CHAPTER THREE

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

Studies utilising the social interaction and light-dark emergence models of anxiety with assessment of ataxia using the rotarod.

3.1. Introduction

The current chapter aims to extend the work conducted in Chapter 2 by testing the generality of the findings observed in the predatory odour avoidance, open area avoidance and the conditioned USV models of anxiety. Chapter 2 provided evidence showing that Lewis rats were less susceptible to the anxiogenic effects of CP 55,940 in comparison to Wistar rats as assessed by these animal models of anxiety. This provides indirect evidence in support of the hypothesis that Lewis rats may be more susceptible to the rewarding effects of cannabinoids (Lepore et al., 1996a) because they are less susceptible to the anxiogenic effects of such drugs. Anxiety is a heterogeneous phenomenon and it has been suggested that different animal models of anxiety may assay different types of anxiety (File, 1996a; File et al., 1996b; Rodgers, 1997). Thus, two further models will be utilised in the current chapter, namely, the social interaction test and light-dark emergence paradigm to assay any distinct effects CP 55,940 may have on anxiety-like behaviour in these models.

Previous research into the effects of cannabis and Δ^9-THC on the social behaviour of animals, for the most part, is limited to studies on aggression. These studies demonstrate that aggression in rats, mice and non-human primates is decreased by the administration of Δ^9-THC (Miczek, 1978; Sieber, 1982; van Ree,

Niesink, & Nir, 1984). Studies specifically investigating the effects of cannabinoids on non-aggressive social interactions are scant, in fact, only one study has been published. This study demonstrated that friendly social interactions (comprised of scores for crawling over another rat and social grooming) were reduced in rats administered Δ^9-THC but not cannabidiol (van Ree et al., 1984). One problem with this study is the stressful nature of the social isolation imposed on rats seven days before being administered Δ^9-THC. Given that stress may interact with Δ^9-THC to produce unusual baseline levels of aggression (Bac, Pages, Herrenknecht, & Paris, 1998; Carlini, 1977) the current study employed a less contrived methodology.

The social interaction test used in the current chapter involves two rats being placed in a common arena. While in this arena an experimenter records the total time the rats spend socially interacting (File, 1980; Guy & Gardner, 1985). These rats are not familiar to each other and have not been socially isolated. The model offers face validity based on the rationale that novelty of environment and partner provide anxiogenic stimuli (Guy & Gardner, 1985). In addition, the social interaction test has been shown to have predictive validity because drugs that have anxiolytic and anxiogenic effects on humans, also respectively increase or decrease the total time rats spend socially interacting (File, 1980; File & Pellow, 1984; Guy & Gardner, 1985). The present study tested the effects of CP 55,940 on the non-aggressive social behaviour of Lewis and Wistar rats.

The social interaction test may be particularly useful in providing novel insight into CP 55,940-induced anxiety for two reasons. First, a previous study has reported that Lewis rats exhibit *less* drug-free anxiety-like behaviours than Wistar rats in the social interaction test (Rex et al., 1996). However, previous research and the results of Chapter 2 showed that Lewis rats exhibit *more* drug-free anxiety-like behaviours as assessed by models of anxiety such as the EPM, open area avoidance and the light-dark emergence test (Berton et al., 1997; Rex et al., 1996). Thus, the administration CP 55,940 in the social interaction test may provide different results to those made in Chapters 2 because this model offers a baseline strain difference in

anxiety that is opposite to that found in most unconditioned animal models of anxiety. Second, at least one example exists where the administration of a drug, that is methylenedioxymethamphetamine (MDMA), produces anxiogenic effects as assessed by the EPM and the light-dark emergence tests. However, it produces an anxiolytic effect as assessed by the social interaction test (Morley, 1999). Thus, the social interaction test may be a prime candidate for measuring whether distinct types of CP 55,940-induced anxiety are produced by cannabinoid intoxication.

The present study also tested the effects of CP 55,940 on anxiety-like behaviour in rats as measured by the light-dark emergence test. This model provides an ethologically valid measure of anxiety that takes advantage of the natural tendency of rats to avoid novel and brightly-lit open spaces (Rodgers, 1997). In the current chapter this test was conducted under lower light conditions than is usually employed in the literature in an attempt to reduce the possibility of observing floor and ceiling effects on the anxiety-like behaviour of Lewis rats (see Chapter 2).

Previous studies have documented anxiogenic effects of cannabinoid compounds in the light-dark emergence model (Navarro et al., 1993; Rodriguez de Fonseca et al., 1997; Rodriguez de Fonseca et al., 1996). However, one of these studies showed that the cannabinoid receptor agonist HU-210 has an anxiolytic effect when rats were tested in an unfamiliar light-dark emergence apparatus (Rodriguez de Fonseca et al., 1996). Thus, the current chapter further tests the assertion that CP 55,940-induced anxiety is a heterogeneous phenomenon by using a methodology that assays a rarely reported and distinct effect of cannabinoids on anxiety. To do this, rats will be tested in an unfamiliar light-dark emergence apparatus.

