistar) and dose of CP 55,940 (vehicle versus 50 µg/kg and 250 µg/kg of CP 55,940). To examine whether 50 µg/kg of CP 55,940 affected *c-fos* expression, locomotor activity, catalepsy and axillary temperature in its own right, an individual contrast compared vehicle to 50 µg/kg of CP 55,940 for both strains combined. Data analysed for axillary temperature were an average of the first and second body temperature measures taken before and after a 30 min locomotor activity test.

To assess for strain differences in baseline *c-fos* expression the data for vehicle-treated Lewis and Wistar rats were analysed by a one-factor ANOVA where strain was treated as the factor and the number of Fos-labeled cells counted in discrete areas of the brain were the dependent variables.

As in Chapters 2 and 3, body temperature and behavioural data (axillary temperature, locomotor activity and catalepsy) were also assessed for the effects of CP 55,940 within strains. Therefore, separate one-factor ANOVA for Lewis and Wistar rats were conducted followed by Dunnett's post-hoc to assess the effects of various CP 55,940 doses within a strain. Furthermore, to assess baseline differences in any of these measures, vehicle-treated Lewis and Wistar rats data were compared using a one-factor ANOVA where strain was treated as the factor and data for body temperature and behaviour was treated as the dependent variable.

Figure 4.2. goes here ie illustrator picture with location of where brain regions were counted.

4.2.2. Results

4.2.2.1. Catalepsy. Wistar rats were more susceptible to the cataleptic effects of 250 µg/kg of CP 55,940 than Lewis rats (see Figure 4.3.). Two-factor ANOVA revealed a significant effect of strain [$F(1,24) = 6.75$, $p < 0.05$], dose [$F(1,24) = 35.62$, $p < 0.001$] and a significant strain by dose interaction [$F(1,24) = 5.27$, $p < 0.05$]. An individual post-hoc comparison between all rats given vehicle and all rats given 50 µg/kg of CP 55,940 showed no significant cataleptic effect ($F < 1$). Within strain post-hoc analysis confirmed these findings showing that only 250 µg/kg of CP 55,940 promoted catalepsy in either strain (see Figure 4.3.) ($p < 0.05$). No significant difference was observed between vehicle-treated Lewis and Wistar rats in baseline catalepsy scores [$F(1,8) = 2.77$, $p = 0.13$].

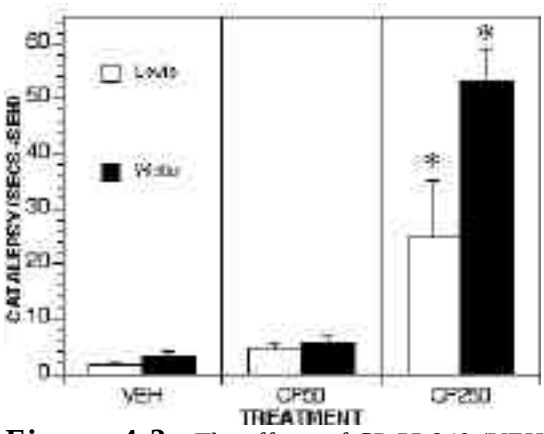

Figure 4.3. The effects of CP 55,940 (VEH = 0 µg/kg, CP50 = 50 µg/kg and CP250 = 250 µg/kg) on catalepsy in Lewis and Wistar rats (n = 5 per group). * signifies the CP 55,940 group is significantly more than the respective vehicle group within strain ($p < 0.05$).

4.2.2.2. Axillary temperature. Wistar rats were more susceptible to the hypothermic effects of CP 55,940 than Lewis rats (see Figure 4.4.). Two-factor ANOVA revealed a significant effect of dose [$F(2,24) = 1.10$, $p < 0.001$] and a significant strain by dose interaction [$F(2,24) = 5.08$, $p < 0.05$]. Both doses of CP 55,940 were effective in lowering body temperature. This was supported with a significant post-hoc comparison between all rats given vehicle and 50

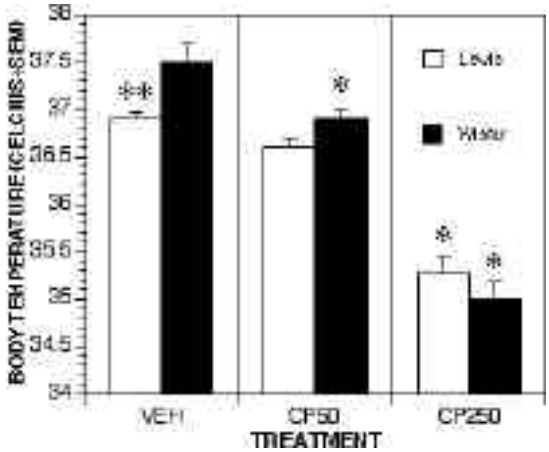

Figure 4.4. The effects of CP 55,940 (VEH = 0 µg/kg, CP50 = 50 µg/kg and CP250 = 250 µg/kg) on axillary temperature in Lewis and Wistar rats (n = 5 per group). * signifies the CP 55,940 group has a significantly lower temperature than the respective vehicle group within strain ($p < 0.05$). ** signifies that Lewis rats (VEH) have a significantly lower temperature than Wistar rats (VEH) ($p < 0.05$).

µg/kg CP 55,940 [$F(1,24) = 9.65$, $p < 0.01$]. The reduction of axillary temperature at the 50 µg/kg dose of CP 55,940 is probably largely attributable to Wistar rats not Lewis rats. This was supported by within strain post-hoc analyses which revealed that this dose significantly reduced axillary temperature only in Wistar rats ($p < 0.05$) (see Figure 4.4.). Lewis rats treated with vehicle showed significantly lower axillary temperature than Wistar rats treated with vehicle [$F(1,8) = 8.05$, $p < 0.05$].

4.2.2.3. Locomotor activity. Wistar rats treated with CP 55,940 showed a dose-dependent reduction in locomotor activity. However, CP 55,940 appeared to have no effect on the Lewis strain of rat (see Figure 4.5.). Two-factor ANOVA revealed a significant effect of strain [$F(1,24) = 18.72$, $p < 0.001$], dose [$F(2,24) = 22.06$, $p < 0.001$] and a significant strain by dose interaction [$F(2,24) = 14.05$, $p < 0.001$]. An individual post-hoc comparison between vehicle and 50 µg/kg of CP 55,940 showed that this dose significantly reduced locomotor activity [$F(1,24) = 21.52$, $p < 0.001$]. The reduction of locomotor activity at 50 and 250 µg/kg of CP 55,940 is probably attributable to Wistar rats not Lewis rats. This was supported by within strain post-hoc analyses which revealed that these doses significantly reduced locomotor activity only in Wistar rats ($p < 0.05$) (see Figure 4.5.). Lewis rats treated with vehicle showed significantly lower locomotor activity than Wistar rats treated with vehicle [$F(1,8) = 18.32$, $p < 0.01$], see Figure 4.5.

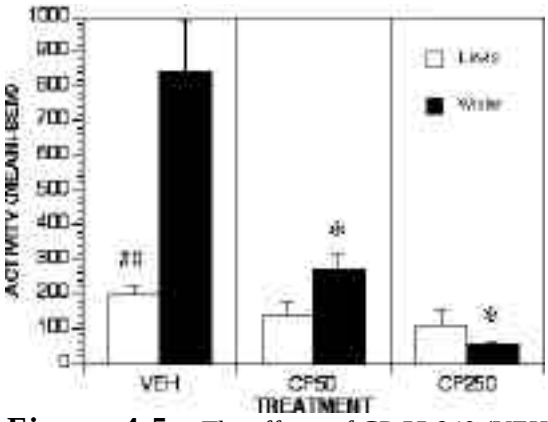

Figure 4.5. The effects of CP 55,940 (VEH = 0 µg/kg, CP50 = 50 µg/kg and CP250 = 250 µg/kg) on locomotor activity in Lewis and Wistar rats (n = 5 per group). * signifies the CP 55,940 group is significantly less than the respective vehicle group within strain ($p < 0.05$). ## signifies that Lewis rats (VEH) are significantly less than Wistar rats (VEH) ($p < 0.01$).

