CHAPTER TWO

Effects of CP 55,940 on anxiety-like behaviours in Lewis and Wistar rats: Part A

Studies utilising the predatory odour avoidance, open area avoidance and conditioned ultrasonic vocalization models of anxiety.

2.1. Introduction

Cannabis is the most widely used illicit drug in the world. However, little is known about the specific mechanisms underlying its habit-forming nature. Cannabis and cannabinoids are also an emerging class of therapeutic compounds. Thus there is an interest in isolating therapeutically useful cannabinoid compounds with little or no abuse liability. As discussed in section 1.5.4., one serious impediment to both of the above endeavours has been the failure to find an adequate animal model of the habit-forming effects of cannabis in humans. In contrast to most other recreational drugs that have been tested, cannabis and cannabinoids have usually failed to support self-administration in laboratory animals. It is hypothesised that this may result from cannabinoids having predominately aversive effects on rodents. One explanation for cannabinoid-induced aversion in rats is based on observations that cannabinoids increase anxiety-like behaviour in this species.

As discussed in section 1.6., a recent twin-study has suggested that the rewarding (pleasant) or aversive (unpleasant) effects of cannabis intoxication in humans are, in part, genetically determined (Lyons et al., 1997). This finding is consistent with research performed in the animal domain which suggests that Lewis strain rats may provide an exception to the general rule of aversive effects of cannabinoids in rats (Gardner & Lowinson, 1991; Gardner et al., 1988; Lepore, et al., 1996a; Lepore et al.,

1995). Lewis rats are found to have a high vulnerability to the rewarding effects of most recreational drugs, including alcohol, opiates and cocaine (Camp et al., 1994; Kosten et al., 1997; Suzuki et al. 1988a; Suzuki et al., 1988b). Furthermore, Lewis rats may be unique in that they are the only rat strain that finds Δ^9-THC rewarding as assessed by the intracranial self-stimulation model (Gardner et al., 1988; Lepore et al., 1996a). No such effects were seen in the Fischer 344 strain and only a marginal facilitatory effect was seen in the Sprague-Dawley strain (Lepore et al., 1996a). Thus, it is hypothesised that Lewis rats find Δ^9-THC rewarding because they are less susceptible to the anxiogenic effects of cannabinoids in comparison to other rat strains (see section 1.6.).

As outlined in section 1.6., Lewis rats may be less susceptible to the anxiogenic effects of cannabinoids because they are less susceptible to stress in general. This is based primarily on the observation that Lewis rats have a hyporesponsive HPA axis in comparison to other strains. Thus, when challenged with a mild stressor, Lewis rats secrete less plasma adrenocorticotropic hormone (ACTH) and corticosterone than other rat strains including spontaneously hypertensive, Fischer 344 and Wistar rats (Chaouloff et al., 1995; Dhabhar et al., 1993, 1997; Dhabhar et al., 1995; Oitzl et al., 1995; Shurin et al., 1995; Sternberg et al., 1989a; Sternberg et al., 1989b). Further, Lewis rats exhibit less release of ACTH and corticosterone in comparison to Fischer 344 rats when administered drugs such as the muscarinic receptor agonist arecoline or the α_1 adrenergic receptor agonist methoxamine (Calogero et al., 1992). As CRH, the precursor to the release of corticosterone, is critical to cannabinoid-induced anxiety (Rodriguez de Fonseca et al., 1996) the effects of cannabinoids on anxiety-like behaviours of Lewis rats may be reduced in comparison to other rat strains (see section 1.6.).

The current chapter concentrates on the effects of the synthetic cannabinoid receptor agonist CP 55,940 (see section 2.2.1.3.) on anxiety-like behaviours in Lewis and Wistar rats. Inbred Wistar rats were chosen as the comparator strain in the current study and throughout the thesis (see Chapters 3, 4 and 5) because inbred Lewis rats

derive from this strain of rat (Oitzl et al., 1995). When attempting to discern strain differences in the effects of CP 55,940 on anxiety it is important to consider differences in baseline levels of anxiety-related behaviours (see section 2.2.1.5.). In general, Lewis rats exhibit higher baseline levels of anxiety-like behaviours than Wistar rats in unconditioned animal models of anxiety such as the EPM (Berton, Ramos, Chaouloff, & Mormede, 1997; Rex, Sondern, Voigt, Franck, & Fink, 1996). However, these findings conflict with a report of low baseline levels of anxiety as measured by the social interaction test (see Chapter 3), where Lewis rats showed significantly higher levels of baseline social interaction than Wistar rats (Rex et al., 1996). The current chapter tests whether the administration of CP 55,940 increases or decreases baseline levels of anxiety-like behaviour.

Anxiety is not a unitary phenomenon and distinct types of anxiety disorders are thought to exist in humans, such as generalised anxiety disorder, phobia and panic disorder (A.P.A., 1994). Further, it has been proposed that different rodent tests of anxiety may relate to these various types of clinical anxiety found in humans, or at the very least, be representative of distinct rodent states of anxiety (File, 1996a; File, Andrews, & Hogg, 1996b; Olivier et al., 1996; Rodgers, 1997). Following this rationale it is feasible that the effects of CP 55,940 exposure established in one model, which is sensitive to one type of anxiety, will not be found in another model that reflects a distinct anxiety state. Thus, the current chapter tests three distinct animal models of anxiety that may represent different anxiety states in the rodent.

The predatory odour avoidance model relies upon the apparently innate fear that rodents have for the odour of their natural predators, such as cats and foxes (Astic & Cattarelli, 1982; Blanchard, Yudko, Rodgers, & Blanchard, 1993; Perrot-Sinal, Heale, Ossenkopp, & Kavaliers, 1996; Zangrossi & File, 1992, 1994). Rats tend to avoid such odours and engage in a variety of defensive behaviours in their presence. These defensive behaviours are systematically altered by administration of drugs which produce anxiolysis in humans such as the benzodiazepine, midazolam, or the tricyclic antidepressant, imipramine (Blanchard, Shepherd, Rodgers, Magee, &

Blanchard, 1993; Dielenberg, Arnold, & McGregor, 1999; McGregor & Dielenberg, 1999). It has been claimed that the anxiety-like behaviour of rats provoked by exposure to cat odour bears some resemblance to phobic anxiety in humans since it does not habituate over repeated exposure to the odour and has been shown to be benzodiazepine-insensitive (Zangrossi & File, 1992, 1994). However, this is contradicted by recent studies that employ a novel methodology to measure the anxiety-like behaviours during exposure to cat odour (Dielenberg et al., 1999; Dielenberg & McGregor, 1999). This model may offer a more sensitive measure of cat odour-induced anxiety because the rat is given the option to hide in a small enclosed box. Previous studies were conducted in the rats' homecage (Zangrossi & File, 1992, 1994) or in a long, narrow alley (Blanchard et al., 1993a) allowing only an ambiguous assessment of approach to and sheltering from the cat odour. Thus, the apparatus and methodology developed by Dielenberg et al. (1999 a, b) was used in the current chapter.

The open area avoidance model relies on the tendency of rats to avoid an open area in favour of a small enclosed area. This model is similar to other natural conflict models such as the light-dark emergence or EPM. In these paradigms the exploratory behaviour of the rat is measured upon introduction to an unfamiliar environment. These measures are essentially based on the conflict between the rats tendency to explore and its tendency to avoid such a novel environment (Martin, 1998). However, the open area avoidance model is slightly different, because the test is conducted under low light conditions and the rat is exposed to the environment before drug testing. Rats were pre-exposed to the testing arena in the current study for two reasons: 1) avoidance of an open area is reliably increased by cannabinoid receptor agonists, such as HU-210, in familiar but not unfamiliar environments (Rodriguez de Fonseca et al., 1996); and 2) it provides a useful comparison to the effects of a cannabinoid on predatory odour avoidance, because the open area avoidance test is identical apart from the absence of cat odour.

