CHAPTER FIVE

Effects of pre-exposure and coadministration of CP 55,940 on behavioural sensitization to cocaine in Lewis and Wistar rats

5.1. Introduction.

Chapters 2, 3 and 4 may help to explain why Lewis rats are the only rat strain so far tested to show a rewarding effect of Δ^9-THC on MFB self-stimulation (Lepore et al., 1996a). The present chapter provides research on a behavioural assay of the addictive potential of cannabis. Furthermore, it provides an animal model of the "gateway hypothesis", that is, the notion that prior exposure to cannabis increases an individual's vulnerability to using other drugs of abuse (Robinson & Berridge, 1993) (see section 1.7.).

Sensitization of mesolimbic DA efflux during repeated intermittent exposure to drugs of abuse has been hypothesized to be a key neural adaptation underlying the development of pathological drug craving (Robinson & Berridge, 1993; Stewart & Badiani, 1993). The most widely used behavioural index of such sensitization is the progressively greater locomotor activity response elicited in rats and mice to repeated administration of drugs of abuse. As discussed in section 1.7., recent studies support the involvement of behavioural sensitization in increasing the drug-seeking behaviour of rodents (De Vries et al., 1998; De Vries et al., 1999; Mendrek et al., 1998; Vanderschuren et al., 1999). Furthermore, behavioural sensitization to amphetamine has been shown to enhance appetitive associative learning (Harmer & Phillips, 1998, 1999a,b). This is significant because associative learning of cues related to drug use is thought to be responsible for the cognitive and behavioural phenomena exhibited by drug abusers that are associated with drug craving and relapse (O'Brien, Childress,

Ehrman, & Robbins, 1998; O'Brien, Childress, McLellan, & Ehrman, 1992, 1993; Robbins, Ehrman, Childress, Cornish, & O'Brien, 2000; Robbins, Ehrman, Childress, & O'Brien, 1999a). Thus behavioural sensitization may play a pivotal role in the enhanced sensitivity of drug abusers to drug-related cues that help maintain chronic drug use.

Sensitization of locomotor activity has been documented to a wide range of drugs of abuse including nicotine, amphetamine, morphine, heroin, cocaine and phencyclidine (Robinson & Berridge, 1993; Stewart & Badiani, 1993). However, to our knowledge, no study has yet looked at whether similar sensitization may be obtained with cannabinoids. This absence of studies in the literature is difficult to explain in light of the many studies that have documented sensitization to other drugs of abuse. One possible explanation is based on cannabinoids generally producing depressant effects on locomotor activity (Compton et al., 1993; Little et al., 1988), and so it may be thought that the chances of observing cannabinoid-induced increases in locomotor activity, even with repeated exposure, are slight. However, close scrutiny of the literature uncovers some reports of low dose stimulatory effects of cannabinoids on locomotor activity in rodents (Little et al., 1988; McGregor et al., 1996c). Furthermore, it is evident that drugs such as nicotine and bromocriptine, which produce initial locomotor suppression, are still able with repeated administration, to produce increases in locomotor activity that become progressively greater over time (Hoffman & Wise, 1992, 1993; Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994).

An interesting phenomenon that offers a neurobiological explanation of polydrug abuse and the "gateway" hypothesis is cross-sensitization. Cross-sensitization is whereby pre-exposure to one drug increases the incentive motivational, rewarding or locomotor activating effects of another drug (Cunningham, Finn, & Kelley, 1997; Stewart & Badiani, 1993). Using locomotor activity as a dependent variable, cross-sensitization is found between many drugs of abuse, for example, cocaine and amphetamine (Bonate, Swann, & Silverman, 1997; Vanderschuren et al.,

1999), amphetamine and morphine (Cunningham et al., 1997; Vanderschuren et al., 1999; Vanderschuren et al., 1997; Vezina, Giovino, Wise, & Stewart, 1989), morphine and cocaine (Vanderschuren et al., 1999), ethanol and cocaine (Itzhak & Martin, 1999) or amphetamine and phencyclidine (Greenberg & Segal, 1985). Thus, cross-sensitization constitutes one of the ways in which the gateway hypothesis might be tested with respect to cannabinoids.