4.2.2.4. C-fos expression. Throughout the brain significant strain differences were observed in CP 55,940-induced *c-fos* expression. Specific counts for 33 regions of interest are shown in Table 4.2. The locations of where these counts

were made are represented in Figure 4.2. Wistar rats showed significantly greater CP 55,940-induced *c-fos* expression than Lewis rats in 15 brain regions. In the forebrain this was observed in the shell and core of the NAS, ICjM, the ventral lateral septum (LS), CEA and the intra-amygdaloid and dorsal area of the lateral division of the BNST. In the hypothalamus, greater CP 55,940-induced *c-fos* expression was observed in Wistar rats in the MnPO, SCh and PVN compared to Lewis rats. In the basal ganglia more Fos-labelled cells were observed in the GP of Wistar rats administered CP 55,940 than Lewis rats. In addition, clear strain differences in the expression of *c-fos* were observed in parts of the midbrain, including the VTA, dorsolateral and ventrolateral PAG and PPTg.

CP 55,940 significantly increased *c-fos* expression in some brain regions without having a differential effect on Lewis and Wistar rats (see Table 4.2.). These areas include the dorsolateral and central CPU, AH, MPO and supraoptic nucleus of the hypothalamus (SO), PV, anterior VTA and the lateral region of the PAG. In addition, CP 55,940 did not affect *c-fos* expression in either strain of rat in some brain regions. These areas include the MPC, septohippocampal nucleus, the medial, dorsal and ventral CPU, the dorsal LS, the medial preoptic area (MPA), the central medial nucleus of the thalamus, the SN and the dorsomedial PAG.

Lewis rats expressed less baseline *c-fos* than Wistar rats in certain brain structures (see Table 4.2.). One-factor ANOVA showed that significantly less Fos-labeled cells were observed in the MPC, PV, central medial nucleus of the thalamus, the intra-amygdaloid nucleus of the BNST, the dorsolateral PAG and the ventrolateral PAG of vehicle–treated Lewis rats when compared to vehicle-treated Wistar rats. All other areas showed no significant differences in baseline *c-fos* expression between Lewis and Wistar rats.

Table 4.2. The effects of CP 55,940 (VEH = 0, CP50 = 50 and CP250 = 250 µg/kg) on the number of Fos-labeled cells in various brain regions of Wistar and Lewis rats. Two-factor ANOVA revealed: a = a significant difference between strains ; b = a significant overall effect of CP 55,940 doses (CP50 and CP250 combined) compared to VEH; c = a significant strain by dose interaction; d = a significant overall difference between VEH and CP50; e = a significant difference between VEH treated Lewis and Wistar rats. A significance level of $p < 0.05$ was adopted for all tests.

REGION	Bregma	WISTAR RATS			LEWIS RATS			Stats
		VEH	CP50	CP250	VEH	CP50	CP250	
Forebrain								
1. Medial Prefrontal Cortex	+3.7	93 ± 23	79 ± 37	146 ± 26	24 ± 15	29 ± 9	45 ± 18	a, e
2. Nucleus Accumbens Shell	+1.0	9 ± 3	15 ± 3	39 ± 8	3 ± 1	11 ± 3	14 ± 0.8	a, b, c, d
3. Nucleus Accumbens Core	+1.0	4 ± 1	1 ± 0.7	8 ± 3	0.2 ± 0.2	0.6 ± 0.4	0.2 ± 0.2	a, b, c
4. Septohippocampal Nucleus	+1.0	0.2 ± 0.2	0.2 ± 0.2	0.5 ± 0.5	0 ± 0	0 ± 0	2 ± 1	
5. Islands of Calleja, major	+1.0	0.6 ± 0.6	3 ± 2	104 ± 15	0.5 ±0.3	10 ± 4	36 ±16	a, b, c, d
6. Lateral Septum, dorsal	+1.0	16 ± 3	10 ± 1	15 ± 5	5 ± 4	3 ± 0.7	6 ± 3	a
7. Lateral Septum, ventral	+0.7	63 ± 12	104 ± 31	217 ± 9	48 ± 8	68 ± 16	88 ± 14	a, b, c
8. BNST lat div, dorsal	-0.26	7 ± 3	68 ± 2	94 ± 13	2 ± 0.6	8 ± 0.8	13 ± 3	a, b, c, d
Hypothalamus/Preoptic								
9. Median Preoptic Nucleus	-0.26	5 ± 1	12 ± 3	28 ± 5	2 ± 0.8	5 ± 2	9 ± 2	a, b, c
10. Medial Preoptic Area	-0.26	21 ± 6	23 ± 3	24 ± 12	13 ± 4	23 ± 3	32 ± 8	
11. Medial Preoptic Nucleus	-1.3	32 ± 7	55 ± 7	82 ± 9	29 ± 5	44 ± 6	57 ± 8	a, b, d
12. Suprachiasmatic Nucleus	-1.4	25 ± 7	29 ± 9	89 ± 40	16 ± 4	20 ± 3	16 ± 6	a, b, c
13. Paraventricular Nucleus	-1.8	14 ± 5	83 ± 16	185 ± 30	18 ± 3	31 ± 6	63 ± 8	a, b, c, d
14. Anterior Hypothalamic Area	-1.8	19 ± 8	48 ± 9	44 ± 8	22 ± 5	25 ± 6	31 ± 6	b
15. Supraoptic Nucleus	-1.8	2 ± 0.6	10 ± 4	7 ± 2	0.4 ± 0.2	3 ± 2	1 ± 0.6	a, b
Thalamus								
16. Paraventricular Nucleus	-1.8	55 ± 5	100 ± 14	113 ± 11	16 ± 1	52 ± 8	79 ± 11	a, b, d, e
17. Central Medial Nucleus	-2.56	16 ± 6	13 ± 6	14 ± 3	0.4 ± 0.2	7 ± 3	7 ± 2	a, e
Amygdala								
18. Central Nucleus	-2.56	3 ± 1	77 ± 17	82 ± 10	0.6 ± 0.6	29 ± 9	25 ± 4	a, b, c, d
19. BNST intra-amygdaloid	-2.56	3 ± 1	8 ± 3	28 ± 28	0.2 ± 0.2	7 ± 1	4 ± 0.9	a, b, c, d, e
Basal Ganglia								
20. Caudate/Putamen, medial	+1.0	8 ± 2	6 ± 3	8.5 ± 4	1 ± 0.4	0.2 ± 0.2	6 ± 2	a
21. Caudate/Putamen, dorsal	+1.0	3 ± 2	7 ± 2	20 ± 4	0 ± 0	0.8 ± 0.6	3 ± 1	a
22. Caudate/Putamen, dorsolat	+1.0	2 ± 0.8	3 ± 1	8.5 ± 3	0.2 ± 0.2	0.2 ± 0.2	3 ± 2	a, b
23. Caudate/Putamen, central	+1.0	0.4 ± 0.2	1 ± 0.4	13 ± 6	0 ± 0	0 ± 0	5 ± 3	b
24. Caudate/Putamen,ventral	+1.0	1 ± 0.8	0.2 ± 0.2	1 ± 0.3	0 ± 0	0.8 ± 0.6	0 ± 0	
25. Globus Pallidus	-0.8	0.4 ± 0.4	1 ± 0.5	15 ± 3	0.2 ± 0.2	0 ± 0	3 ± 1	a, b, c
26. Substantia Nigra, reticulata	-5.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.4	0 ± 0	
Ventral Tegmental Area								
27. Anterior	-5.8	2 ± 1	2 ± 0.6	5 ± 0.8	0.2 ± 0.2	2 ± 0.6	2 ± 0.5	a, b
28. Posterior	-6.3	0.4 ± 0.4	4 ± 0.9	8 ± 0.7	2 ± 0.7	5 ± 2	8 ± 1	b, c, d
Periaqueductal Gray								
29. Dorsomedial Nucleus	-6.72	16 ± 4	19 ± 5	27 ± 7	10 ± 2	8 ± 2	9 ± 2	a
30. Dorsolateral Nucleus	-6.72	16 ± 3	17 ± 5	41 ± 6	8 ± 2	12 ± 4	15 ±2	a, b, c, e
31. Lateral Nucleus	-6.72	12 ± 3	17 ± 5	28 ± 8	7 ± 2	11 ± 3	16 ± 4	b
32. Ventrolateral Nucleus	-8.00	22 ± 3	18 ± 3	67 ± 18	4 ± 2	10 ± 9	27 ± 17	a, b, c, e
Other Brainstem Area								
33. Pedunculopontine Tegmental Nucleus	-8.3	2 ± 0.6	3 ± 0.8	12 ± 1	1 ± 0.5	5 ± 2	5 ± 0.5	a, b, c, d