USVs may serve as an indicator of emotion in the rodent (Martin, 1998; Rodgers, 1997; Vivian & Miczek, 1999). Specifically, USVs in the range of 18-30 kHz may indicate negative emotional states because rodents emit these calls when experiencing aversive situations. Examples of such situations include: exposure to a predator (Blanchard, Blanchard, Agullana, & Weiss, 1991; Shepherd, Blanchard, Weiss, Rodgers, & Blanchard, 1992); inescapable electric footshocks (Rowan, Cullen, & Moulton, 1990; Van der Poel, Noach, & Miczek, 1989); and withdrawal from chronic drug intake (Knapp, Duncan, Crews, & Breese, 1998; Mutschler & Miczek, 1998a,b). Pharmacological studies concentrating on anxiety have previously measured whether the emission of USVs to footshock are modulated by anxiolytic or anxiogenic drugs (De Vry, Benz, Schreiber, & Traber, 1993; Rowan et al., 1990; Van der Poel et al., 1989). However, these studies are limited because they cannot differentiate between anxiety and pain modulating effects of the compound tested (De Vry et al., 1993; Kaltwasser, 1991).

The conditioned USVs model escapes such limitations because the rat is administered the drug in the absence of footshock. This model relies on the fact that rats re-exposed to an environment in which they have received shock emit vocalizations in the range of 18-30 kHz (Groenink, Mos, Van der Gugten, & Olivier, 1996; Molewijk, van der Poel, Mos, van der Heyden, & Olivier, 1995). Conditioned USVs are thought to be an index of anxiety, since drugs that have anxiolytic effects in humans systematically modulate them (Molewijk et al., 1995). Interestingly, conditioned USVs seem particularly modulated by drugs that are effective in treating panic disorder in humans, leading to the suggestion that they have some validity as an animal model of panic disorder (Molewijk et al., 1995). Since cannabis has been reported to provoke panic attacks in some human users (Thomas, 1996) this model was thought to be particularly appropriate for use in the present study.

The aim of the current chapter is to shed some light on why the Lewis strain of rat shows a uniquely rewarding effect of Δ^9-THC in the intracranial self-stimulation model (Lepore et al., 1996). Cannabinoids such as Δ^9-THC may have rewarding

effects on Lewis rats because they are less susceptible to the aversive effects of cannabinoids. Thus, it was hypothesised that Lewis rats will show a reduced anxiogenic response to the synthetic analogue of Δ^9-THC, CP 55,940, compared to Wistar rats in the three animal models of anxiety used.

2.2. Experiment 2A. The effects of CP 55,940 on predatory odour avoidance in Lewis and Wistar rats

2.2.1. Methods

2.2.1.1. Subjects. The subjects were 40 inbred male Lewis albino rats (ARC, Perth, Australia) and 40 inbred male Wistar albino rats (SPF, Sydney, Australia). Rats from each strain were 75 days old at the start of the experiment. Lewis rats weighed between 250 and 350 g and Wistar rats weighed between 300 and 400 g at the time of testing. The rats were group housed in large plastic tubs with 8 subjects per tub. Each tub contained rats from the same strain. The rats were maintained on a 12 h reverse light-dark cycle (lights off at 8.30 am) with food and water available *ad libitum*. All testing occurred during the dark cycle. Each rat was handled for 2 min per day on each of the four days prior to the start of the experiment. All rats were randomly allocated to groups to receive vehicle or 10, 25 or 50 µg/kg CP 55,940 (n = 10). The University of Sydney Animal Care and Ethics Committee approved all experiments conducted in the current thesis unless otherwise stated.

2.2.1.2. Apparatus. Testing took place in four chambers as depicted in Figure 2.1. (Dielenberg et al., 1999; Dielenberg & McGregor, 1999; McGregor & Dielenberg, 1999). The chambers comprised a rectangular arena with perspex walls [60 cm (L) x 26 cm (W) x 36 cm (H)] and a metal grid floor which was raised 2 cm above a tray containing wood shavings. At one end of the chamber was a small wooden box [21 cm (L) x 24 cm (W) x 22 cm (H)] termed the "hide box". On the front wall of the hide box was a small square hole (6 cm x 6 cm) that allowed rats to enter the box. On the opposite wall of the apparatus to the hide box was an alligator clip positioned 4 cm above the metal grid floor. During testing, a piece of cat collar was attached to the clip. A domestic cat wore this cat collar for a period of three weeks before the start of the experiment. The domestic cat was a female tabby that was 8 years old at the time of testing. On removal from the cat, the collar had been placed in

an airtight plastic container and was stored in a refrigerator at 4°C. The collar was cut into four equivalent pieces with one piece being used in each of the four test chambers. Before the beginning of trials requiring exposure to cat odour, the collar was attached to the clip and left to stand for 30 min before testing. The cat collar was always handled with plastic gloves.

Photocell detectors were placed at opposite ends of the chamber (see Figure 2.1.) which fed their output to a Macintosh™ computer running WorkbenchMac™ data acquisition software (McGregor, 1996a). The placement of the photocells allowed determination of: 1) the amount of time the rats spent in close vicinity to the cat collar ("approach time"); and 2) the amount of time spent in the hide box ("hide time"). During testing, the room in which the chambers were located was illuminated by a 40W red light suspended 1.5 m above the apparatus.

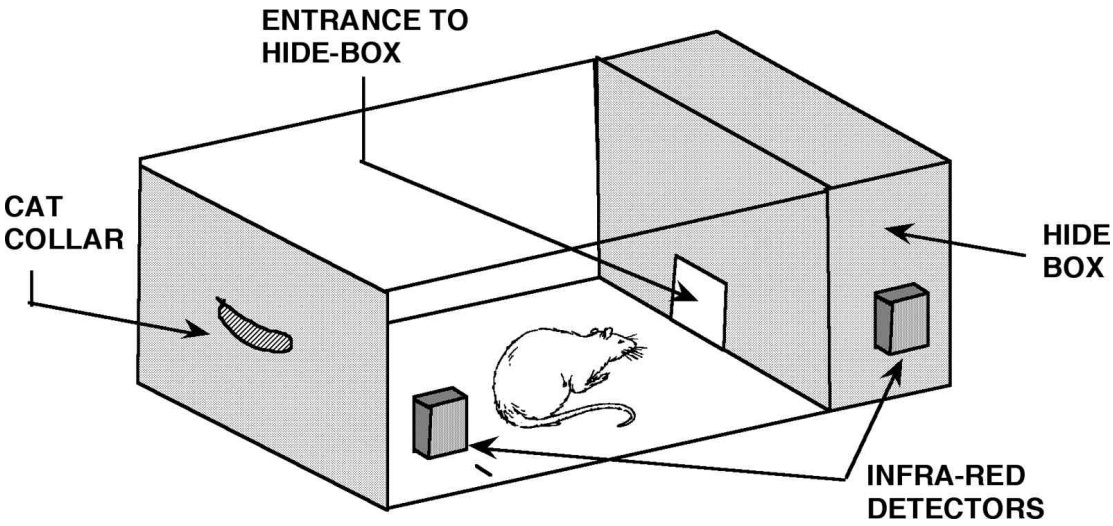

Figure 2.1. The predatory odour avoidance apparatus.

2.2.1.3. Drugs. The synthetic cannabinoid receptor agonist CP 55,940 ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol) (a generous gift from Pfizer Ltd, Sydney) is an easier pharmacological tool to use than Δ^9-THC. Δ^9-THC must be ordered in from the U.S.A. where it is not always available and is expensive to obtain. Moreover, cannabinoid research has been plagued by unreliable results based on the unstable and troublesome chemical nature of Δ^9-THC (Dewey, 1986). For example, Δ^9-THC is degraded when exposed to light or

air (Chesher, Christie, & Morgan, 1994). Further, Δ^9-THC is formulated as a "gummy-type" substance which does not dissolve readily in aqueous solutions and often sticks to glassware (Dewey, 1986). Thus, the actual dose of Δ^9-THC used in different studies is likely to vary considerably and contribute to the lack of consensus between studies. However, using CP 55,940 reduces these problems. CP 55,940 is formulated as a powder in a way which: 1) makes it easy to dissolve in ethanol and aqueous solutions; and 2) minimises its degradation upon exposure to light and air.