The current chapter determines whether pre-exposure to a cannabinoid renders an animal hypersensitive to the locomotor activating effects of cocaine. Interestingly, some recent studies show that the endogenous cannabinoid system may be intrinsically linked to reward circuitry. First, the cannabinoid receptor antagonist SR 141716A was shown to block induction of unbiased conditioned place preference to cocaine, morphine or food (Chaperon et al., 1998). Second, a recent study showed that CB_1 receptor stimulation reduced cocaine intake in rats possibly by enhancing the rewarding effects of cocaine (Fattore, Martellotta, Cossu, Mascia, & Fratta, 1999). The present study tested whether interactions occur between the effects of cannabinoids and cocaine by observing if cross-sensitization exists between CP 55,940 and cocaine.

Previous studies have shown that Lewis rats show greater behavioural sensitization to cocaine and methamphetamine than other rat strains and this effect has been correlated with greater drug-induced stimulation of extracellular DA levels in the NAS (Camp et al., 1994; Kosten et al., 1994; Ortiz et al., 1995). Furthermore, evidence suggests that cannabinoids may be acutely rewarding in Lewis rats, with Δ^9-THC facilitating brain stimulation reward (Gardner & Lowinson, 1991; Lepore et al., 1996) and increasing the release of DA from the NAS (Chen et al., 1991; Tanda et al., 1997). However, the question of whether the effects of cannabinoids on Lewis rats become sensitized over repeated cannabinoid exposure has never been answered and therefore will be tested in the present investigation.

Repeated exposure to stress or corticosterone has been shown to sensitize rats to the stimulant properties of drugs such as cocaine or amphetamine (Deroche et al.,

1995; Deroche, Piazza, Casolini, Le Moal, & Simon, 1993; Johnson, Svensson, Engel, & Soderpalm, 1995; Kalivas & Duffy, 1989; Marinelli, Le Moal, & Piazza, 1996; Ortiz et al., 1995; Piazza et al., 1994; Rivet, Stinus, LeMoal, & Mormede, 1989). As stress and corticosterone have been shown to facilitate behavioural sensitization to drugs of abuse it is conceivable that Wistar rats may also be susceptible to behavioural cross-sensitization between CP 55,940 and cocaine. In Chapters 2 and 3 it was shown that Wistar rats treated with CP 55,940 showed more anxiety-related behaviours than Lewis rats. Moreover, in Chapter 4 it was shown that Wistar rats showed heightened CP 55,940-induced *c-fos* expression in stress and anxiety-related areas of the brain, such as the CEA and PVN, in comparison to Lewis rats. The enhanced stressor-like effects of CP 55,940 on Wistar rats is consistent with evidence which demonstrates that cannabinoids activate the HPA-axis and cause the release of adrenal hormones, such as corticosterone (Rodriguez de Fonseca et al., 1997; Rodriguez de Fonseca et al., 1991; Rodriguez de Fonseca et al., 1996; Weidenfeld et al., 1994; Wenger et al., 1997). In addition, chronic administration of CP 55,940 increases CRH gene expression in the PVN of rats (Corchero, Fuentes, & Manzanares, 1999). Thus, if CP 55,940 does act as a stressor in Wistar rats, or it does act to increase the release of corticosterone over days, then repeated exposure to it may provide a mechanism whereby cross-sensitization occurs between CP 55,940 and cocaine.

Rather than producing cross-sensitization, some drugs may actually hinder the development of sensitization to another drug. For instance the following drugs all block the development of behavioural sensitization to cocaine: the inhibitor of neuronal nitric oxide synthase, 7-nitroindazole (Haracz, MacDonall, & Sircar, 1997; Itzhak, 1997); the DA receptor antagonist, haloperidol (Mattingly, Rowlett, Ellison, & Rase, 1996b); and the *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK-801 (Kalivas & Alesdatter, 1993; Kim, Park, Jang, & Oh, 1996; Li et al., 1999; Wolf & Jeziorski, 1993). Furthermore, sensitization to psychomotor stimulants is associated with an altered sensitivity to glutamate in mesolimbic neurons (White, Hu, Zhang, & Wolf,