Figure 4.6 goes here

Figure 4.7

Fig 4.8

Fig 4.9

Fig 4.10

Figure 4.11

4.3. Experiment 4B. Cocaine-induced *c-fos* expression and locomotor activity in Lewis and Wistar rats

In Experiment 4A it was shown that Lewis rats exhibit less CP 55,940-induced *c-fos* expression than Wistar rats in many different brain structures. Thus, it became important to test whether Lewis rats are generally subsensitive to drug-induced *c-fos* expression. Stated in another way, it is possible that Lewis rats may generally express less *c-fos* compared to Wistar rats irrespective of what class of drug is administered. To test this hypothesis Experiment 4B assessed the effects of cocaine on *c-fos* expression in Lewis and Wistar rats. The present study did not aim to provide an exhaustive overview of the effects of cocaine on *c-fos* expression, therefore only a subset of areas reported in Experiment 4A were examined.

4.3.1. Methods

4.3.1.1. Subjects. The subjects were 4 Lewis rats and 4 Wistar rats matched for age (75-90 days old). All rats weighed within the same range as stated in section 4.2.1.1. The rats were housed and maintained under the same conditions as stated as in section 4.2.1.1.

4.3.1.2. Drug. Cocaine hydrochloride (Australian Pharmaceutical Industries) was dissolved in 0.9% saline and injected at a dose of 15 mg/kg in a volume of 1 ml/kg immediately before behavioural testing. This dose was chosen based on previous studies that have assessed the effects of cocaine on *c-fos* expression (Harlan and Garcia, 1998) and because this dose will be used in Chapter 5.

4.3.1.3. Procedure. It should be noted, that in Experiment 4B all animals were administered cocaine and no respective control groups were utilised. As the experiment was conducted in the same fashion as Experiment 4A it was assumed that using the results from vehicle-treated Lewis and Wistar rats in Experiment 4A was sufficient for the purposes of comparing cocaine-treated with vehicle-treated animals.

4.3.1.3.1. ***Behavioural testing.*** Exactly the same procedures were used in Experiment 4B as in Experiment 4A, except on the test day where all animals were injected with 15 mg/kg of cocaine (n = 4 per group) rather than CP 55,940 before being tested for catalepsy, axillary temperature and locomotor activity (see section 4.2.1.3.1.).

4.3.1.3.2. ***Immunohistochemistry.*** Immunohistochemistry was conducted using the same procedure as in Experiment 4A (see section 4.2.1.3.2).

4.3.1.3.3. ***Counting of labeled cells.*** The amount of *c-fos* expression to 15 mg/kg of cocaine was quantified in 8 regions or subregions using the same procedure as in Experiment 4A (see section 4.2.1.3.3.). These regions are the: MPC, NAS (shell and core), MnPO, PVN, PV, CEA and dorsolateral PAG. These areas were chosen to provide a contrast to the areas counted in Experiment 4A and also because they include areas where cocaine-induced *c-fos* expression had previously been observed (Harlan and Garcia, 1998).

4.3.1.4. Data Analysis. Data for *c-fos* expression and locomotor activity were analysed using a two-factor ANOVA, with the two factors being strain (Lewis versus Wistar) and dose (vehicle versus 15 mg/kg of cocaine).

4.3.2. Results

4.3.2.1. Catalepsy. All rats administered 15 mg/kg cocaine immediately removed their forepaws from the bar to explore the bench below. Thus, cocaine had no cataleptic effects on either strain of rat.

4.3.2.2. Axillary temperature. Unfortunately, rats administered 15 mg/kg of cocaine were hyperactive and difficult to handle. This made reliable measurements of axillary temperature impossible. Thus, data for body temperature were not recorded in Experiment 4B.

4.3.2.3. Locomotor activity. The effects of cocaine on locomotor activity in Lewis and Wistar rats are shown in Figure 4.12. 15 mg/kg of cocaine significantly increased locomotor activity in both strains. Statistical analysis revealed a significant effect of cocaine [$F(1,14) = 33.88$, $p < 0.0001$] but no significant differences between strains ($F = 4.14$, $p = 0.061$). Lewis rats showed a trend toward increased responsivity to 15 mg/kg of cocaine when compared to Wistar rats due to Lewis rats having lower baseline locomotor activity than Wistar rats (see Figure 4.12.). However, the strain by dose interaction fell just short of statistical significance difference ($F = 4.25$, $p = 0.058$).

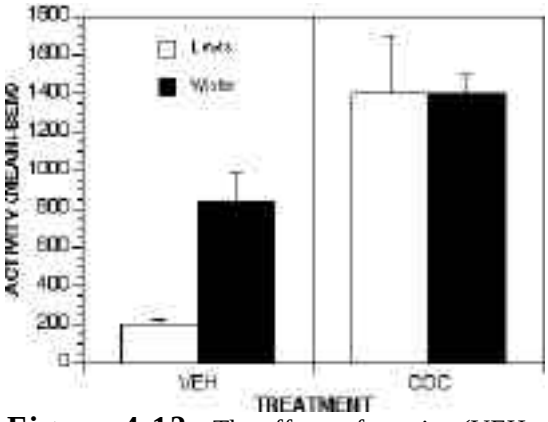

Figure 4.12. The effects of cocaine (VEH = 0 µg/kg, COC = 15 mg/kg) on locomotor activity in Lewis and Wistar rats (n = 4 per group).

4.3.2.4. *C-fos* expression. Specific counts for 8 regions of interest are shown in Table 4.3. Where these counts were made is represented in Figure 4.2. No significant strain differences were identified in the amount of *c-fos* expressed to 15 mg/kg of cocaine in any of the eight brain regions counted. Cocaine clearly increased *c-fos* expression in both strains in the following areas: the shell and core of the NAS, MnPO, PVN, PV, CEA and the dorsolateral PAG. Cocaine appeared to have a larger

effect on *c-fos* expression in the MPC of Lewis strain rats as indexed by a significant strain by dose interaction. However, this occurred in the absence of any effect of dose.

Table 4.3. The effects of 15 mg/kg of cocaine (COC15) on the number of Fos-labeled cells counted in various brain regions of Lewis and Wistar rats (n = 4 per group). Two-factor ANOVA revealed: a = a significant difference between strains ; b = a significant difference between cocaine and vehicle; c = a significant strain by cocaine interaction. A significance level of $p < 0.05$ was adopted for all these tests. Vehicle (VEH*) data from Experiment 4A were used for the purposes of statistical comparison (n = 5 per group).