From the results in Chapter 2 it could be argued that Lewis rats are merely subsensitive to the effects of CP 55,940 because only a low to moderate dose range was used. To counter this possible problem, the present chapter employed a higher dose of CP 55,940 to observe whether the Lewis rats subsensitivity to the anxiogenic effects of CP 55,940 could be overcome by increasing the dose of CP

55,940 administered. However, using a higher dose of CP 55,940 may introduce the problem of motor impairment. Thus, the measurement of anxiety-like behaviour may be confounded by the motor impairing effects of cannabinoids at higher doses. Therefore, the current investigation assessed the motor impairing effects of CP 55,940 by using the rotarod test. The rotarod tests motor coordination by measuring the ability of rodents to maintain balance on a rotating spindle. Previous studies have shown that the administration of cannabinoids, such as Δ^9-THC, resulted in motor incoordination, or decreased latency to fall from the rotarod (Meng, Manning, Martin, Fields, 1998; Pryor et al., 1977; Pryor, Larsen, Husain, & Braude, 1978; Siemens & Doyle, 1979). The current chapter extends this work by testing whether strain differences exist in the ataxic effects of CP 55,940.

3.2. Experiment 3A. The effects of CP 55,940 on the behaviour of Lewis and Wistar rats in the social interaction test

3.2.1. Methods

3.2.1.1. Subjects. The subjects were 64 inbred male Lewis albino rats (ARC, Perth, Australia) and 64 inbred male Wistar albino rats (Concord Hospital, Sydney, Australia). Rats from each strain were approximately 75 - 90 days old at the start of the experiment. The rats were group housed in large plastic tubs with eight 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.

3.2.1.2. Apparatus. The social interaction test was conducted in a clear perspex box [52 cm (L) x 52 cm (W) x 40 cm (H)] (see Figure 3.1.). The floor was marked into quarters. A miniature video camera with a fish eye lens monitored the activity of rats from vertically above the box. This sent a signal to a video recorder located in a sound proof box within the testing room. The apparatus was illuminated under low red light using a 40 W red light bulb. The floor of the room contained black sheets of plastic that were spread out under and around the apparatus.

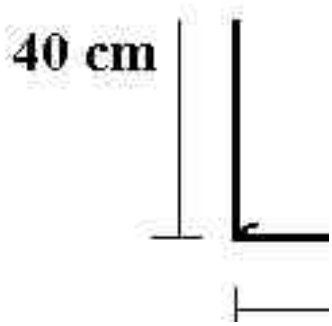

Figure 3.1. The social interaction apparatus.

3.2.1.3. Drug. CP 55,940 (Tocris™) was prepared as described in section 2.2.1.3. It 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.

CP 55,940 was injected i.p. in an injection volume of 1 ml/kg 30 min prior to behavioural testing. Doses of CP 55,940 used were 0, 25, 50 and 75 µg/kg.

3.2.1.4. Procedure. Rats were first habituated to the rotarod (see section 3.4.1.3.) before being grouped into pairs of equal weight where the members of each pair were taken from a different home cage. Both rats were then randomly allocated to the same experimental group (either vehicle, 25, 50 or 75 µg/kg of CP 55,940) with 8 pairs per experimental group. 30 min after injection of either vehicle or CP 55,940 (25, 50 or 75 µg/kg) pairs of rats were tested in the social interaction model.

Pairs of rats were placed in the social interaction test for 10 min. In between tests all testing apparatus were thoroughly cleaned with a 20% ethanol solution. The total duration of social interaction and aggression was recorded for each pair. Data were scored under the rubric of either social behaviours or aggressive behaviours. These categories were comprised of 11 different behaviours in total. That is, the different social behaviours were sniffing partner, mutual grooming, genital investigation, following and crawling over or under the partner. Aggressive behaviours were kicking, biting, boxing, aggressive groom, jumping on and wrestling (File, 1980; Guy & Gardner, 1985; Morley, 1999). The different social behaviours were summed together to produce aggregate scores as it is these that are conventionally reported in the literature (File, 1980; Guy & Gardner, 1985). In addition, aggregated scores provide more sensitive measures of either social interaction or aggressive interaction. The behaviour of the rats in the social interaction test were recorded on video and scored at a later date by the author who was blind to group assignment. The scoring comprised of the scorer observing and timing the duration of the different social behaviours and adding them together to gain a total social interaction time.

3.2.1.5. Data Analysis. The effects of CP 55,940 on aggressive behaviour were not analysed as Lewis rats did not exhibit any aggressive behaviours and only 3 Wistar rats showed aggressive behaviour. For the 3 Wistar rats that were aggressive, this was not related to treatment with CP 55,940.

The total time spent performing different social behaviours (sniffing partner, mutual grooming, genital investigation, following and crawling over or under the partner) were added together to give a total social interaction time (see section 3.2.1.4.) that was used as the basis for statistical analysis. A significance level of 0.05 was adopted for all tests.

Strain differences in drug-free social interaction. To assess whether any baseline differences existed between Lewis and Wistar rats, data from vehicle-treated rats in both strain were compared using an independent sample t-test where strain (Lewis versus Wistar) was treated as a factor and the total time spent in social interaction was treated as the dependent variable.