1995) as well as enhanced glutamate and aspartate release in the NAS (Pierce, Bell, Duffy, & Kalivas, 1996; Reid, Ho, & Berger, 1996; Robinson et al., 1997). Interestingly, cannabinoids, including CP 55,940, inhibit the release of glutamate in cultured hippocampal neurons via a presynaptic G-protein mediated mechanism (Shen et al., 1996). If cannabinoids also inhibit excitatory amino acid transmission in reward-relevant areas such as the VTA and the NAS, then this may provide a mechanism whereby CP 55,940 could prevent behavioural sensitization. The possibility that cannabinoids may prevent the development of behavioural sensitization to cocaine was also tested in the present study by examining whether coadministration of CP 55,940 with cocaine might inhibit behavioural sensitization to cocaine.

In Experiment 5A, to optimise the parameters of drug administration for promoting sensitization, a protocol for producing behavioural sensitization to cocaine was established. In Experiment 5B, the ability of CP 55,940 to sensitize locomotor activity was then tested using a similar protocol. Further tests examined whether pre-exposure to CP 55,940 enhances the locomotor-stimulant effects of cocaine and cocaine-induced behavioural sensitization. Experiment 5B also tests whether chronic pre-exposure to cocaine modulates the effects of an acute dose of CP 55,940. Taken together, Experiment 5B tests cross-sensitization in both the cannabinoid-cocaine and cocaine-cannabinoid directions. Experiment 5C tests whether the coadministration of CP 55,940 and cocaine was able to influence the development of cocaine-induced behavioural sensitization.

5.2. Experiment 5A. Behavioural sensitization to cocaine in Lewis and Wistar rats

5.2.1. Methods

5.2.1.1. Subjects. A total of 32 rats were used as subjects. The rats were aged 90 days at the time of testing. Sixteen subjects were male Albino Wistar rats (SPF, Sydney, Australia) while the other 16 were male Lewis rats (ARC, Perth, Australia). The rats were group housed (8 per box) with each strain housed separately. All rats were maintained on a 12 h reversed light-dark cycle (light off at 8.30 am) with food and water available *ad libitum*. All testing occurred during the dark cycle. All rats were handled for 2 min per day on each of the four days prior to the start of the experiment.

5.2.1.2. Apparatus. Locomotor activity was measured in eight Coulbourn operant cages as previously described in Chapter 4 (see section 4.2.1.3.1.).

5.2.1.3. Procedure. The procedure involved three different phases. Phase 1 was a "habituation" phase in which the rats were placed in the testing chambers in the drug-free state for 1 h on two consecutive days. This allowed the dissipation of exploratory behaviour in the novel context prior to drug testing. In the second "sensitization " phase, starting 48 h after the final habituation session, the rats were injected with either cocaine (15 mg/kg) or saline before being placed in the activity cages for 60 min. This procedure was repeated every 48 h to give a total of 7 sensitization sessions. Eight rats from each strain received cocaine during this phase while the other 8 received saline. In the final "weekly probe" phase, the duration of drug induced sensitization was examined by re-testing activity for 60 min following cocaine (15 mg/kg) or saline injection at 1 week and 2 weeks following the final test of the "sensitization" phase. This allowed determination of the persistence of the sensitization phenomenon in both strains, often thought to be long-lasting (Robinson & Berridge, 1993).

5.2.1.4. Drug. Cocaine hydrochloride (Australian Pharmaceutical Industries, Sydney) was dissolved in 0.9% saline and injected i.p. at a dose of 15 mg/kg in a volume of 1 ml/kg.