Apart from CP 55,940 being a more potent and reliable pharmacological tool than Δ^9-THC, it is used in the current chapter and throughout the thesis (see Chapters 3, 4, 5 and 6) because it has a very similar pharmacological profile to Δ^9-THC (Little, Compton, Johnson, Melvin, & Martin, 1988). CP 55,940 mimics the effects of Δ^9-THC in numerous behavioural tests (Little et al., 1988; Mechoulam, Devane, & Glaser, 1992) and totally substitutes for Δ^9-THC when evaluated in a drug discrimination paradigm (Gold, Balster, Barrett, Britt, & Martin, 1992). Moreover, cross-tolerance is found between CP 55,940 and Δ^9-THC (Fan, Compton, Ward, Melvin, & Martin, 1994; Pertwee, Stevenson, & Griffin, 1993).

Prior studies have used exceedingly large doses of cannabinoids which call into question their relevance to recreational human use and also their validity when attempting to interpret the effects of cannabinoids on animal behaviour (McGregor, Arnold, Weber, Topple, & Hunt, 1998; Miyamoto, Yamamoto, Ohno, & Watanabe, 1997; Miyamoto et al., 1996; Porcella, Gessa, & Pani, 1998; Rodriguez de Fonseca et al., 1997). In the current chapter and throughout the thesis (see Chapters, 3, 4, 5 and 6), a dose range of CP 55,940 was chosen which approximates the amount of cannabis ingested by human users. This dose range was also chosen as it does not confound the study of rodent behaviour by simply impairing motor performance.

Previous investigators have estimated that a marijuana cigarette or "joint" weighing 1 g and containing 2% of Δ^9-THC when smoked by a human would be equivalent to 0.2 mg/kg of Δ^9-THC being absorbed when taken orally by a rat (Gardner & Lowinson, 1991; Rosenkrantz, Sprague, Fleischman, & Braude, 1975). This is

consistent with the dose range (0.5 – 2 mg/kg) that has been reported to be effective in modulating reward in rats (Gardner & Lowinson, 1991). Further, this is equivalent to the amount smoked by recreational users where it has been reported that users smoke between 0.2 and 40 "joints" per day with a median of 2 "joints" per day (Reilly, Didcott, Swift, & Hall, 1998). Because CP 55,940 is approximately 30 times as potent as Δ^9-THC, it was calculated that the lowest dose (10 µg/kg) approximates the strength of one "joint" in humans while the highest dose (50 µg/kg) is below that known to induce catalepsy or pronounced sedation in rats (McGregor et al., 1996c).

CP 55,940 was prepared in the current chapter and throughout the thesis as follows. CP 55,940 was first dissolved in absolute ethanol and then diluted in saline and Tween 80 to make a final vehicle solution of 2.5% Tween 80, 2.5% ethanol and 95% saline. The drug was injected intraperitoneally (i.p) in an injection volume of 1 ml/kg 30 min prior to behavioural testing.

2.2.1.4. Procedure. The experiment consisted of two phases that were spaced 24 h apart (see Dielenberg et al, 1999). At the beginning of all phases rats were placed in the middle of the apparatus facing the cat collar (see Figure 2.1.). In the first phase ("habituation") all rats were administered 1 ml/kg of 0.9 % saline and 30 min later were exposed to the testing apparatus for 20 min in the presence of an unworn cat collar (a collar that had never been worn by a cat). This allowed rats to habituate to the novel apparatus and gave an indication of any strain differences in exploration of the novel environment. In the second phase ("test") the rats from the respective strains were split into four groups of 10 rats. 30 min after injection of either vehicle or CP 55,940 (10, 25, or 50 µg/kg), each group was exposed to the apparatus for 20 min in the presence of the cat collar. In between all phases, the testing chambers were thoroughly cleaned with a 20% ethanol solution and the wood shavings under the grid floor were replaced.

2.2.1.5. Data Analysis. The effects of CP 55,940 on anxiety-like behaviour in Lewis and Wistar rats were based on two measures: 1) the amount of time

the rats spent in close vicinity to the predatory odour ("approach time") and; 2) the amount of time spent in the hide box ("hide time"). The anxiety-like behaviour induced by predatory odour was indexed for each rat by using difference scores. These difference scores were derived by subtracting the hide times and approach times during habituation from the equivalent scores during test. This was done to reduce variation caused by individual differences in exploration that were evident in the habituation phase that may contribute to differences observed in the effects of cat odour on anxiety-like behaviour in the test phase. A significance level of 0.05 was adopted for all tests.

The effects of cat odour. To test whether cat odour had an effect on anxiety-like behaviour, a one-factor analysis of variance (ANOVA) compared either hide time or approach time of vehicle-treated rats in the test phase to the respective hide time or approach time of vehicle-treated rats in the habituation phase. This was done separately for each strain (Lewis and Wistar rats).

Strain differences in the effects of cat odour. This analysis tested strain differences in the effects of cat odour on anxiety-like behaviour. A one-factor ANOVA was used, where strain (Lewis and Wistar) was treated as a factor and the difference scores for hide time and approach times for vehicle-treated rats in the test phase were treated as the dependent variables.

Strain differences in the effects of CP 55,940 on cat odour induced anxiety. Inferences drawn concerning strain differences in the effects of CP 55,940 on cat odour induced anxiety-like behaviours were based on two different statistical approaches. The first analysed the effects of CP 55,940 *within* each strain. However, this approach can only reliably infer strain differences when no strain differences are found in the effects of cat odour alone. The second statistical approach to assess strain differences in effects of CP 55,940 on cat odour induced anxiety could do so irrespective of baseline differences in anxiety-like behaviours of Lewis and Wistar rats. This approach analysed the effects of CP 55,940 *between* each strain. However, the disadvantage of this approach compared to the first one is that less information is acquired relevant to the effects of particular doses of CP 55,940.

Previous researchers have combined these approaches as a highly informative method of analysing the effects of varying doses of a compound on the behaviour of phenotypically distinct animals (Kopp et al., 1999; Simar et al., 1996).

1) Within strains. The first approach analysed the effects of particular doses of CP 55,940 (that is, 10, 25 and 50 µg/kg) in comparison to vehicle within each strain. A one-factor ANOVA followed by Dunnett's post-hoc test was performed on the difference scores in order to assess the effects of the various drug doses. This was done separately for each strain (Lewis and Wistar rats).

2) Between strains. In the second approach, a two-factor ANOVA was performed on test phase data where strain (Lewis and Wistar) and dose (vehicle and CP 55,940 doses) were the factors. This analysis allowed the calculation of a strain by dose interaction effect that assessed strain differences in the effects of CP 55,940 while taking into account baseline differences in anxiety-like behaviours.