Strain differences in the effects of CP 55,940 on social interaction. Similar to what was described in section 2.2.1.5., two different statistical approaches were used to assess strain differences in the effects of CP 55,940 on anxiety-like behaviour in the social interaction test:

1) Within strains. The first approach compared the individual doses of CP 55,940 (that is, 25, 50 and 75µg/kg) with vehicle. The social interaction data for Lewis and Wistar rats were analysed separately using a one-factor ANOVA followed by Dunnett's post-hoc tests to assess the effects of the various CP 55,940 doses.

2) Between strains. The second approach to assess strain differences in the effects of CP 55,940 on social interaction behaviour used a two-factor ANOVA where strain and dose were the factors and total time spent in social interaction was the dependent variable.

3.2.2. Results

No strain differences in drug-free social interaction. No significant differences were observed between Lewis and Wistar vehicle-treated animals in the total time spent in social interaction [t(14) = 3.79, p = 0.072]. While this comparison did not reach significance, Lewis rats tended to show lower levels of social interaction than Wistar rats (see Figure 3.2.).

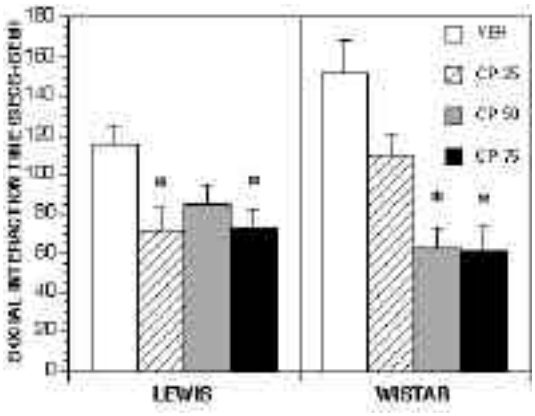

Figure 3.2. The effects of vehicle (VEH) and 25, 50 and 75 µg/kg of CP 55,940 (CP25, CP 50 and CP 75) on the mean total time Lewis (left) and Wistar (right) rats engaged in social behaviours (n = 8 per condition) in the social interaction test. * signifies that the CP 55,940 group is significantly different to the vehicle group ($p < 0.05$).

Strain differences in the effects of CP 55,940 on social interaction.

1) Within strains. In Wistar rats a dose-dependent reduction in total social interaction time was observed with CP 55,940 administration. However, in Lewis rats this reduction in social interaction time in rats administered CP 55,940 followed more of a step-down function. One-factor ANOVA showed that CP 55,940 significantly reduced social interaction in Lewis and Wistar rats respectively [$F(3,28) = 4.11$, $p < 0.05$; $F(3,28) = 11.78$, $p < 0.001$]. Lewis rats exhibited significant reductions in total social interaction time at the 25 and 75 µg/kg doses, whereas, Wistar rats showed significant reductions in total social interaction time at the 50 and 75 µg/kg doses.

2) Between strains. Analysis of social interaction time by two-factor ANOVA revealed a strain by dose interaction [$F(3,56) = 3.87$, $p < 0.05$] suggesting that the strains responded differently to CP 55,940, with Lewis rats showing a smaller reduction in social interaction time with the administration of CP 55,940 in comparison to Wistar rats. In addition, the two-factor ANOVA showed an overall effect of dose [$F(3,56) = 13.75$, $p < 0.0001$] but not strain [$F(1,56) = 1.70$, $p = 0.20$].

3.3. Experiment 3B. The effects of CP 55,940 on the anxiety-like behaviour of Lewis and Wistar rats in the light-dark emergence test

3.3.1. Methods

3.3.1.1. Subjects. The same subjects were used as described in section 3.2.1.1. Sixteen rats were allocated to each experimental group, that is, vehicle, 25, 50 or 75 µg/kg of CP 55,940.

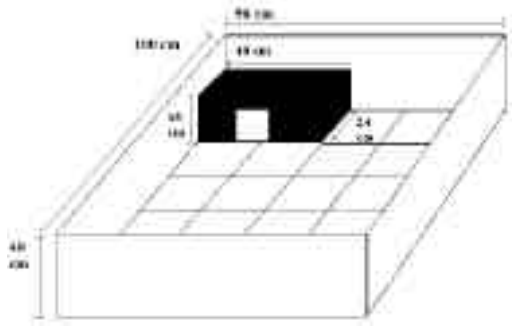

Figure 3.3. The emergence apparatus.

3.3.1.2. Apparatus. The light-dark emergence test was conducted in a square-shaped arena [(L) 100 cm x 96 (W) cm] (see Figure 3.3.). This arena was enclosed by a 40 cm high chipboard wall. The floor was divided into 16 marked squares and a black wooden hide box [(L) 40 cm x (W) 24 cm x (H) 15 cm]. This box contained a square hole that was oriented out toward the open area. A video camera monitored the activity of the rats from above the box. This sent a signal to a video recorder located in an adjacent room. The emergence apparatus was illuminated under more intense light than that used in the social interaction test or open area avoidance test by using a 60 W light bulb that was draped with red cellophane.