5.2.1.5. Data Analysis. Activity counts were totaled within each 60 min test to give a single activity count for each rat for each test. Planned contrasts compared the drug-free locomotor activity across groups (cocaine versus vehicle) within each strain on each of the two habituation days to ensure no baseline differences in activity prior to drug administration. The occurrence of sensitization in each strain was assessed using a repeated measures ANOVA where group (cocaine versus saline) was the factor and day (days 3 versus 15) was the repeated measure. This analysis was performed separately for each strain. The group by day interaction allowed a comparison of the activity elicited by cocaine with that elicited by vehicle on the first versus the last day of the sensitization phase. To assess the persistence of sensitization, data for the one week and two week probe tests were again compared with activity on the first day of the sensitization phase (day 3) for vehicle versus cocaine groups within each strain. A significance level of $p < 0.05$ was adopted for all tests.

5.2.2. Results

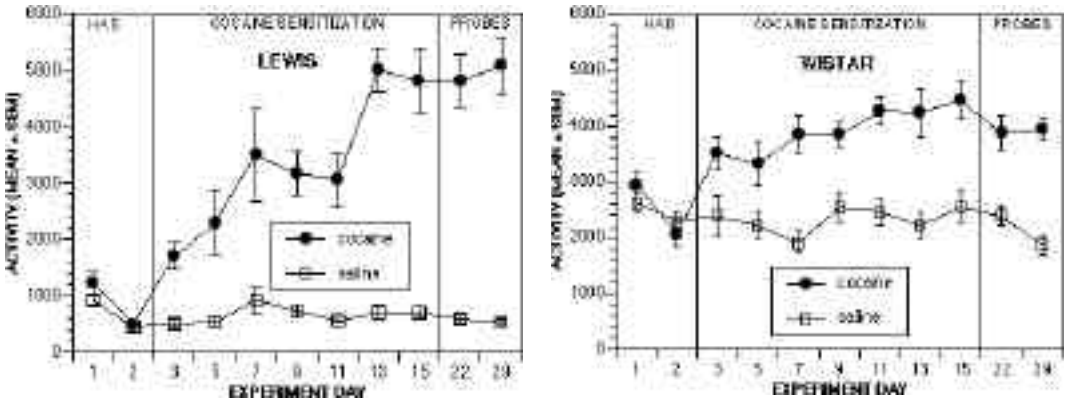

Figure 5.1. Behavioural sensitization to 15 mg/kg of cocaine in Lewis (left) versus Wistar rats (right). Mean locomotor activity scores are shown across the three experimental phases for the groups administered either cocaine or saline (n=8 per group).

5.2.2.1. Habituation phase. The locomotor activity data for Experiment 5A are shown in Figure 6.1. On both habituation days there were no significant differences in locomotor activity between experimental groups (cocaine versus saline) in either strain of rat (F*s* < 1.60). However, Wistar rats were more active than Lewis rats during the habituation phase.

5.2.2.2. Sensitization phase. When comparing activity on the first and seventh day of cocaine treatment, a significant group by day interaction was found for the Lewis strain [F(1,14) = 36.17, $p < 0.001$]. However, the same interaction effect was not significant in the Wistar strain [F(1,14) = 2.41, $p = 0.14$].

5.2.2.3. Weekly probes. A significant group by day interaction in the Lewis rats was also seen when comparing activity on the first cocaine day with activity on the first weekly probe test [F(1,14) = 41.71, $p < 0.001$]. There was no such effect in the Wistar rats (F < 1). A significant group by day interaction was also evident on the second probe test in the Lewis rats [F(1,14) = 63.18, $p < 0.001$] and the Wistar rats [F(1,14) = 4.95, $p < 0.05$]. This significant Wistar result appeared to reflect a drop off in activity in the vehicle group, and not from any increase in responsivity to cocaine in the cocaine group (see Figure 6.1.).

5.3. Experiment 5B. CP 55,940 pre-exposure and behavioural sensitization to cocaine

5.3.1. Methods

5.3.1.1. Subjects. The subjects were a total of 64 rats consisting of 32 male Wistar rats aged 65 days and 32 male Lewis rats aged 67 days. These strains were obtained from the same sources as described in Experiment 5A. Rats were housed under identical conditions to Experiment 5A and each rat was handled for at least 2 min daily for five days prior to the start of the experiment.