2.2.2. Results

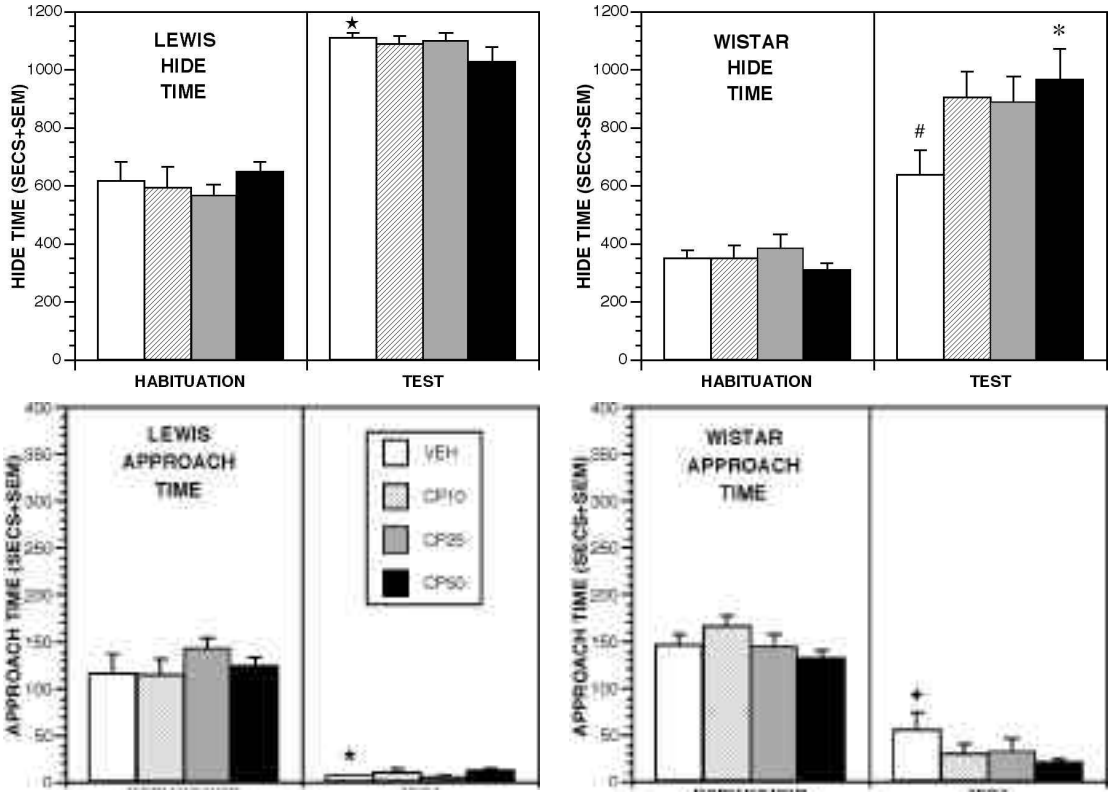

Figure 2.2. Effects of either vehicle (VEH) or 10, 25 or 50 µg/kg of CP 55,940 (CP10, CP25 and CP50, respectively) on predatory odour avoidance. Mean scores for hide times (upper) and approach times (lower) for Lewis (left) and Wistar (right) rats are shown. * signifies the CP 55,940 group is significantly different to the vehicle group ($p < 0.05$). Symbols located above the vehicle group in the test phase indicate that this group is significantly different from the respective vehicle group in the habituation phase (# denotes $p < 0.01$; ★ denotes $p < 0.001$; and ✦ denotes $p < 0.0001$).

Cat odour-induced anxiety. The data for hide time and approach time for each strain in each phase of the experiment are shown in Figure 2.2. Exposure to the cat odour clearly increased hide time [$F(1,18) = 53.99$, $p < 0.001$; $F(1,18) = 10.61$, $p < 0.01$] and decreased approach time [$F(1,18) = 16.51$, $p < 0.001$; $F(1,18) = 29.60$, $p < 0.0001$] in both Lewis and Wistar rats respectively.

No strain differences in the effects of cat odour. Exposure to cat odour had no differential effect upon Lewis or Wistar rats. That is, one-factor ANOVA showed no significant differences between the difference scores of vehicle-treated Lewis and Wistar rats in terms of hide time [$F(1,18) = 3.62$, $p = 0.073$]. Although there was a tendency for Lewis rats to hide more, this failed to reach significance. No significant difference was found between vehicle-treated Lewis and Wistar rats in the difference scores for approach time [$F(1,18) = 0.43$, $p = 0.52$].

Strain differences in the effects of CP 55,940 on cat odour-induced anxiety.

1) Within strains. Analysis of difference scores for Wistar rats revealed a significant overall difference between groups for hide time [$F(3,36) = 2.95$, $p < 0.05$] but not approach time [$F(3,36) = 1.99$, $p = 0.13$]. Post-hoc analysis revealed that the group given 50 µg/kg of CP 55,940 hid significantly more than the group treated with vehicle. Analysis of difference scores for the Lewis rats revealed no significant differences between CP 55,940-treatment groups for either hide time or approach time ($Fs < 1.25$).

2) Between strains. Analysis by two-factor ANOVA revealed a significant strain by dose interaction for hide time [$F(1,72) = 3.21$, $p < 0.05$] but not for approach time [$F(1,72) = 1.84$, $p = 0.15$]. Effects of strain were observed on both hide time [$F(3,72) = 22.56$, $p < 0.0001$] and approach time [$F(3,72) = 15.03$, $p < 0.001$]. However, no effect of dose was observed on either hide time [$F(1,72) = 1.58$, $p = 0.20$] or approach time [$F(1,72) = 1.01$, $p = 0.39$].

2.3. Experiment 2B. The effects of CP 55,940 on open area avoidance in Lewis and Wistar rats

2.3.1. Methods

2.3.1.1. Subjects. The subjects were 36 Lewis and 36 Wistar experimentally naive rats acquired from the same sources as Experiment 2A (see section 2.2.1.1.). Rats from both strains were approximately 75 days old at the start of the experiment and were housed in an identical fashion to Experiment 2A. Each rat was handled for 2 min per day on each of the six days prior to the start of the experiment. All rats were randomly allocated to groups to receive vehicle or 10, 25 or 50 µg/kg of CP 55,940 (n = 9 per group).

2.3.1.2. Apparatus. The apparatus used was identical to that used in Experiment 2A (see section 2.2.1.2.).

2.3.1.3. Drug. CP 55,940 was prepared as outlined in section 2.2.1.3.

2.3.1.4. Procedure. The procedure used was the same as for Experiment 2A (see section 2.2.1.4.) except no cat odour was present in the test phase of the experiment.

2.3.1.5. Data Analysis. Data analyses were based on the same measures of hide time and approach time as referred to in section 2.2.1.5. A significance level of 0.05 was adopted for all tests.

Strain differences in avoidance of a novel and open area. Strain differences in anxiety-like behaviour in response to a novel and open area were assessed in Experiment 2B. To do this hide times and approach times in Lewis and Wistar were analysed by using all data collected in the habituation phase of Experiment 2B (n = 40). A one-factor ANOVA was performed where strain was the factor and the dependent variable was hide time or approach time.

Strain differences in avoidance of an open area from habituation to test. To assess any strain differences in the changes of avoidance behaviour from habituation to test a one-factor ANOVA was used. In this analysis strain (Lewis and Wistar) was treated as a factor and the difference scores (test-habituation) of hide time and approach time of vehicle-treated rats were treated as the dependent variables.

Strain differences in the effects of CP 55,940 on open area avoidance. Two different statistical approaches were employed to assess strain differences in the effects of CP 55,940 on open area avoidance. This was done for the same reasons as outlined in section 2.2.1.5. These are:

1) Within strains. A one-factor ANOVA of difference scores (test - habituation) followed by Dunnett's post-hoc tests was used to analyse treatment differences in hide times and approach times. This analysis was applied separately to each strain.

2) Between strains. The second approach used to assess strain differences in CP 55,940-induced anxiety using test phase data and was based on a two-factor ANOVA where strain and dose were the factors.