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

3.3.1.4. Procedure. Immediately following the social interaction test (see section 3.2.), each rat was taken to the emergence apparatus for a 5 min test before being tested on the rotarod (see section 3.4.). At the start of each trial rats were placed inside the hide box. Rats were scored for emergence latency (time elapsed before leaving the hide box to explore the open arena), time spent hiding (where hiding was defined as all four paws being present inside the hide box), time in the open field

(where open field exploration was defined as all four paws being present in the open field), and the number of times the rat emerged from the hide box to being in the open field. From these measures the mean time spent in the hide box per entry was calculated (Rodriguez de Fonseca et al., 1996). The behaviour of the rats in the light-dark emergence test was recorded on video and scored at a later date by an experienced scorer who was blind to the experimental conditions.

3.3.1.5. Data analysis. The effects of CP 55,940 on anxiety-like behaviour of Lewis and Wistar rats in the light-dark emergence test were based on 4 different measures: 1) emergence latency (time elapsed before leaving the hide box to explore the open arena); 2) time spent in the open field; 3) number of times the rat emerged from the hide box; and 4) the mean time spent in the hide box per entry (see section 3.3.1.4.). Unlike previous analyses the data gained from the light-dark emergence test were not normally distributed. Thus, nonparametric statistics were employed to analyse the results. A significance level of 0.05 was adopted for these tests.

Strain differences in drug-free light-dark emergence. To assess whether any baseline differences exist between Lewis and Wistar rats, data from vehicle-treated rats in both strains were compared using a Mann-Whitney U test where strain was treated as a factor and the four measures were separately treated as the dependent variables.

Strain differences in the effects of CP 55,940 on light-dark emergence. A within strain approach was used to analyse strain differences in the effects of CP 55,940 on light-dark emergence behaviour (see section 2.2.1.5). First, a Kruskal-Wallis one-factor ANOVA-by-ranks test was used to assess whether CP 55,940 had any overall effect on any of the light-dark emergence measures. This analysis was applied separately to each strain where dose was treated as the factor and the four measures were separately treated as the dependent variables. In addition, Mann-Whitney U tests were used to analyse separately the effects of the various CP

55,940 doses in comparison to vehicle. Again these analyses was applied separately to each strain.

3.3.2. Results

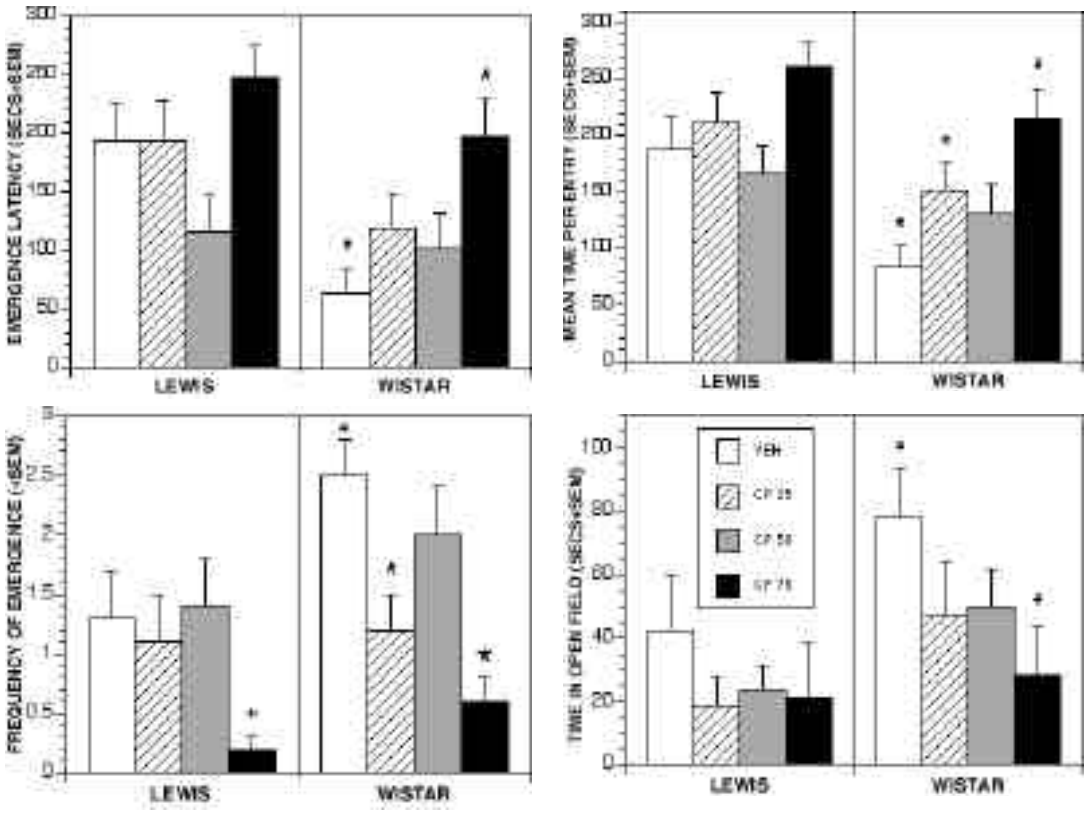

Figure 3.4. Effects of vehicle (VEH) and 25, 50 and 75 µg/kg of CP 55,940 (CP25, CP50 and CP75 respectively) on the light-dark emergence behaviour of Lewis and Wistar rats (n = 16 per group). *, #, or ★ signifies that CP 55,940 group is significantly different to the vehicle group for that strain of rat ($p < 0.05$, 0.01 or 0.001 respectively). * or # located above the bar representing the vehicle group for Wistar rats indicates that this group is significantly different to the respective vehicle group for Lewis rats ($p < 0.05$ or 0.01 respectively).