REGION	Bregma	WISTAR RATS		LEWIS RATS		Stats
		VEH*	COC15	VEH*	COC15	
Forebrain						
1. Medial Prefrontal Cortex	+3.7	93 ± 23	83 ± 9	24 ± 15	81 ± 3	a, c
2. Nucleus Accumbens Shell	+1.0	9 ± 3	37 ± 4	3 ± 1	48 ± 10	b
3. Nucleus Accumbens Core	+1.0	4 ± 1	51 ± 7	0.2 ± 0.2	42 ± 7	b
Hypothalamus/Preoptic						
9. Median Preoptic Nucleus	-0.26	5 ± 1	20 ± 6	2 ± 0.8	16 ± 2	b
13. Paraventicular Nucleus	-1.8	14 ± 5	76 ± 5	18 ± 3	90 ± 9	b
Thalamus						
16. Paraventricular Nucleus	-1.8	55 ± 5	107 ± 9	16 ± 1	91 ± 8	a, b
Amygdala						
18. Central Nucleus	-2.56	3 ± 1	36 ± 7	0.6 ± 0.6	43 ± 3	b
Periaqueductal Gray						
30. Dorsolateral	-6.72	16 ± 3	38 ± 3	8 ± 2	32 ± 2	a, b

Figure 4.13

4.4. Discussion

Clear strain differences were observed in CP 55,940-induced *c-fos* expression in several brain regions. In general, CP 55,940 produced significantly more Fos-labeled cells in Wistar rats than Lewis rats. These strain differences in *c-fos* expression are consistent with the strain differences observed in the effects of CP 55,940 on catalepsy, body temperature and locomotor activity. In addition, the effects of CP 55,940 on *c-fos* expression in Lewis and Wistar rats appears distinct from the effects of cocaine, where no strain differences were observed in any brain region of interest. Taken together, these data illustrate that Lewis rats are less affected by CP 55,940 than Wistar rats which is particularly interesting given the equivalent effects that cocaine has on these two strains of rat.

The current investigation also reports for the first time strain differences in the effects of cannabinoids on body temperature and catalepsy. Consistent with prior studies, CP 55,940 promoted catalepsy, reductions in axillary temperature and reductions in locomotor activity (Fan et al., 1994; McGregor et al. 1996c; Pertwee et al., 1993; Rubino et al., 1994). CP 55,940 had less effect on Lewis rats than Wistar rats in all of these tests. However, the strain differences in the effects of CP 55,940 on locomotor activity are somewhat ambiguous because of the significantly lower baseline (i.e. drug-free) locomotor activity observed in Lewis rats. That is, as drug-free Lewis rats showed very little locomotion, Lewis rats treated with CP 55,940 did not have much room to exhibit a typical cannabinoid-induced locomotor depression. However, the observation of clear strain differences in the cataleptic effects of 250 µg/kg of CP 55,940 provides independent confirmation that this drug has divergent effects on the motor behaviour of Lewis and Wistar rats.

In Chapter 3, CP 55,940 (25, 50 and 75 µg/kg) had a significantly greater ataxic effect on Lewis rats compared to Wistar rats as assessed by the rotarod test (see section 3.4.2.). However, in the current chapter CP 55,940 administered within this dose-range appeared to have more of an effect on the motor performance of Wistar rats than Lewis rats as assessed by the locomotor activity test. Further, a greater

cataleptic effect was seen on the bar test in Wistar rats given 250 µg/kg of CP 55,940. As previously discussed, the locomotor activity test results are hard to interpret because vehicle-treated Lewis rats displayed extremely low levels of locomotor activity (see Figure 4.5.). Thus, reductions in locomotor activity that may occur in response to CP 55,940 treatment could not be discerned. However, the problem of vehicle-treated animals exhibiting extremely low levels of locomotor activity does not apply to Wistar rats. Interestingly, Wistar rats treated with 50 µg/kg of CP 55,940 ambulated less than Wistar rats treated with vehicle in the locomotor activity test (see Figure 4.5.). However, Wistar rats treated with 50 µg/kg of CP 55,940 were able to ambulate and maintain balance on a rotating spindle no differently from Wistar rats treated with vehicle in the rotarod test.

These discrepant results may arise because measures of locomotor activity and rotarod performance are considerably different in: 1) how they assess motor behaviour and 2) in what they are assessing in terms of the functioning of brain structures thought to underly the different aspects of motor behaviour. First, the locomotor activity test measures the level of free ambulation in an open environment. In contrast, the rotarod test measures the ability to coordinate movement and maintain balance on a rotating spindle. Thus, Wistar rats under the influence of CP 55,940 may be less motivated to ambulate in an open arena, but are motivated to ambulate, and can do so without impediment, when challenged by the rotarod test. It is possible this occurs because Wistar rats under the influence of CP 55,940 find falling from the rotating spindle more aversive than Lewis rats administered this compound. Thus, Wistar rats are more likely than Lewis rats to avoid falling by coordinating and maintaining ambulation. Second, locomotor activity tests and rotarod performance may assay the function of different neural structures. Specifically, the rotarod test is thought to assay cerebellar function whereas the locomotor activity test is thought to reflect functioning of the mesolimbic DA system or structures within the basal ganglia (Goodlett, Thomas, & West, 1991; Hauber, Lutz, & Munkle, 1998; Husain, Agrawal, Hasan, & Seth, 1994; Triarhou, Zhang, & Lee, 1996). Thus, the

seemingly contradictory effects of CP 55,940 on measures of locomotor activity and rotarod performance observed between Lewis and Wistar rats may reflect differences between these strains in the distribution and sensitivity of CB_1 receptors. Thus, Lewis rats may have more sensitive CB_1 receptors in the cerebellum than Wistar rats. However, Wistar rats may have more sensitive CB_1 receptors in the basal ganglia than Lewis rats. Interestingly, this is consistent with the strain differences observed in CP 55,940-induced *c-fos* expression in the basal ganglia, where Wistar rats showed increased Fos-labeled cells in the GP in comparison to Lewis rats (at 250 µg/kg). Unfortunately, not enough cerebellar tissue was harvested in the current study to allow assessment of CP 55,940-induced *c-fos* expression in this area. Future studies could test the hypothesis that Lewis rats show more CP 55,940-induced *c-fos* expression in the cerebellum than Wistar rats.

The current investigation underscores the importance of using a dose range that approximates the amount of cannabis ingested recreationally by human users and that does not greatly impair rodent behaviour. The highest dose administered in the present chapter (250 µg/kg) is similar to the doses of Δ^9-THC and synthetic analogues used in previous studies (McGregor et al., 1998; Miyamoto et al., 1997; Miyamoto et al., 1996; Porcella et al., 1998; Rodriguez de Fonseca et al., 1997). Such doses produce profound catalepsy and disruption of motor activity that is rarely, if ever, seen in human cannabis users. However, the 50 µg/kg dose of CP 55,940 used approximates doses used by humans and does not grossly disrupt rodent behaviour (see section 2.2.1.3.).

Significant increases in *c-fos* expression in brain regions induced by 250 µg/kg of CP 55,940 were not obtained with 50 µg/kg of CP 55,940. Regions only significantly activated by the higher dose included the core of the NAS, dorsal and dorsolateral CPU, ventral LS, MPO, SCh, GP, and the lateral, ventrolateral and dorsolateral PAG. Thus, cannabinoid-induced *c-fos* expression in some brain areas previously reported may be an artifact of the large doses used and therefore lack ecological validity. For example, Glass *et al* (1995) showed that 2.5 mg/kg of CP 55,940 could only induce sparse amounts of Fos protein in the striatum (see Table

4.1.). This dose is 50 times larger than the dose of CP 55,940 (50 µg/kg) used in the current chapter thought to reflect the doses used by humans (see section 2.2.1.3.). Thus, the relevance of previous studies to human cannabis use is questionable. In addition, if researchers are interested in the biochemical-behavioural correlates of cannabinoid effects, then using high doses of cannabinoids should be avoided as such doses confound behavioural measures in animal models.