2.3.2. Results

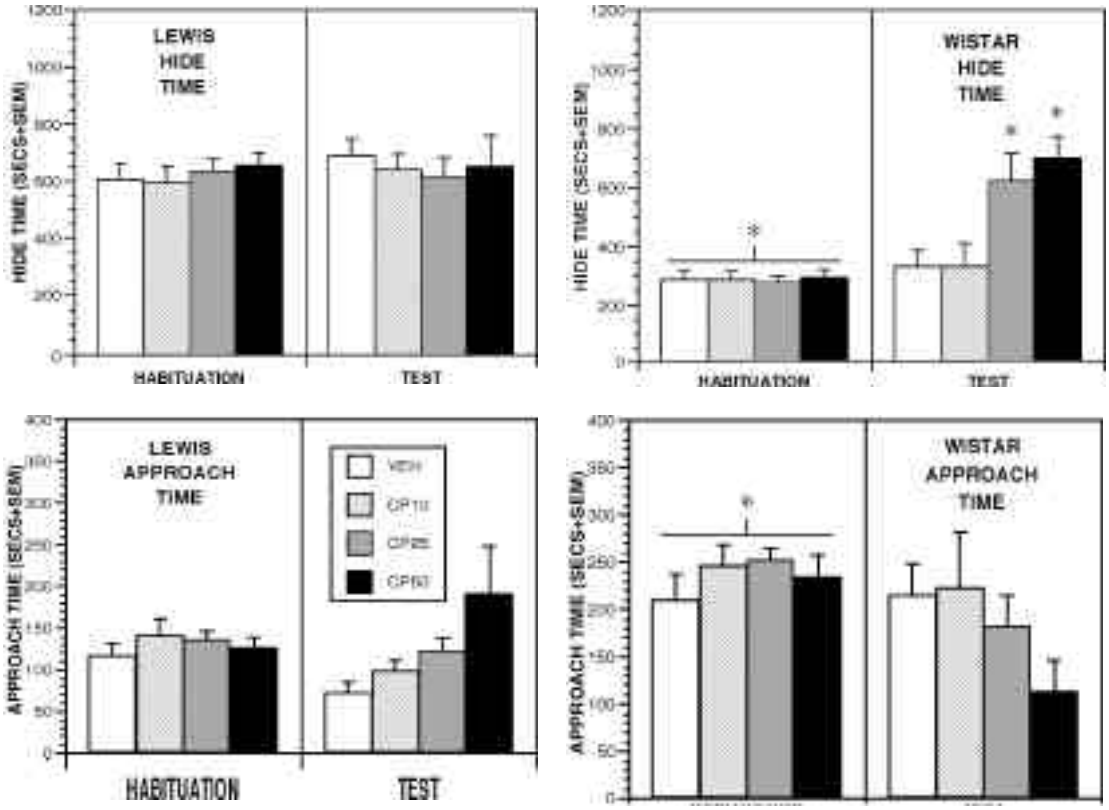

Figure 2.3. Effects of vehicle (VEH) or 10, 25 or 50 µg/kg of CP 55,940 (CP10, CP25 and CP50, respectively) on open area avoidance. Mean scores for hide times (upper) and approach times (lower) for Wistar (left) and Lewis (right) rats are shown. * signifies that within a strain the CP 55,940 group is significantly different to the vehicle group ($p < 0.05$). In addition, the * in the habituation phase for Wistar rats indicates that these scores are significantly different to the respective scores in the habituation phase for Lewis rats for either hide time or approach time ($p < 0.05$).

Strain differences in avoidance of a novel and open area. Strain differences were observed between Lewis and Wistar rats in anxiety-like behaviour exhibited during the habituation phase. Lewis rats hid significantly more [$F(1,70) = 6.51$, $p < 0.05$] and approached significantly less [$F(1,70) = 5.20$, $p < 0.05$] than Wistar rats.

No strain differences in avoidance of an open area from habituation to test. A one-factor ANOVA found no significant differences in anxiety-like behaviour between vehicle-treated Lewis and Wistar rats in the difference scores (test – habituation) for either hide time ($F < 1$) or approach time [$F(1,16) = 2.11$, $p = 0.17$].

Strain differences in the effects of CP 55,940 on open area avoidance.

1) Within strains. CP 55,940 dose-dependently increased hide time [$F(3,32) = 5.77$, $p < 0.01$] but did not significantly decrease approach time [$F(3,32) = 1.62$, $p > 0.2$] in Wistar rats. Post-hoc analysis revealed that the 25 and 50 µg/kg groups hid significantly more than rats treated with vehicle. The difference scores for Lewis rats revealed no significant differences between any CP 55,940-treatment group and vehicle for hide time ($F < 1$) or approach time [$F(3,32) = 2.74$, $p = 0.06$].

2) Between strains. Analysis by two-factor ANOVA revealed a significant strain by dose interaction for hide time [$F(1,64) = 3.63$, $p < 0.05$] and approach time [$F(1,64) = 3.59$, $p < 0.05$] when only considering data from the test phase. In addition, two-factor ANOVA revealed significant effects of strain on both hide time [$F(1,64) = 7.71$, $p < 0.01$] and approach time [$F(1,64) = 5.60$, $p < 0.05$]. However, no effects of dose were observed on either hide time [$F(1,64) = 2.70$, $p = 0.056$] or approach time ($Fs < 1$).

2.4. Experiment 2C. The effects of CP 55,940 on conditioned USVs and immobility in Lewis and Wistar strain rats

2.4.1. Methods

2.4.1.1. Subjects. The subjects were the same 40 Lewis and 40 Wistar rats used in Experiment 2A (see section 2.2.1.1.). Sixteen rats, 8 Lewis and 8 Wistar, were used in pilot studies to determine an optimal methodology for producing conditioned USVs. This left a total of 32 Wistar and 32 Lewis rats for the experiment proper. A period of five weeks elapsed between the end of experimentation in the predatory odour avoidance model and the start of experimentation in the conditioned USV model. For this experiment, the rats from each strain were randomly reassigned to 4 groups per strain. Again the groups were to receive vehicle or 10, 25 or 50 µg/kg CP 55,940 (n=8 per group).

2.4.1.2. Apparatus. The apparatus consisted of a standard Coulbourn operant chamber [30 (L) x 50 (H) x 25.5 (W) cm] with aluminium side and back walls and a perspex front wall. The floor of the chamber consisted of 16 metal bars that were connected to a Coulbourn shock generator. Two passive infra-red motion detectors (Jaytech, Sydney) were located at ground level, one in the centre of the left aluminium wall and the second directly opposite this in the right wall. The detectors were customized so that they triggered whenever the rat moved and were sensitive to relatively small movements of the head and body of a rat. A Macintosh™ computer running WorkbenchMac™ data acquisition software (McGregor, 1996a) received the output of these detectors and allowed the total number of seconds spent moving for each minute of testing to be determined.

An ultrasound microphone (Ultrasound Advice, London, U.K.) was embedded in the aluminium roof of the test chamber. The microphone was connected to a S-25 BAT detector (Ultrasound Advice, London, U.K.) which sent its high-frequency output to a customised signal detection device. This device generated separate digital

outputs whenever it detected an incoming signal in the frequency bands of 20-30, 30-40, 40-50, 50-60 and 60-80 KHz. A Macintosh™ computer running WorkbenchMac™ software received these digital outputs and counted the number of signals (regardless of duration) received in each frequency band for each minute of testing. The equipment was calibrated prior to each test session by sending pure tones of different ultrasonic frequencies into the test chamber using a signal generator, ultrasound amplifier and ultrasound loudspeaker (Ultrasound Advice, London, U.K.).

2.4.1.3. Drugs. CP 55,940 was prepared as outlined in section 2.2.1.3.

2.4.1.4. Procedure. The procedure involved two phases, a "conditioning" phase followed by a "test" phase with each phase lasting 10 min and separated by 90 min. In both phases USVs and immobility were recorded. In the conditioning phase rats received a 1 mA shock of 1 sec duration at 2, 4, 6 and 8 min into the 10 min session. After this session they were returned to their home cage. One hour later, the rats were injected either with vehicle or 10, 25 or 50 µg/kg CP 55,940 and after a further 30 min were placed back in the chamber for the 10 min test phase. No shock was delivered during the test phase. Since only one test chamber was available and a total of 64 rats were to be tested, the experiment was run across consecutive days in four replicates of 16 rats each. Equal numbers of rats from each strain and drug treatment group were included in each replicate.