5.3.1.2. Apparatus. The same apparatus was used as in Experiment 5A. The apparatus is described in section 4.2.1.3.1.

5.3.1.3. Procedure. Rats from each strain were randomly allocated to 4 different treatment groups (n=8 per group). These were to receive either vehicle or CP 55,940 at three different dose levels (10, 25 or 50 µg/kg). Doses of CP 55,940 were chosen so that the highest dose (50 µg/kg) had a clear locomotor depressant effect in Wistar rats (see Chapter 4), while the lowest dose had the possibility of producing locomotor stimulation (McGregor et al., 1996c). Due to the large number of subjects, rats were tested on alternate days in two separate replicates of 32 rats. Strain and drug condition were equally represented within each replicate. The running of replicates on alternate days meant that for each rat, each test session in each phase was separated by 48 h. The time of day of testing was counterbalanced across strain and treatment groups to control for possible time of day effects on activity.

The experimental procedure involved six different phases as follows. Phase 1 ("habituation") consisted of a single habituation test lasting 60 min where rats were placed in the testing cages in the absence of any drug treatment. In phase 2 ("CP 55,940 phase"), starting 48 h later, rats were injected with doses of CP 55,940 or vehicle according to their experimental group. 5 min post-injection they were placed in the activity cages for a 60 min test. Ten such sessions were performed, one session every 48 h. In phase 3 ("conditioning test 1") all rats were injected with vehicle 5 min

before activity testing. This phase was included to assess whether any locomotor stimulant or depressant effects of CP 55,940 could be conditioned to the test environment in which the drug state was experienced, as has been shown for many other drugs of abuse (Stewart & Badiani, 1993). In phase 4 ("cocaine phase") all rats were injected with 15 mg/kg of cocaine 5 min prior to testing in the activity cages. This was repeated every 48 h for a total of six sessions. The first of these cocaine injections served as a test to see whether rats pre-exposed to various doses of CP 55,940 would show a different locomotor response to cocaine than rats pre-exposed to vehicle. The five subsequent tests with cocaine determined whether pre-exposure to cannabinoids could modulate the induction of behavioural sensitization to cocaine. In phase 5 ("conditioning test 2"), all rats were again administered saline and given a single 60 min test. Comparison of this phase with the results of the conditioning test 1 allowed assessment of whether any hyperactivity seen to cocaine would be conditioned to the test environment. In the final phase ("CP 55,940 probe"), the rats were given the same dose of CP 55,940 or vehicle that they received during phase 2 and again tested for 60 min. This phase allowed assessment of whether intermittent exposure to cocaine might modify the locomotor response to CP 55,940.

5.3.1.4. Drugs. CP 55,940 (Pfizer) was prepared in the same way as throughout the thesis (see section 2.2.1.3.). Cocaine hydrochloride (Glaxo) was prepared as in Experiment 5A. All drugs were injected i.p. in an injection volume of 1 ml/kg.

5.3.1.5. Data Analysis. As in Experiment 5A, activity counts were summed within each 60 min test to give a single activity count for each rat for each test. Groups within each strain were compared for their activity in the habituation phase using planned contrasts. These contrasts involved comparing each of the drug groups (10, 25 and 50 µg/kg CP 55,940) with the vehicle group. In this and all subsequent contrasts involving such multiple drug group and vehicle group

comparisons, Bonferroni corrections modified the threshold of statistical significance to $p < 0.0166$.

The occurrence of sensitization to CP 55,940 was assessed by comparing the activity on the first day of the intermittent CP 55,940 phase with that seen on the final day of that phase for the vehicle group versus each of the three CP 55,940 groups in each strain. To determine whether CP 55,940 effects on activity were conditioned to the test environment, the activity data for the vehicle group in phase 3 were compared with data for each of the groups pre-exposed to CP 55,940 within each strain. To determine whether pre-exposure to CP 55,940 altered the initial response to cocaine on the first test of phase 4, planned contrasts were used to compare the activity induced by cocaine in vehicle pre-treated rats with each of the groups of rats receiving pre-treatment with CP 55,940. To assess whether behavioural sensitization to cocaine was different in vehicle compared to CP 55,940 pre-treated rats, the vehicle and CP 55,940 pre-treated groups in each strain were compared for their change in locomotor activity from the first to the sixth cocaine administration in the cocaine phase. To determine whether the cocaine effect on activity was conditioned to the test environment, the activity data in phase 5 were compared with the equivalent phase 3 data for all groups within each strain using planned contrasts. Finally, to determine whether repeated exposure to cocaine modified the response to CP 55,940, the data for phase 6 were compared with the data for the final drug test of phase 2 for each group within each strain.