The current investigation, showed cannabinoid-induced *c-fos* expression in brain regions that had not been previously reported to occur as a result of the administration of classical and non-classical cannabinoid receptor agonists such as Δ^9-THC and CP 55,940. These areas include the ICjM, GP, MPO, AH, the dorsolateral, ventrolateral and lateral PAG, and the PPTg. The identification of these areas as Fos positive in the present study, but not in previous studies, is most likely a consequence of the lack of a comprehensive mapping attempt in these previous studies (see Table 4.1.). For example, most studies have only assessed cannabinoid-induced *c-fos* expression in the striatum (Glass & Dragunow, 1995; Miyamoto et al., 1997; Miyamoto et al., 1996). The most comprehensive study to date analysed a total of 20 structures covering regions from the cortex to the brainstem using the non-classical cannabinoid receptor agonist HU-210 (Rodriguez de Fonseca et al., 1997). As the current investigation analysed 33 different brain regions it therefore provides the most comprehensive mapping of the distribution of exogenous cannabinoid-induced *c-fos* expression to date.

It may of course be possible that some of the novel findings of the present study reflect the use of CP 55,940 rather than Δ^9-THC, HU-210 and anandamide used in previous studies (Mailleux et al., 1994; McGregor et al., 1998; Miyamoto et al., 1997; Miyamoto et al., 1996; Patel, Moldow, Patel, Wu, & Chang, 1998; Porcella et al., 1998; Rodriguez de Fonseca et al., 1997; Wenger et al., 1997). This may result because of slight differences in the actions of cannabinoid receptor agonists on the CNS. First, these cannabinoid compounds differ in potency as assessed in different behavioural tests. Second, the maximum effect that these compounds have in different

bioassays are not always the same (Martin et al., 1999; Pertwee, 1997). That is, cannabinoid compounds sometimes act as partial agonists depending on the assay being used. It has been suggested these slight differences in the effects of cannabinoid compounds may rely on the CB_1 receptor being coupled to multiple effector systems (Bonhaus, Chang, Kwan, & Martin, 1998; Martin et al., 1999). Further, recent evidence suggests that the binding characteristics of the cannabinoid receptor agonist used determines what effector systems will be coupled to the CB_1 receptor. Therefore this phenomenon, otherwise known as agonist specific trafficking of intracellular responses (Bonhaus et al., 1998; Kenakin, 1997), may be responsible for the novel findings found here with CP 55,940 in comparison to previous studies using Δ^9-THC, HU-210 and anandamide.

Nonetheless, a number of the present findings agree well with previous reports (see Table 4.1.). Thus increased *c-fos* expression has been previously reported to be found in the CPU, NAS (McGregor et al., 1998; Miyamoto et al., 1997; Miyamoto et al., 1996; Porcella et al., 1998; Rodriguez de Fonseca et al., 1997), BNST (Patel et al., 1998; Rodriguez de Fonseca et al., 1997), VTA (Rodriguez de Fonseca et al., 1997), LS (McGregor et al., 1998), CEA and various nuclei of the hypothalamus such as the PVN, the SO and SCh (McGregor et al., 1998; Rodriguez de Fonseca et al., 1997).

It is possible that CB_1 receptor activation alone is responsible for the pattern of *c-fos* expression observed in the current chapter. This seems likely by noting that cannabinoid-induced increases in *c-fos* expression occurs in many areas which contain at least low levels of CB_1 receptors. These areas include the: NAS, VTA, CPU, GP, LS, BNST, CEA, PVN and the PAG (Herkenham et al., 1991a,b; Pettit et al., 1998; Tsou et al., 1998). However, consistent with earlier findings, CP 55,940 did not affect *c-fos* expression in the SN, an area that contains many CB_1 receptors. Furthermore, the increase in *c-fos* expression observed in the GP, another area rich in CB_1 receptors, was relatively sparse and restricted to the medial portion of this structure (see Figure 4.2.).

The lack of *c-fos* expression in areas rich with CB_1 receptors may be due to the CB_1 receptor's negative link to adenylate cyclase and the suppressant consequences this has on cAMP production. When cAMP production is inhibited, *c-fos* expression is more likely to be suppressed than promoted (Hughes & Dragunow, 1995). However, it has been shown that cannabinoids may also increase cAMP production (Bonhaus et al., 1998). In the current investigation, some of the *c-fos* expressed could be due to activation of CB_1 receptors that are positively linked to adenylate cyclase, increasing cAMP production. However, CP 55,940 is markedly less efficacious in stimulating the production of cAMP than in suppressing its formation (Bonhaus et al., 1998). Thus it is likely that the lack of *c-fos* expression in areas rich in CB_1 receptors is due to CP 55,940 inhibiting cAMP production. Although, it can not be ruled out that some of the *c-fos* expressed might be accounted for by CP 55,940 increasing the production of cAMP.

In any case, *c-fos* does not always appear to reflect CB_1 receptor activation alone, at least in reward and motor-relevant neural substrates. For example, Δ^9-THC-induced *c-fos* expression in the dorsomedial striatum and the NAS is blocked by the D_1 DA receptor antagonist SCH 23390 (Miyamoto et al., 1996). This illustrates that D_1 receptors play a permissive role in Δ^9-THC-induced *c-fos* expression in the striatum. Consonant with this, Δ^9-THC increased DA efflux in the NAS (Chen et al., 1990; Chen et al., 1991; Tanda et al., 1997). These studies are consistent with the notion that the *c-fos* expression observed in the current study may also reflect transynaptic activation of other neurochemical systems. While it is clear DA plays some role in cannabinoid-induced *c-fos* expression, the role of other neurotransmitters have not yet been tested. In light of recent evidence showing that endogenous opioids play an important role in the rewarding effects of cannabinoids (Chen et al., 1990; Chen et al., 1991; Tanda et al., 1997) and that central CRH receptors are important to the anxiogenic effects of cannabinoids (Rodriguez de Fonseca et al., 1996), future studies could address the role of endogenous opioids and CRH as mediators of cannabinoid-induced *c-fos* expression.

The aforementioned evidence which shows that *c-fos* is probably not due to CB_1 receptor activation alone is consistent with the notion that cannabinoids have a neuromodulatory role in the CNS (Di Marzo et al., 1998). Recent evidence shows that presynaptically located CB_1 receptors gate the release of other neurotransmitters [for example, γ - aminobutyric acid (GABA) or glutamate] (Chan, Chan, & Yung, 1998; Levenes, Daniel, Soubrie, & Crepel, 1998; Shen, Piser, Seybold, & Thayer, 1996; Shen & Thayer, 1999; Szabo, Dorner, Pfreundtner, Norenberg, & Starke, 1998; Szabo, Wallmichrath, Mathonia, & Pfreundtner, 2000; Vaughan, Connor, Bagley, & Christie, 2000; Vaughan, McGregor, & Christie, 1999). This is consonant with evidence which shows that anandamide inhibits presynaptic voltage-sensitive Ca^{2+} channels in hippocampal neurones (Twitchell, Brown, & Mackie, 1997). Thus, in the current study CP 55,940 may have altered the release of other neurotransmitters that may be responsible for the *c-fos* expression observed.

The question of why Lewis rats show less overall cannabinoid-induced *c-fos* expression in comparison to Wistar rats is a difficult one to answer. In contrast to the effects of cocaine, where no differences were observed between strains, the strain differences in CP 55,940-induced *c-fos* expression were profound. One possible reason could be that Lewis rats have fewer central CB_1 receptors than Wistar rats and are thus subsensitive to cannabinoid effects. Alternatively, strain differences may also exist in CP 55,940's affinity for the CB_1 receptor. Thus, future studies might usefully compare CB_1 receptor binding to see if any strain differences in ligand binding affinity or CB_1 receptor number exist. A related hypothesis is that Lewis rats may have less G proteins in a pool utilised by CB_1 receptors. This is partially supported by evidence showing that Lewis rats have less G proteins in the NAS in comparison to Fischer 344 rats (Brodkin et al., 1998). Another possibility is that pharmacokinetic differences exist between the strains. For example, Lewis rats may more efficiently metabolise CP 55,940 than Wistar rats. Future studies could test whether the administration of CP 55,940 leads to differences in blood plasma levels of CP 55,940 between Lewis and Wistar rats.