2.4.1.5. Data Analysis. The effects of CP 55,940 on anxiety-like behaviour in Lewis and Wistar rats were based on two different measures: 1) the total number of USVs emitted and 2) the total time spent immobile. The scores for activity (seconds spent moving in the 10 min session) were subtracted from 600 to give an index of "immobility" which was then used in statistical analysis. Immobility provides an index of freezing that is sometimes claimed to be a useful model of anxiety (Conti, Maciver, Ferkany, & Abreu, 1990) because rats exposed to shock or an aversive environment show pronounced freezing.

The data retrieved in the test phase produced markedly unequal variances across experimental groups hinting at a possible violation of the homogeneity of variance assumption of ANOVA. Therefore the data were transformed logarithmically according to the equation: $y = \log_{10}(x + 1)$. Logarithmic transformation is a standard technique used to homogenise variance and was especially fitting in this case as the standard deviations were proportional to the means (Howell, 1987).

Strain differences in unconditioned USVs and immobility. Unconditioned USVs and immobility in Lewis and Wistar were assessed by using all data collected in the conditioning phase of Experiment 2C. A one-factor ANOVA analysed this data, where strain (Lewis and Wistar rats) was treated as a factor and the number of USVs and immobility for the conditioning phase were treated as the dependent variables.

Strain differences in conditioned USVs and immobility. To determine strain differences in baseline conditioned USVs and immobility a one-factor ANOVA was performed. In this analysis, strain was treated as a factor (Lewis versus Wistar rats) and the difference scores for the number of USVs and immobility for vehicle-treated rats were treated as the dependent variables.

Strain differences in the effects of CP 55,940 on conditioned USVs and immobility. Difference scores were calculated for each rat (test - conditioning) for both immobility and USVs and these difference scores were used as the basis for statistical analysis. Difference scores were calculated for each rat (test - conditioning) for both immobility and USVs and these difference scores were used as the basis for statistical analysis. The data were analysed consistent with the two different approaches explained in section 2.2.1.5. These are:

1) Within strains. Data was compared across groups (that is, vehicle or 10, 25, and 50 µg/kg of CP 55,940) using one-factor ANOVA followed by Dunnett's post-hoc tests. Separate analyses were constructed for Lewis and Wistar rats.

2) Between strains. Data were assessed by a two-factor ANOVA on test phase data where strain and dose were the factors. This analysis allowed the calculation of a

strain by dose interaction effect that provided another method of assessing strain differences in the effects of CP 55,940 on anxiety.

2.4.2. Results

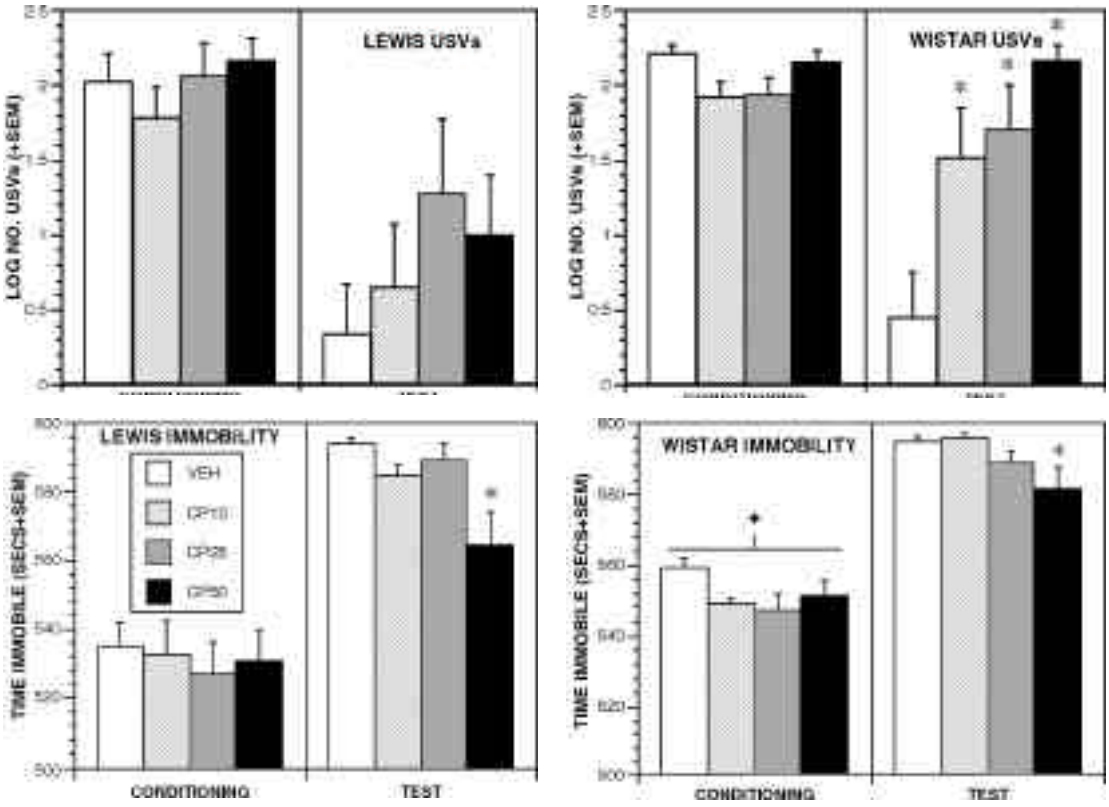

Figure 2.4. Effects of either vehicle (VEH) or 10, 25 or 50 µg/kg of CP 55,940 (CP10, CP25 and CP50, respectively) on conditioned USVs and immobility. Mean scores for number of log-transformed USVs (upper) and immobility (lower) for Wistar (left) and Lewis (right) rats in the conditioned USV model are shown. Note that the y-axis scale in the immobility graphs start from 500 secs to enhance clarity of exposition. * signifies that the CP 55,940 group is significantly different to the vehicle group ($p < 0.05$). In addition, the ✦ located in the conditioning phase for drug-free Wistar rats denotes that these scores are significantly different to the respective scores in the conditioning phase for drug-free Lewis rats ($p < 0.0001$).

Strain differences in unconditioned immobility but not USVs. Data for USVs and immobility are shown in Figure 2.4. No significant differences were observed in unconditioned USVs as measured in the conditioning phase ($F < 1$) between the strains. However, Lewis rats showed significantly less unconditioned immobility than Wistar rats [$F(1,56) = 22.75$, $p < 0.0001$].

Strain differences in conditioned immobility but not USVs. No significant difference was found between vehicle-treated Lewis and Wistar rats in the number of conditioned USVs [$F(1,13) = 1.41$, $p = 0.26$]. However, Lewis vehicle-treated rats showed significantly higher levels of conditioned immobility than vehicle-treated Wistar rats [$F(1,13) = 9.17$, $p < 0.01$] when unconditioned levels of immobility were taken into account.

Strain differences in the effects of CP 55,940 on conditioned USVs and immobility. Rats that produced less than 10 USVs in the conditioning phase were removed from the experiment since it was known from pilot studies that such rats were unlikely to vocalize during the test session regardless of drug treatment. One Wistar rat from the 25 µg/kg CP 55,940 group was removed according to this criterion and one vehicle, two 10 µg/kg, one 25 µg/kg and one 50 µg/kg group rat from the Lewis strain.

1) Within strains. Analysis showed a clear effect of CP 55,940 on the number of conditioned USVs in Wistar rats [$F(3,27) = 8.67$, $p < 0.001$]. Post-hoc tests revealed that Wistar rats treated with 10, 25 and 50 µg/kg of CP 55,940 all vocalized significantly more than vehicle-treated rats. In contrast, treatment with CP 55,940 had no significant effect upon conditioned USVs in Lewis rats ($F < 1$). Significant overall effects were seen for conditioned immobility in both Wistar, [$F(3,23) = 4.97$, $p < 0.01$] and Lewis, [$F(3,27) = 3.65$, $p < 0.05$] strains. Post-hoc analysis revealed that rats treated with 50 µg/kg of CP 55,940 showed decreased levels of immobility when compared to vehicle in both strains of rat.