Strain differences in drug-free light-dark emergence. The results from the light-dark emergence test are shown in Figure 3.4. In terms of drug-free levels of anxiety-like behaviours, Lewis rats appeared more anxious as assessed by all measures used in the light-dark emergence test. Mann-Whitney tests showed that vehicle-treated Lewis rats had significantly increased emergence latency ($p < 0.01$) and mean time per entry ($p < 0.05$) compared to vehicle-treated Wistar rats. In addition, vehicle-treated Lewis rats showed significantly reduced frequency of emergence ($p < 0.05$) and time spent in the open field ($p < 0.05$) than vehicle-treated Wistar rats.

Strain differences in the effects of CP 55,940 on light-dark emergence. Kruskal-Wallis tests showed that in Wistar rats the administration of CP 55,940 resulted in a dose-dependent increase in emergence latency ($p < 0.05$) and mean

time per entry ($p < 0.01$), and a dose-dependent decrease in frequency of emergence ($p < 0.001$) and time spent in the open area ($p < 0.05$). In Wistar rats, Mann-Whitney U tests revealed that 75 µg/kg of CP 55,940 significantly increased emergence latency ($p < 0.01$) and that 25 and 75 µg/kg increased the mean time per entry ($p < 0.05$ and $p < 0.01$ respectively). Wistar rats administered either 25 or 75 µg/kg of CP 55,940 demonstrated significant reductions in the frequency of emergence ($p < 0.01$ and $p < 0.001$ respectively). Further, Wistar rats treated with 75 µg/kg of CP 55,940 showed significantly decreased time in the open field ($p < 0.01$).

Kruskal-Wallis tests showed that CP 55,940 had no effect on Lewis rats in terms of emergence latency ($p = 0.07$), mean time per entry ($p = 0.09$) or time spent in the open area ($p = 0.12$). However, Kruskal-Wallis analysis showed that CP 55,940 reduced the frequency of emergence in Lewis rats ($p < 0.05$). Mann-Whitney U tests revealed that it was only the Lewis rats administered 75 µg/kg of CP 55,940 that showed a significant reduction in the frequency of emergence ($p < 0.05$).

3.4. Experiment 3C. The effects of CP 55,940 on the rotarod performance of Lewis and Wistar rats

3.4.1. Methods

3.4.1.1. Subjects. The same subjects were used as described in section 3.2.1.1. and 3.3.2.1.

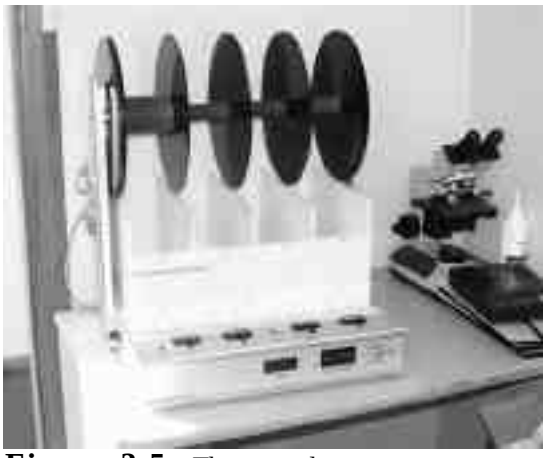

Figure 3.5. The rotarod apparatus.

3.4.1.2. Apparatus. The rotarod (Accurotor™, Accuscan Instruments Inc., Ohio, U.S.A.) consisted of four lanes (see Figure 3.5.). Each lane was separated from one another with white perspex. The lanes contained a rotating spindle (diameter = 8 cm) that was elevated by 40 cm. All testing on the rotarod was conducted one rat at a time on the spindle depicted on the far left hand side of the apparatus (see Figure 3.5.). The rotarod contained a control interface that allowed the rotating spindle speed to be set in revolutions per minute (r.p.m.) and also the time to maximum r.p.m. to be set in sec. The apparatus was automated so that it recorded the elapsed time before the rat fell to the base of the apparatus from the elevated rotating spindle.

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

3.4.1.3. Procedure. Rats were initially trained on the rotarod to ensure that the latency to fall from the apparatus was of sufficient duration in the test to enable any ataxic effects of CP 55,940 to be discerned. Training occurred on the same day of the test but prior to the rats being administered CP 55,940 and tested in the social interaction and the light-dark emergence tests (see section 3.2. and 3.3.). In training, rats were placed on the rotarod for a total of 10 min. Pilot studies showed that a rotation speed of 12 r.p.m. was an effective training speed. The rats were tested for the

entire 10 min if they repeatedly fell off the rotating spindle. If the rats stayed on the rotating spindle for a duration of 120 sec without falling, it would be removed from the apparatus to avoid the confounding influence of fatigue on test performance.