4.4.1. Novel cannabinoid-activated brain regions. Strain differences were observed in the hypothermic effects of CP 55,940 with Lewis rats showing a significantly smaller hypothermic effect. Consistent with this, CP 55,940 significantly increased *c-fos* expression in preoptic and hypothalamic regions thought to be involved in thermoregulation. These areas include the MnPO, MPO and AH (Scammell, Price, & Sagar, 1993; Smith, Jansen, Gilbey, & Loewy, 1998; Travis & Johnson, 1993; Yamada, Cho, Coleman, & Richelson, 1995). Interestingly, previous studies have shown that injection of Δ^9-THC into the preoptic area of the anterior hypothalamus caused significant reductions in body temperature (Fitton & Pertwee, 1982; Pertwee, Hedley, McQueen, & Gentleman, 1988).

Consonant with the strain difference observed in CP 55,940-induced hypothermia, strain differences were observed in *c-fos* expression in the MnPO and the PVN. Previous studies assessing the expression of *c-fos* have shown that a hot environment increases *c-fos* expression in the MnPO (Scammell et al., 1993). Moreover, the administration of endothelin, which reduces body temperature and is known to regulate cardiovascular function, increased *c-fos* expression in the MnPO (Zhu & Herbert, 1996). The role of the PVN in thermoregulation is highlighted by an experiment showing that stimulation of the PVN with the excitatory amino acid glutamate increased the temperature of intercapsular brown adipose tissue (Amir, 1990). Conversely, lesions of the PVN decreased the activity of sympathetic innervations of this brown adipose tissue (Sakaguchi, Bray, & Eddlestone, 1988). It must be noted that no significant increase in CP 55,940-induced *c-fos* expression was observed in the MPA in the current study. This was somewhat surprising in light of previous studies which indicate that the MPA regulates body temperature and contains thermosensitive neurons (Basta, Tzschentke, & Nichelmann, 1997; Kobayashi, 1986).

The ICjM is another area of the brain in which cannabinoid-induced *c-fos* expression has not previously been reported. The ICjM's function remains largely a mystery. It is possible that the ICjM might also be involved in the hypothermic effect of cannabinoids. The current study showed clear strain differences in the hypothermic

effects of CP 55,940 that correlated with strain differences observed in *c-fos* expression in the ICjM. A recent study has shown that intracranial administration of D_3 receptor agonists into the ICjM causes hypothermia, an effect which was potentiated by D_1 agonists (Barik & de Beaurepaire, 1998). Thus, at the level of the ICjM cannabinoids may act like D_3 receptor agonists that promote hypothermia. D_3 receptor agonists and cannabinoid receptor agonists have many common effects such as hypothermia (Barik & de Beaurepaire, 1998; Van den Buuse, 1995), locomotor inhibition (Kling-Petersen, Ljung, & Svensson, 1995a; Sautel et al., 1995) and reward modulation (Caine & Koob, 1993; Kling-Petersen, Ljung, Wollter, & Svensson, 1995b; Rivet, Audinot, Gobert, Peglion, & Millan, 1994). In light of this, it is interesting to speculate upon the role of the ICjM in the rewarding effects of cannabinoids. A role for the ICjM in reward appears plausible based on the anatomical proximity of the ICjM and the NAS. The ICjM lies adjacent to the shell of the NAS in the ventral striatum, which is an outflow of the mesolimbic DA system. Thus, it is possible that functional interactions occur between the ICjM and the shell of the NAS. Interestingly, many other recreationally used drugs promote *c-fos* expression in the ICjM such as MDMA and cocaine (Stephenson et al., 1999; Young, Porrino, & Iadarola, 1991). Future studies need to address if the ICjM has a functional role in the rewarding effects of drugs such as cannabis.

The present study showed for the first time that a cannabinoid increases *c-fos* expression in the PPTg. This area has been implicated in a wide range of functions including reinforcement, reward, motor behaviour, control of sleep-wake cycles, antinociception and cognitive processing (Inglis & Winn, 1995; Winn, Brown, & Inglis, 1997). Interestingly, cannabinoids affect all of these functions (Ameri, 1999; Feinberg, Jones, Walker, Cavness, & March, 1975; Gardner & Lowinson, 1991; Gardner & Vorel, 1998; Mechoulam et al., 1997). It is unlikely that the effects of CP 55,940 on *c-fos* expression in the PPTg are mediated via direct activation of CB_1 receptors as there appear to be none in this area (Herkenham et al., 1991b; Pettit et al., 1998; Tsou et al., 1998). Therefore it is likely the effects of CP 55,940 are mediated

by transynaptic activation from other brain areas. The PPTg has extensive connectivity with the basal ganglia (Inglis & Winn, 1995). Specifically, afferent and efferent connections between the PPTg and areas rich in CB_1 receptors, such as the GP and the SN have been described (Inglis & Winn, 1995). However, behavioural evidence argues against a functional role of the PPTg in locomotor activity. Thus PPTg lesions do not affect locomotor activity or stimulant-induced hyperactivity (Inglis et al., 1994b; Inglis, Dunbar, & Winn, 1994a).

Recently, converging evidence has strongly implicated the PPTg as part of the neural circuit underlying reward. It has been proposed that the PPTg may provide another important interface between incentive motivation and behaviour (Winn et al., 1997). This is supported by anatomical connections between the limbic system and the PPTg (Inglis & Winn, 1995). Specifically, the PPTg provides efferents to structures where the administration of CP 55,940 increased the number of Fos-labeled cells such as the LS and the amygdala (Hallanger & Wainer, 1988). In addition, Fos-labeled cells have also been observed in the reward-relevant NAS which makes direct and indirect afferent connections to the PPTg (Groenewegen, Berendse, & Haber, 1993; Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991; Inglis & Winn, 1995). This anatomical evidence is consolidated by behavioural evidence which shows that lesions of the PPTg reduced heroin self-administration (Olmstead, Munn, Franklin, & Wise, 1998), responding for rewarding brain stimulation (Lepore & Franklin, 1996b) and the development of conditioned place preferences to opiates, stimulants or food (Nader, Bechara, & van der Kooy, 1997; Olmstead & Franklin, 1993). The current investigation provides preliminary evidence that cannabinoids also act on neural substrates which underlie a reward circuit that includes the PPTg.

4.4.2. The challenge of dissociating the neural substrates for the aversive, analgesic and rewarding effects of cannabinoids. CP 55,940 induced *c-fos* expression in a range of brain structures known to be involved in anxiety, fear and defensive behaviour. These include the CEA, BNST and the PAG. The

pattern of *c-fos* expression in these areas, particularly in Wistar rats, may highlight a neural circuit that underpins an integrated fear or anxiety response to cannabinoid administration. The divergent effects on *c-fos* expression across strains are consistent with the effects of CP 55,940 on Lewis and Wistar rats in a variety of different models of anxiety as demonstrated in Chapters 2 and 3. In these models Lewis rats show markedly less anxiety-like behaviours than Wistar rats when administered CP 55,940 (10 - 75 µg/kg).

The CEA is a pivotal site in a putative neural circuit that may underpin an integrated anxiety response to the administration of cannabinoids. The CEA is thought to be an important output pathway which modulates many aspects of unconditioned and conditioned fear (eg. bradycardia, freezing, hypoalgesia and activation of the HPA axis) (Rosen & Schulkin, 1998; Swanson & Petrovich, 1998). It provides inputs and outputs to the BNST and output to the PVN (involved in stress and the HPA) and the PAG (involved in defensive behaviour, cardiovascular function and pain transmission) (Alheid, de Olmos, & Beltramino, 1995; Swanson & Petrovich, 1998). Interestingly, in the current investigation all of the above areas (CEA, BNST and PVN) showed CP 55,940-induced *c-fos* expression, with significantly more Fos-labeled cells counted in Wistar rats in comparison to Lewis rats. Consistent with an anxiogenic effect, Δ^9-THC directly infused into the CEA caused mice to spend less time exploring the open arms of an EPM (Onaivi et al., 1996). In addition, *c-fos* expression in the amygdala appears functionally critical to anxiety, as *c-fos* antisense oligonucleotides infused into the amygdala abolished anxiety as assessed by the Vogel conflict test (Heilig et al., 1993).