2) Between strains. Analysis by two-factor ANOVA revealed no significant strain by dose interactions for the number of conditioned USVs [$F(3,48) = 1.21$, $p = 0.32$] or conditioned immobility [$F(3,48) = 2.16$, $p = 0.10$]. In addition, no effects of strain were observed on the number of conditioned USVs [$F(3,48) = 3.96$, $p = 0.052$]. However, effects of strain were observed on conditioned immobility [$F(3,48) = 4.19$, $p < 0.05$]. Further, effects of dose were observed on both the number of conditioned USVs [$F(3,48) = 4.34$, $p < 0.01$] and the time spent immobile [$F(3,48) = 9.03$, $p < 0.0001$].

2.3. Discussion

The present chapter indicates that Lewis rats display less anxiety-like behaviour than Wistar rats when administered the synthetic cannabinoid receptor agonist, CP 55,940. This was highlighted in three different animal models of anxiety, namely, the predatory odour avoidance, open area avoidance and conditioned USV paradigms. This provides evidence, albeit indirect, in support of the theory that Lewis rats may be more susceptible to the rewarding effects of Δ^9-THC because they are less susceptible to the anxiogenic effects of cannabinoids in comparison to other rat strains (Gardner & Lowinson, 1991; Gardner et al., 1988; Lepore et al., 1996; Lepore et al., 1995).

The results of Experiment 2A (section 2.2.2.) concur with recent reports which show that exposure to cat odour promotes hiding behaviour in rats (Blanchard et al., 1993; Dielenberg et al., 1999; Dielenberg & McGregor, 1999; File, Zangrossi, Sanders, & Mabbutt, 1993; McGregor & Dielenberg, 1999; Zangrossi & File, 1994). This is highlighted by comparing the results of Experiment 2A (anxiety-like behaviour tested in presence of cat odour) with Experiment 2B (anxiety-like behaviour tested in the absence of cat odour). In Experiment 2A a dramatic increase in hide time and decrease in approach time was observed from habituation to test in either strain (see Figure 2.3.). However, these changes in anxiety-related behaviours were not observed from habituation to test in Experiment 2B because the cat odour was not present in the test phase (see Figure 2.2.).

No strain differences were observed in cat odour-induced anxiety-like behaviour (see section 2.2.2.). However, there was a tendency for Lewis rats to hide more than Wistar rats in response to cat odour exposure. This indicates that the delineation of a strain difference may be possible in future studies. Strain differences in cat odour-induced anxiety may be observed if: 1) more appropriate experimental controls are used; 2) more subjects are allocated to each experimental group; 3) and a more extensive length of time is spent measuring anxiety-like behaviour using more sensitive measures. This final improvement may aid in avoiding the clear ceiling (hide time) and floor

effects (approach time) of the cat odour on avoidance behaviour that was observed in the Lewis rats (see Figure 2.2.).

The observed strain-independent increase in hiding behaviour to cat odour exposure in Experiment 2A (section 2.2.2. and Figure 2.2.) was magnified by the administration of CP 55,940 in Wistar rats but not Lewis rats. This extends previous findings of aversive and anxiogenic effects of cannabinoids on rodents and suggests that strain differences may exist in this response (McGregor, 1996b,c; Onaivi et al., 1990; Parker & Gillies, 1995; Rodriguez de Fonseca et al., 1997; Rodriguez de Fonseca et al., 1996; Sanudo-Pena et al., 1997). However, the failure of Experiment 2A to find an increase in anxiety-related behaviours in Lewis rats administered CP 55,940 must be treated with caution due to the very high levels of hiding observed in this strain. Thus, the presence of ceiling and floor effects in vehicle-treated Lewis rats during the test phase may have obscured any possible effects of CP 55,940 (see Figure 2.2.).

Experiment 2B (see section 2.3.) re-assessed the possible effects of CP 55,940 on anxiety by using a method that avoided the problem of the behavioural measures reaching ceiling and floor levels in Lewis rats. By using the same apparatus as Experiment 2A, without presenting the cat odour during the test phase, the elucidation of the effects of CP 55,940 within this strain became possible. The results of Experiment 2B (section 2.3.2.) further confirm the results seen in Experiment 2A (section 2.2.2.) where low to moderate doses of CP 55,940 increased anxiety-like behaviour in Wistar but not Lewis rats. Clearly, even with ample headroom for an effect to be determined, there was no increase in open area avoidance behaviour observed with the administration of CP 55,940 in Lewis rats. If anything there was a trend toward less hide time and more approach time that is indicative of increased exploration of the open area (see Figure 2.3.). In contrast, Wistar rats treated with CP 55,940 exhibited a clear dose-dependent increase in open area avoidance suggesting an anxiogenic effect of the drug. Thus, Experiment 2B provides evidence of a strain difference in the effects of CP 55,940 on open area avoidance behaviour in rats.

Lewis rats showed higher avoidance of a novel and open area in comparison to Wistar rats (see section 2.3.2. and habituation phase data in Figure 2.3.). 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). These studies suggest that Lewis rats are a particularly anxious rat strain compared to other strains such as Fischer 344 rats, spontaneously hypertensive rats and Wistar rats. However, conflicting evidence shows that Lewis rats are less anxious in comparison to Wistar rats as measured by the social interaction test (Rex et al., 1996). In this test, Lewis rats placed in a novel environment showed increased social interaction (for example, sniffing and following another rat) in comparison to Wistar rats. Chapter 3 will examine if the previously reported strain difference in the social interaction test can be reproduced. In addition, the effects of CP 55,940 on social interaction behaviour of Lewis and Wistar rats will be tested.

Much preclinical research indicates that central CRH systems are important to the endocrine, autonomic and behavioural consequences of anxiety (Arborelius, Owens, Plotsky, & Nemeroff, 1999). For instance, recent studies have shown that CRH receptor knockout mice display less drug-free anxiety than mice with intact CRH receptors (Contarino et al., 1999; Heinrichs, Lapsansky, Lovenberg, De Souza, & Chalmers, 1997; Smith et al., 1998; Timpl et al., 1998). The findings that Lewis rats have a hypoactive HPA axis are inconsistent with the high baseline anxiety observed in this strain here and elsewhere (Berton et al., 1997; Rex et al., 1996). However, it is possible that CRH systems in the HPA axis have no role in determining the Lewis rat's high baseline level of anxiety relative to other strains. It is possible that this strain difference may be explained by differences in another neurochemical involved in anxiety such as 5-hydroxytryptamine (5-HT).

The reduced levels of CP 55,940-induced anxiety-like behaviours observed here in Lewis rats is consistent with previous studies that have shown Lewis rats to release less ACTH and corticosterone to a variety of other drugs (Calogero et al., 19921) (see section 2.1.). One possible explanation of these findings is that there is poor cross-talk

between neurochemical systems in general, including the endogenous cannabinoid system, and those in the HPA axis of Lewis but not Wistar rats. Thus, when a drug activates areas of the brain in Lewis rats, little cross-talk may occur between areas of the brain involved in anxiety, and CRH systems within the HPA axis. Future studies should aim to more clearly elucidate the role that CRH has in baseline and drug-induced anxiety.