Lewis and Wistar rats were later tested for the effects of CP 55,940 on rotarod performance. This was conducted immediately after the rats had completed the social interaction and light-dark emergence tests (see sections 3.2. and 3.3.). In the rotarod test, rats were timed for their latency to fall from the rotating spindle. The spindle was initially set at a rotating speed of 5 r.p.m. and gradually accelerated to a maximum speed of 40 r.p.m. where the maximum speed of rotation was reached in 200 sec. Rats were given two trials and the average latency to fall from the rotating spindle was taken as their score. A score was not accepted as a trial unless the rat stayed on the rotarod for more than 10 sec.

3.4.1.4. Data analysis. The dependent variable used was the latency of rats to fall from the rotating spindle. A significance level of 0.05 was adopted for all tests.

Strain differences in drug-free rotarod performance. To assess whether any baseline differences existed between Lewis and Wistar rats, data from vehicle-treated rats in both strains were compared using a one-factor ANOVA where strain (Lewis versus Wistar) was treated as a factor and the latency to fall time (in sec) as the dependent variable.

Strain differences in the effects of CP 55,940 on rotarod performance. Two different statistical approaches were again used to assess strain differences in the effects of CP 55,940 on rotarod performance (see section 2.2.1.5.):

1) Within strains. The latency to fall time for Lewis and Wistar rats were analysed separately using a one-factor ANOVA followed by a Dunnett's post-hoc test to assess the effects of the various CP 55,940 doses (that is, 25, 50 and 75µg/kg).

2) Between strains. The second approach to assess strain differences in the effects of CP 55,940 on rotarod performance used a two-factor ANOVA where strain

and dose were the factors and the latency to fall time (in sec) was the dependent variable.

3.4.2. Results

No strain differences in drug-free rotarod performance. The data for average latency to fall for each strain are shown in Figure 3.6. No differences were observed between Lewis and Wistar vehicle-treated animals ($F < 1$) showing that the two strains performed equivalently on the rotarod when not administered CP 55,940.

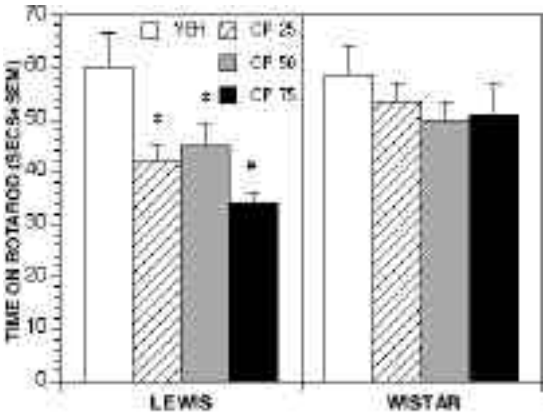

Figure 3.6. The effects of vehicle (VEH) and 25, 50 and 75 µg/kg CP 55,940 (CP25, CP50 and CP75) on mean time on the rotarod in Lewis (left) and Wistar rats (right) (n = 16 per group). * signifies the CP 55,940 group was significantly lower than the vehicle group ($p < 0.05$).

Strain differences in the effects of CP 55,940 on rotarod performance.

1) Within effects. One-factor ANOVA confirmed that Lewis rats treated with CP 55,940 showed significantly reduced time on the rotarod [$F(3,60) = 6.7$, $p < 0.001$]. Dunnett's post-hoc analysis showed that all doses (25, 50 and 75 µg/kg of CP 55,940) produced significantly reduced rotarod performance when each was compared separately to vehicle. However, in Wistar rats CP 55,940 was found to have no effect on time spent on the rotarod at any dose tested ($F < 1$).

2) Between effects. Analysis by two-factor ANOVA revealed no significant strain by dose interaction ($F = 1.54$, $p = 0.21$). However, a significant effect of strain [$F(1,119) = 6.53$, $p < 0.05$] and dose [$F(3,119) = 5.19$, $p < 0.01$] was revealed.

3.5. Discussion

The results from the current chapter support the notion that Lewis rats are less susceptible than Wistar rats to the anxiogenic effects of CP 55,940. In the current chapter this was most clearly shown in the results of the social interaction test and less clearly demonstrated in the results of the light-dark emergence test. These data corroborate the studies conducted in Chapter 2 which showed that only Wistar rats, not Lewis rats, were affected by CP 55,940 as measured in a predatory odour avoidance model, an open area avoidance model and a conditioned USV model of anxiety.

CP 55,940 dose-dependently reduced the total time Wistar rats spent in social interaction in a linear fashion. In contrast, CP 55,940 reduced the total time Lewis rats spent in social interaction with this decrease being uniform across all doses. Thus, unlike the experiments in Chapter 2, CP 55,940 (25 µg/kg) was shown to have at least a small anxiogenic effect on Lewis rats. However, in Lewis rats the decrease in total time spent in social interaction was not magnified by higher doses of CP 55,940 (that is, 50 and 75 µg/kg), whereas in Wistar rats the decrease in social interaction was magnified by these higher doses. This suggests that Lewis rats may be less susceptible to CP 55,940-induced anxiety than Wistar rats.