The BNST is another brain region that may be crucial to the expression of CP 55,940-induced anxiety. As part of the extended amygdala, the BNST in thought to be a rostral extension of the CEA (Alheid et al., 1995). The BNST has a very similar structure to the CEA and contains similar transmitters, cell morphology and has similar efferent connections (Alheid et al., 1995; Swanson & Petrovich, 1998). The BNST is proposed to be an important site for the expression of anxiety rather than fear (Davis, 1998). This proposition is based on studies which show that lesions of the BNST are

selectively effective in disrupting the startle reflex enhanced by more ambiguous cues such as the administration of CRH or exposure to a threatening environment. However, lesions of this area are ineffective in reducing the startle reflex to explicit cues such as tones (which relies on the functional integrity of the CEA) (Davis, 1998). Based on the longer duration of exposure and the ambiguity of the cues it was concluded that the BNST may be vital for anxiety rather than fear. In light of this, it is hypothesised that the administration of CP 55,940 may elicit anxiety via cannabinoid-induced CRH release in the BNST. Future studies could address the role of the BNST in cannabinoid-enhanced startle and more generally cannabinoid-induced anxiety.

It is possible that the differential effects CP 55,940 has on Lewis and Wistar rats could be attributable to differences in cannabinoid-induced activation of the HPA between these strains. Consonant with this, the Lewis rats HPA axis is hyporesponsive to various stimuli and releases less basal and stress-induced corticosterone (Brodkin et al., 1998; Rivest & Rivier, 1994). This appears to be a consequence of a deficiency in the ability of the hypothalamus to synthesise and secrete CRH in Lewis rats (Sternberg et al., 1989a; Sternberg et al., 1989b). These findings are consistent with the observation of less cannabinoid-induced *c-fos* expression in the PVN of Lewis rats compared to Wistar rats. The PVN produces CRH, a precursor to corticosterone release (Antoni et al., 1983). In addition, the effects of CRH on *c-fos* expression shows commonalities with the current investigation with increased Fos-labeled cells observed in PVN, CEA, LS, BNST, NAS and the CPU (Arnold et al., 1992). Furthermore, administration of the selective CRH antagonist, D-Phe CRF_{12-41}, attenuated the anxiogenic effects of the cannabinoid receptor agonist HU-210 in a defensive-withdrawal model (Rodriguez de Fonseca et al., 1996). In light of these studies it appears CRH plays some role in the stressful or anxiogenic effects of cannabinoids.

In light of Lewis rats exhibiting decreased CP 55,940-induced *c-fos* expression in neural substates relevant to stress and anxiety, it was interesting to observe whether CP 55,940 had any differential effects on *c-fos* expression in reward-relevant brain

areas such as the VTA and the NAS. Interestingly, Lewis rats again appeared less susceptible to CP 55,940-induced *c-fos* expression compared to Wistar rats, with strain differences found in the shell and core of the NAS. In both strains of rat, CP 55,940 promoted more *c-fos* expression in the shell in comparison to the core of the NAS. This is interesting, because the shell of the NAS has been delineated as a structure critical to the rewarding effects of drugs of abuse. Consistent with this finding, Δ^9-THC increased extracellular DA concentrations preferentially in the shell of the NAS (Tanda et al., 1997). However, the strain differences observed in CP 55,940-induced *c-fos* expression in the shell of the NAS can not be easily reconciled with the DA theory of reward. That is, as Lewis rats are the only rat strain to show a rewarding effect when administered Δ^9-THC, the DA theory of reward would predict that Lewis rats show enhanced DA efflux in the NAS in comparison to Wistar rats. Based on the finding that DA is critical to cannabinoid-induced *c-fos* expression in the striatum (Miyamoto et al., 1996) it is reasonable to assume that *c-fos* acts as a marker for cannabinoid-induced dopaminergic transmission in the shell of the NAS. Therefore, the DA theory of reward would predict that Lewis rats show increased *c-fos* expression in the shell of the NAS in comparison to Wistar rats. However, the opposite was found in the current investigation.

This finding appears to be more easily reconciled by the increasing body of evidence suggesting that dopaminergic transmission in the NAS is also produced by stressful or anxiogenic stimuli (Joseph et al., 1996; Salamone, Cousins, & Snyder, 1998). Interestingly, the CEA projects to the shell of the NAS via the lateral BNST (Alheid et al., 1995). Thus if the shell of the NAS is crucial for the expression of cannabinoid-induced anxiety, then anxiety-relevant information may be transmitted via the BNST from the CEA. This could be seen as further evidence to support the notion that the NAS is part of a circuit which underlies cannabinoid-induced anxiety rather than reward, or a circuit which attributes the incentive value of both pleasurable and aversive stimuli.

The ambiguity of whether the NAS is involved in the aversive and rewarding effects of cannabinoids sheds light on an important issue which may be important not only to the understanding of the appetitive effects of cannabinoids. This issue involves working out the exact relationship between reward and aversion, and the implication this relationship may have for appetitive behaviour. For example, while cocaine has clearly rewarding effects in humans, it also has aversive effects (Benowitz, 1992; Williamson et al., 1997). Cocaine is also at once rewarding and anxiogenic in rats (Blanchard et al., 1998; Ettenberg & Geist, 1991; Mantsch & Goeders, 1998; Rogerio & Takahashi, 1992; Yang et al., 1992). In addition, as shown in the current investigation, cocaine promotes *c-fos* expression in similar areas to CP 55,940, including areas implicated in both reward and anxiety, such as the shell and core of the NAS, CEA, PVN and the PAG. For cocaine, the rewarding and anxiogenic effects have been dissociated in an elegant study (Ettenberg & Geist, 1991). Rats avidly self-administering cocaine over days in a runway task showed more retreats than controls from the goal box. As the rats retreated only a short distance and continued to avidly self-administer cocaine, this observation was proposed to reflect a conflict situation for the rat between positive and negative aspects of cocaine's effects. In addition, the anxiolytic compound diazepam, dose-dependently reduced this retreat behaviour. Thus, for cocaine it appears the conflict between its aversive and rewarding effects has implications for cocaine self-administration. Future studies will hopefully address the relationship between aversion and reward and what implication this has for appetitive behaviour relevant to cannabinoids.

CP 55,940 appeared to have approximately equivalent effects on *c-fos* expression in the VTA of Lewis and Wistar rats. If anything, CP 55,940 had less of an effect on *c-fos* expression in the VTA of Lewis rats than Wistar rats consistent with the distinct effects that CP 55,940 had on *c-fos* expression in the shell of the NAS. One proposed mechanism which might mediate cannabinoid-induced DA release in the NAS is disinhibition of DA cell firing in the VTA via inhibition of GABA interneurons (Ameri, 1999). This theory is based on the finding that cannabinoids increase the

spontaneous firing of neurons within the VTA (French, 1997; French et al., 1997; Gessa, Mascia, Casu, & Carta, 1997). Distinctively, this effect is opposite to the effects that other drugs of abuse have on spontaneous VTA firing (Einhorn, Johansen, & White, 1988; Henry, Greene, & White, 1989; Seutin, Verbanck, Massotte, & Dresse, 1991). The effects of cannabinoids on *c-fos* expression in the VTA may provide an explanation for the long-lasting plastic changes that affect this area with repeated drug administrations. It is possible that *c-fos*, which regulates gene expression, controls the production of proteins responsible for the plastic changes involved in drug abuse. Interestingly, protein inhibitors directly infused into the VTA attenuate behavioural sensitization to cocaine and amphetamine (Karler, Finnegan, & Calder, 1993; Sorg & Ulibarri, 1995). However, not too much can be inferred from the data of the current study because only sparse cannabinoid-induced *c-fos* expression was observed in the VTA.