The increase in USVs in Wistar rats administered CP 55,940 in a shock-paired context further confirms the anxiogenic effects of the drug established in the predatory odour avoidance and the open area avoidance paradigms (see section 2.4.2. and Figure 2.4.). The effect was dose-dependent with the largest potentiation of USVs observed in Wistar rats injected with 50 µg/kg of CP 55,940. The results for the Lewis rats were again less clear cut than the results for the Wistar rats in this model. Although there was a tendency towards increased USVs in the Lewis rats administered 25 µg/kg of CP 55,940 this effect was not significant, and clearly not an effect of the same order of magnitude as that seen in Wistar rats given the same dose. While this study provides further support for the hypothesis that Lewis rats are less susceptible to the anxiogenic effects of CP 55,940 in comparison to Wistar rats, it could be improved. Because the number of rats allocated to each experimental group in Experiment 2C was small, and the measures taken in the conditioned USV paradigm were highly variable, more subjects were needed to increase statistical power. Therefore, Experiment 2C could be improved by increasing the number of subjects to approximately 12 to 16 rats allocated per experimental group.

The data for immobility in the conditioned USV paradigm were less impressive, due to the near-ceiling levels of immobility seen in all rats. The only exception to this was the unexpected decline in immobility in Lewis and Wistar rats given 50 µg/kg of CP 55,940. The reasons for this are unclear, particularly since other results show that this dose of CP 55,940 inhibits spontaneous locomotor activity in Wistar rats (see Chapter 4 and 5). However, one possible explanation arises from an analysis of the wide spectrum of behaviours that rodents perform in different aversive situations. In

rodents, fear and anxiety are thought to lie on a continuum from low anxiety that is associated with behavioural inhibition (for example, freezing) and high anxiety which is associated with behavioural activation (for example, fight or flight) (Blanchard et al., 1993; Rodgers, 1997). In conditioned models, where rats undergo footshock, it is common to observe behavioural activation, in the form of a post-shock activity burst, which is followed by freezing (Rodgers, 1997). Therefore, vehicle-treated rats placed in a context previously paired with footshock exhibit high immobility or freezing. However, CP 55,940-treated rats may exhibit less immobility or freezing within this unique context because the drug has magnified their anxiety sufficiently to induce behavioural activation.

On a methodological level, Experiment 2C provides a useful model for demonstrating anxiogenic effects of drugs. The combination of a schedule of footshock coupled with a short (90 min) delay between conditioning and test, caused high levels of immobility in rats re-exposed to the chamber but little by way of USVs in vehicle-treated rats. However, when CP 55,940 was administered, a clear potentiation of USVs was produced in Wistar rats but not Lewis rats. Obviously, if an anxiolytic drug were to be tested, increased levels of shock or reduced conditioning-test intervals would have to be employed to increase the baseline levels of USV during test (Molewijk et al., 1995).

While some USV paradigms have utilized procedures where the drug is administered at the same time as shock (Cullen & Rowan, 1994; De Vry et al., 1993; Nielsen & Sanchez, 1995) this approach suffers from the potential confound of a drug decreasing nociceptive sensitivity to shock itself. This is particularly problematic when using cannabinoid receptor agonists, such as CP 55,940, which have well documented antinociceptive effects (Fuentes et al., 1999; Lichtman & Martin, 1991a,b; Walker et al., 1999). The present approach, where CP 55,940 is administered in the shock-paired environment in the absence of shock itself, avoids this potential confound (see Figure 2.4.). In addition, studies interested in strain differences in the effects of shock on behaviour are still open to interpretation. For example, Lewis rats show lower

levels of immobility in response to shock in comparison to Wistar rats (see section 2.4.2. and conditioning phases of Figure 2.4.). Thus, Lewis rats probably respond with post-shock activity bursts of longer duration that results in a decrease in the amount of immobility compared to Wistar rats. However, whether this strain difference is attributable to Lewis rats being more susceptible to anxiety or nociception is difficult to disentangle.

One possible alternative explanation of the strain differences observed in CP 55,940-induced anxiety in the current chapter might be that Lewis rats are generally subsensitive to the effects of cannabinoids. That is, cannabinoids may affect Lewis rats less than other rat strains irrespective of what behaviour is assayed. This explanation implies that Lewis rats should also be subsensitive to the effects of cannabinoids on an array of different tests, such as those testing motor behaviour, reward, analgesia or anxiety. Future studies in this thesis will assess the viability of this explanation by testing the effects of CP 55,940 on Lewis and Wistar rats in other behavioural assays apart from animal models of anxiety. Arguing against the explanation that Lewis rats are merely subsensitive to the effects of cannabinoids is the realisation that the dose range of Δ^9-THC that facilitates self-stimulation in Lewis rats (0.5 - 1.0 mg/kg), but not other rat strains, is relatively low and approximates the potency of the doses of CP 55,940 used in the present chapter (Lepore et al., 1996a). In Chapter 3, a larger dose of CP 55,940 will be used to test whether the Lewis rats lack of sensitivity to the anxiogenic effects of CP 55,940 can be surmounted by increasing the dose.

As described in section 1.5.4., human survey data illustrates that cannabis users commonly report experiences of anxiety, panic, paranoia and fear when intoxicated by the drug (Annis & Smart, 1973; Hall, 1995; Hall & Solowij, 1998; Thomas, 1993; Thomas, 1996). The current chapter addressed whether cannabinoid-induced anxiety is a homogeneous or heterogeneous phenomenon by observing the effects of CP 55,940 in three different animal models of anxiety-related behaviour. It has been argued that these paradigms involve different types of anxiety, with claims that predatory odours elicit anxiety similar to “phobic anxiety” (Zangrossi & File, 1994) while the re-exposure

to a shock-paired environment elicits a state similar to "panic" (Molewijk et al., 1995). Thus, different animal models of anxiety might assess slightly different psychological states, with some measuring behaviours indicative of phobic states and others measuring behaviours indicative of panic states. The consistent anxiogenic effects of CP 55,940 observed in all models of anxiety tested in the current chapter therefore can not resolve whether CP 55,940-induced anxiety is homogeneous or heterogeneous in nature. Future studies could test markedly different animal models of anxiety (see Chapter 3), such as the social interaction test, to observe whether the same effects of CP 55,940 on anxiety-like behaviour can be observed. Further, future studies may attempt to test the effects of different anti-phobic anxiety and anti-panic drugs to see if they have differential effectiveness in alleviating CP 55,940-induced anxiety.

Results of the current chapter also provide a model of genetic susceptibility to the anxiogenic effects of cannabinoids in the human population. As noted earlier, a twin study has shown that the pleasant or unpleasant effects of cannabis are determined in part by genetic disposition (Lyons et al., 1997). Further, the present results have some similarity with a study that claimed a differential anxiogenic response to Δ^9-THC in the EPM across C57BL/6, DBA/2 and ICR strains of mice (Onaivi et al., 1996). It was concluded that only the ICR mice showed heightened Δ^9-THC-induced anxiety on the EPM. However, the problem with this conclusion was that the other mice strains had higher baseline levels of anxiety regardless of drug treatment and these near-ceiling effects left little room for an anxiogenic response of the Δ^9-THC to be discerned. This situation bears some similarity with that seen in the predatory odour avoidance model (see section 2.2.2.), where high levels of baseline anxiety in Lewis rats made a strain difference in CP 55,940-mediated anxiety difficult to discern.

The strain differences reported in the current chapter between Lewis and Wistar rats in cannabinoid-induced anxiety has implications for the study of genetic vulnerability to drug dependence. In humans, individuals who have experienced anxiety as a consequence of cannabis intoxication are more likely to discontinue use of cannabis than individuals who are pleasantly affected by the drug (Lyons et al., 1997;

Thomas, 1996). As Lewis rats are less susceptible to the anxiety-provoking effects of CP 55,940 in comparison to Wistar rats, it is plausible that they are more likely to voluntarily self-administer the drug. Further, it helps to explain why Lewis rats are the only rat strain to show a rewarding effect of Δ^9-THC as assessed using the intracranial self-stimulation model (Lepore et al., 1996). That is, Lewis rats may be more susceptible to the rewarding effects of cannabinoids because they are less susceptible to the anxiogenic effects of these drugs in comparison to other rat strains.