The current study was not able to address the effects of CP 55,940 on aggression because aggressive behaviour was seldom observed in either strain of rat irrespective of CP 55,940 treatment. Previous studies have shown that Δ^9-THC can increase or decrease aggression dependent on the experimental conditions. However, studies which demonstrate that Δ^9-THC increased levels of aggressive behaviour in rodents can be dismissed as irrelevant to the current study. That is, these studies are only successful under contrived conditions where rodents have been random eye movement (REM) sleep-deprived, food-deprived, socially isolated or stressed prior to the administration of Δ^9-THC (Bac et al., 1998; Carlini, 1977; Carlini, Hamaoui, & Martz, 1972; Carlini & Masur, 1969; Fujiwara & Ueki, 1979; Palermo Neto & Carlini, 1972). Conversely, studies which have demonstrated that Δ^9-THC decreased aggressive behaviour in rodents highlight why it is difficult to establish a satisfactory

baseline level of aggression as shown in the present chapter (Miczek, 1978). That is, these studies were successful as they relied on selecting rodents that showed consistent aggressive behaviour under drug-free conditions. Therefore, it is highly unlikely that this study would have documented Δ^9-THC reducing aggression unless the levels of aggression exhibited by vehicle-treated rats were maximised by utilizing rats that showed high drug-free aggression and omitting rats that showed low drug-free aggression. Furthermore, studies interested in the effects of cannabinoids on aggressive behaviour used a slightly different procedure to the current experiment. That is, unlike in the current study where pairs of rodents were introduced into a novel chamber, these experiments used a procedure more conducive to aggressive behaviour where a stranger rodent intrudes upon the homecage of a conspecific rodent or a group of conspecific rodents (Miczek, 1978). Therefore, it is clear that the lack of aggressive behaviours in rats observed in the present study are consistent with conclusions that the effects of Δ^9-THC on aggressive behaviour can only be measured when baseline levels of aggression are maximised (Miczek, 1978).

In the light-dark emergence test, CP 55,940 caused dose-dependent anxiogenic effects on all four measures of behaviour in Wistar rats. However, Lewis rats were affected only by the highest dose of CP 55,940 (75 µg/kg) and only when considering the frequency of emergence. It is difficult to attribute this difference to the effect of CP 55,940 on anxiety because the motor coordination of Lewis rats may have been impaired as indicated in the results of the rotarod test (see section 3.4.2.). Further, CP 55,940 had no effect on the anxiety-like behaviour of Lewis rats on any other measure reported. Thus, it may be concluded that Lewis rats are less susceptible to the anxiogenic effects of CP 55,940 than Wistar rats in the light-dark emergence test. Although closer scrutiny of the data makes it clear that such a conclusion should be made with caution. Like the predatory odour avoidance test in Chapter 2, vehicle-treated Lewis rats tended to show floor or ceiling effects on the respective measures made in the light-dark emergence test. Thus, it could be argued that strain differences

may not actually exist because there is little room left to delineate an anxiogenic effect of CP 55,940 in Lewis rats.

However, the floor and ceiling effects observed in the light-dark emergence test were not as drastic as those observed in the predatory odour avoidance test (see section 2.2.2.). Thus, more room existed for Lewis rats to show an increase in anxiety-like behaviour with the administration of CP 55,940 than in the predatory odour avoidance test. Further, the current chapter employed a higher dose of CP 55,940 than Chapter 2 making it even more likely that anxiogenic effects of CP 55,940 on Lewis rats would be observed if such an effect existed. Therefore, the results for the light-dark emergence test appear to be consistent with the hypothesis that Lewis rats are less susceptible to CP 55,940-induced anxiety in comparison to Wistar rats.

The current chapter again shows that Lewis rats exhibit higher baseline levels of anxiety-like behaviours than Wistar rats. That is, vehicle-treated Lewis rats displayed significantly more anxiety-related behaviours than vehicle-treated Wistar rats as assessed by the light-dark emergence test. These data generally reinforce previous findings and the findings in Chapter 2 where Lewis rats exhibit more anxiety-like behaviours than Wistar rats under drug-free conditions (Berton et al., 1997; Rex et al., 1996). When comparing vehicle-treated Lewis and Wistar rats in the social interaction test there was a trend to show Wistar rats were less anxious than Lewis rats but this failed to reach statistical significance (see section 3.2.2.). This finding conflicts with a previous report that Lewis rats exhibit less drug-free anxiety-like behaviours than Wistar rats in the social interaction test (Rex et al., 1996). One explanation of this result relies on the current study using Lewis and Wistar rats that were bred in Australia not in Europe such as in the Rex *et al* (1996) study. Rex *et al* (1996) observed considerable variation in the anxiety-like response of identical strains of rat that could be influenced by the environment in which the rats were bred. Thus, while Wistar rats bred at vendors such as Charles River or BGVV showed less social interaction than Lewis rats bred at Charles River, Wistar rats bred by the vendor of Winkelmann showed no such differences. Therefore, studies assessing strain differences in anxiety-

like behaviours must carefully consider, not only the strain of rats used, but also what breeding facility the rats were acquired from, when interpreting results. In the current thesis the Lewis and Wistar rats used were reared in different environments, therefore presenting the minor possibility that strain differences observed here may be attributable to breeding facilities rather than strain.