One of the most interesting observations made from the current study is the coincidence of CP 55,940-induced *c-fos* expression in many areas of the brain that mediate both analgesia and reward. Thus, increased *c-fos* expression was noted in the VTA, NAS, PAG and in the PPTg. Recently, Franklin (Franklin, 1998) reviewed the literature and found that a striking number of drugs have both analgesic and rewarding effects. Further, the analgesic and rewarding potencies of such drugs were positively correlated. Morphine and amphetamine are two drugs in which this relationship has been most clearly documented. These drugs appear to elicit their rewarding and analgesic effects by acting on almost the same neural substrates. Thus analgesia and reward is promoted by microinjecting these drugs into brain sites such as the VTA, NAS and the PAG (Franklin, 1998). Interestingly, cannabinoid receptor agonists also affect both of these functions. It is clear that cannabinoids have effects on the mesolimbic DA system that may serve to underlie its rewarding and aversive effects. Future studies could address whether this same substrate is involved in analgesia.

The PAG is an important brain structure involved in cannabinoid-induced antinociception, catalepsy and hypothermia (Lichtman, Cook, & Martin, 1996).

Lichtman *et al* (1996) showed that microinjections of CP 55,940 into the ventrolateral PAG produced all of the above effects. In addition, this study showed that microinjection of CP 55,940 into the dorsolateral PAG had no such effects. Further, the PAG has been implicated in neural circuitry involved in defensive behaviour (Canteras, Chiavegatto, Valle, & Swanson, 1997; Canteras & Goto, 1999). Prior studies indicate that the ventrolateral PAG is involved in freezing and immobility, whereas the dorsolateral PAG is involved in the control of flight behaviours (Behbehani, 1995; Carrive, 1993; Carrive, Leung, Harris, & Paxinos, 1997; De Oca, DeCola, Maren, & Fanselow, 1998; Keay & Bandler, 1993). The current investigation showed that Lewis rats were less susceptible to CP 55,940-induced *c-fos* expression than Wistar rats in the ventrolateral and dorsolateral PAG. Thus it can be seen that the effects of CP 55,940 on *c-fos* expression in the dorsolateral PAG are likely to be consistent with the findings made in Chapters 2 and 3 where Lewis rats were less susceptible to CP 55,940-induced anxiety-related behaviours than Wistar rats. Further, the strain differences observed in the effects of CP 55,940 on *c-fos* expression in the ventrolateral PAG are consistent with Lewis rats being less susceptible to CP 55,940-induced catalepsy and hypothermia as illustrated in the present study. In addition, the strain differences in CP 55,940 induced *c-fos* expression in the ventrolateral PAG may highlight that Lewis rats are also less susceptible to the antinociceptive effects of CP 55,940 in comparison to Wistar rats. Future studies could further address this issue.

4.4.3. *C-fos* expression and cannabinoid-induced locomotor inhibition. The influence of cannabinoids on locomotor activity is thought to be controlled via presynaptic modulation of excitatory and inhibitory inputs to the basal ganglia (Ameri, 1999). Dense CB_1 receptor distribution occurs in the GP and the SN, and moderate CB_1 receptor distribution occurs in the CPU (Herkenham et al., 1991b; Pettit et al., 1998; Tsou et al., 1998). Most receptors in the GP and the SN appear to be localised presynaptically on striatal afferents, which is inferred from *in situ* hybridisation studies which show that cannabinoid receptor mRNA is more abundant in

the striatum than in the GP and the SN, and that lesions of striatal afferents leads to CB_1 degeneration in the GP and SN (Herkenham et al., 1991a; Mailleux & Vanderhaeghen, 1992; Westlake, Howlett, Bonner, Matsuda, & Herkenham, 1994). The locomotor depressant effects of cannabinoids seems to involve modulation of other neurotransmitter systems, such as GABA, DA and glutamate in these brain areas (Ameri, 1999). It is noteworthy that only sparse *c-fos* expression was observed in a small and localised area of the GP of Wistar rats administered the highest dose of CP 55,940. Furthermore, no CP 55,940-induced *c-fos* expression was observed in the SN in the present study. It is likely that CP 55,940 affected these areas, however, cannabinoid transmission alone may not be enough to induce expression of *c-fos*. If excitatory transmitters released in the GP and the SN are important to locomotor activity and the expression of *c-fos* in these areas, then the presynaptic distribution of CB_1 receptors may act to inhibit locomotor activity and the expression of *c-fos* in the GP and SN. If this is true, then *c-fos* expression provides no clear marker for cannabinoid promoted locomotor inhibition.

To add to these perplexing results, little *c-fos* was expressed in the CPU, with only sparse Fos immunoreactivity observed in its central and dorsolateral regions. Arguing against the interpretation that the CPU mediates the locomotor depressant effects of cannabinoids is that strain differences observed in the effects of CP 55,940 on motor behaviour did not correspond with its effects on *c-fos* expression. An alternative brain site that may mediate the effects of cannabinoids on locomotor activity is the core of the NAS. Thus direct infusion of Δ^9-THC into the NAS promoted catalepsy in mice (Onaivi et al., 1996). In the current investigation, the lesser effects of CP 55,940 on locomotor activity and catalepsy in Lewis rats relative to Wistar rats correlated with the differential pattern of *c-fos* expression observed in the core and shell of the NAS between these strains.

4.4.4. Strain differences in baseline *c-fos* expression. Interestingly, Lewis rats showed *less* baseline *c-fos* expression than Wistar rats in areas

implicated in anxiety-like and defensive behaviours such as the MPC, intra-amygdaloid nucleus of the BNST, and the dorsolateral and ventrolateral PAG. In Chapters 2 and 3, vehicle-treated Lewis rats were observed to exhibit *more* anxiety-related behaviours than vehicle-treated Wistar rats as assessed by the open area avoidance apparatus and the light-dark emergence test. This confirms previous strain differences that have been reported using unconditioned animal models of anxiety, such as the exploratory conflict test, EPM and free exploratory paradigm (Berton et al., 1997; Rex et al., 1996). The reason for the negative correlation between baseline *c-fos* expression and anxiety-like behaviour is unclear and underscores the need for a better understanding of how *c-fos* expression relates to behaviour.

4.4.5. Conclusion. The current chapter shows clear strain differences in the effects of CP 55,940 on *c-fos* expression in various regions and subregions. These differences in *c-fos* expression are at least partly correlated with strain differences in the effects of this drug on body temperature and motor behaviour across the two strains. CP 55,940 also promoted *c-fos* expression in areas not previously assessed, including the PPTg, ICjM, PAG and AH. In addition, the current investigation reported for the first time strain differences in the effects of cannabinoids on body temperature and catalepsy. The effects of CP 55,940 on *c-fos* expression in areas implicated in cannabinoid-induced anxiety, such as the CEA, BNST, PVN and PAG were also of interest given the clear strain differences observed in CP 55,940-induced anxiety-like behaviours reported in Chapters 2 and 3.

Thus, the Lewis rat's susceptibility to the rewarding effects of cannabinoids may be explained by their reduced susceptibility to the anxiogenic effects of cannabinoids. However, this idea is less compelling when considering the effects of CP 55,940 on neural substrates which underlie reward. The effects of CP 55,940 on *c-fos* expression in a neural circuit that may underly reward, which includes the NAS, VTA, and PPTg, were reduced in Lewis rats in comparison to Wistar rats. Future investigations could address whether the reduced effects of CP 55,940 on the Lewis rat

are pharmacokinetic or pharmacodynamic in nature, or whether there exists some other inherent difference between the strains such as the number of CB_1 receptors. In addition, future studies could reconcile the pattern of *c-fos* expression observed here with prior reports of the Lewis rat being a unique cannabinoid-preferring rat strain.