Interestingly the differential effects that CP 55,940 has on anxiety-like behaviours, with Wistar rats more sensitive than Lewis rats, is opposite to the effects of CP 55,940 on ataxia, where Lewis rats are more sensitive than Wistar rats. In Lewis rats, CP 55,940 caused a dose-dependent reduction in the latency to fall from the rotarod. This is consistent with previous studies that have shown that Δ^9-THC decreases rotarod performance in rats (Meng et al., 1998; Pryor et al., 1977; Pryor et al., 1978; Siemens & Doyle, 1979). In Wistar rats, CP 55,940 had no significant effect on rotarod performance. Thus, the effects of CP 55,940 on Wistar rats observed in the animal models of anxiety are unlikely to be due to motoric impairment or greater overall sensitivity to CP 55,940. While the Lewis rats administered CP 55,940 were motorically impaired, Lewis rats still showed less anxiety in comparison to Wistar rats as measured by the different animal models of anxiety.

It is interesting to speculate on why Lewis rats were less susceptible to the anxiogenic effects of CP 55,940 when they were clearly affected by the cannabinoid in terms of a reduction in motor coordination. Obviously, anxiety and motor behaviour have different neural substrates. Research shows that CB_1 receptors reside in neural substrates of anxiety and fear such as the CEA, PVN and the bed nucleus of the stria terminalis (BNST) (Herkenham et al., 1991b; Pettit et al., 1998; Tsou et al., 1998). Further, evidence also shows that CB_1 receptors are found in neural substrates of motor behaviour such as the globus pallidus (GP), the substantia nigra (SN), the caudate-putamen (CPU) and the cerebellum (Herkenham et al., 1991a,b; Pettit et al., 1998; Tsou et al., 1998). Thus, CP 55,940 may act differentially on the substrates of anxiety and motor behaviour to produce these distinct effects. The next chapter (Chapter 4) will assess neural activation using *c-fos* immunohistochemistry to observe whether the

administration of CP 55,940 changes the biochemical activity of the above regions of the brain involved in anxiety and motor behaviour.

The finding that Lewis rats may be deficient in their ability to secrete and release CRH has been hypothesized as the possible explanation for why Lewis rats are less susceptible to the anxiogenic effects of CP 55,940 in comparison to Wistar rats. This explanation may aid in attempts to dissociate motor impairment from deficient CRH release as contributing factors in determining the results obtained for Lewis rats in the social interaction test. If CRH is solely responsible, then administration of a CRH antagonist may abolish the small and uniform decrease in time spent in social interaction observed across doses of CP 55,940 administered. Furthermore, the lack of dose-dependency observed in the effects of CP 55,940 on the social behaviour of Lewis rats may be explained by their deficiency in synthesising and secreting CRH. The Lewis rat's CB_1 receptors may just as efficiently "cross-talk" with CRH systems in the hypothalamus to induce anxiety at low doses. For example, 25µg/kg of CP 55,940 caused an anxiogenic-profile in Lewis rats in the social interaction test. However, higher doses of CP 55,940 may not be able to release more CRH because its limited stores have already been depleted, thus restricting the expression of CP 55,940-induced anxiety.

As discussed in section 2.4., this mechanism may only be relevant to drug-induced stress and anxiety rather than being responsible for Lewis rats having higher baseline levels of anxiety in comparison to other strains including Wistar rats. The findings that Lewis rats have a hypoactive HPA axis is inconsistent with the relative baseline anxiety observed between the strains reported in Chapter 2 and those reported previously (Berton et al., 1997; Rex et al., 1996). However, Lewis rats do show reduced levels of drug-induced anxiety such as reported in Chapter 2 and based on a previous study showing that Lewis rats release less ACTH and corticosterone to a variety of other drugs (Calogero et al., 1992) (see section 2.1.). Thus, there may be little drug-induced “cross-talk” between neurochemical systems involved in anxiety, including the endogenous cannabinoid system, and the HPA axis in Lewis rats but not

Wistar rats. Therefore, CRH systems in the HPA axis may have a role in determining the differential effects of CP 55,940 on Lewis and Wistar rats, but not in determining the Lewis rat's higher baseline level of anxiety relative to Wistar rats.

The current chapter further reinforces the studies conducted in Chapter 2 and together provide convincing evidence for the hypothesis that Lewis rats are less susceptible to CP 55,940-induced anxiety than Wistar rats. As the current study showed that Lewis rats were more sensitive than Wistar rats to CP 55,940-induced ataxia, the criticism that Lewis rats may just be globally subsensitive to the effects of CP 55,940 cannot be supported. This chapter provides further indirect support for the hypothesis that Lewis rats are more susceptible to the rewarding effects of cannabinoids, because they are less susceptible to the anxiogenic effects of such drugs. Stated in another way, in Wistar rats the effects of cannabinoids on the neural substrates mediating anxiety far outweigh the effects these drugs have on the neural substrates underlying reward. However, the Lewis strain of rat appears less susceptible to the effects of cannabinoids on neural substrates involved in anxiety that allows the effects of cannabinoids on the neural substrates of reward to be unmasked. The following chapter will test this hypothesis by observing if any genetic differences exist in the effects of CP 55,940 on *c-fos* expression in areas of the brain implicated in reward (for example, the NAS and VTA) or anxiety (for example, the CEA or PVN).