5.3.2. Results

During Experiment 5B, one Lewis rat from the 50 µg/kg CP 55,940 died for unknown reasons (on the last day of phase 2) and data for this rat were removed from the experiment. In addition, eight data points, two data points per experimental group in the Wistar rats, were lost during phase 3 (conditioning test 1) due to a computer malfunction.

5.3.2.1. Habituation phase. The results for Experiment 5B are shown in Figure 6.2. In the habituation phase, prior to drug administration, there were no significant differences between any of the experimental groups in locomotor activity in either strain (Fs < 1).

5.3.2.2. CP 55,940 phase. Planned contrasts based on a repeated measures ANOVA (comparing the first versus last day of CP 55,940 administration) revealed no significant differences between the 10 µg/kg groups and vehicle groups in either strain. There was a significant main effect when comparing the 25 µg/kg and vehicle groups in the Wistar [$F(1,28) = 52.22$, $p < 0.001$] and Lewis strains [$F(1,27) = 8.94$, $p < 0.01$] but no significant interaction effects. This reflects a global inhibition of activity with the 25 µg/kg dose in both strains that did not vary over time.

The 50 µg/kg groups caused a similar significant overall depression of activity relative to vehicle in both the Wistar [$F(1,28) = 91.89$, $p < 0.001$] and Lewis [$F(1,27) = 15.60$, $p < 0.001$] strains. There was also a significant interaction effect when comparing the Lewis rats given the 50 µg/kg dose with the vehicle group [$F(1,27) = 5.54$, $p < 0.05$]. However, consideration of Figure 6.2. suggests that this effect is due to the activity in the vehicle group decreasing over time as opposed to the activity in the 50 µg/kg group increasing.

5.3.2.3. Conditioning test 1. On the first conditioning test, no significant differences were found between the vehicle group and any of the groups

pre-exposed to CP 55,940 in the Lewis (Fs < 1.4) or Wistar (Fs < 2.1) strains. This suggests that the locomotor depressant effect of CP 55,940 seen in the 25 and 50 µg/kg doses did not condition to the test environment.

5.3.2.4. First day of cocaine phase. In both strains, no significant differences were found between the activity of the vehicle group on first exposure to cocaine and any of the groups given prior exposure to CP 55,940 in either strain (Fs < 1.05). This suggests that intermittent pre-exposure to CP 55,940 does not alter the locomotor activation produced by a moderate dose of cocaine.

5.3.2.5. Intermittent cocaine phase. No significant differences were found in the change in activity of the vehicle group relative to any of the CP 55,940 pre-treated groups from day 1 to day 6 of the cocaine phase in either Wistar (Fs < 1) or Lewis rats (Fs < 1.4). This suggests that intermittent pre-exposure to CP 55,940 does not alter the locomotor effects produced by repeated intermittent exposure to cocaine. However, it should be noted that no overall sensitization to the effects of cocaine was evident when activity on day 1 and day 6 of the cocaine phase were compared for all rats within the Lewis [$F(1,27) = 2.41$, $p = 0.13$] or Wistar ($F < 1$) strains.

5.3.2.6. Conditioning test 2. When activity during conditioning test 2 was compared with activity during conditioning test 1 a significant overall decrease in activity across the two conditioning tests was apparent in the Wistar rats [$F(1,23) = 8.50$, $p < 0.01$]. However, a significant overall increase in activity was apparent in the Lewis strain [$F(1,30) = 60.23$, $p < 0.001$]. When the effects of CP 55,940 on activity were compared with vehicle no significant between-group comparisons were found (Fs < 1).

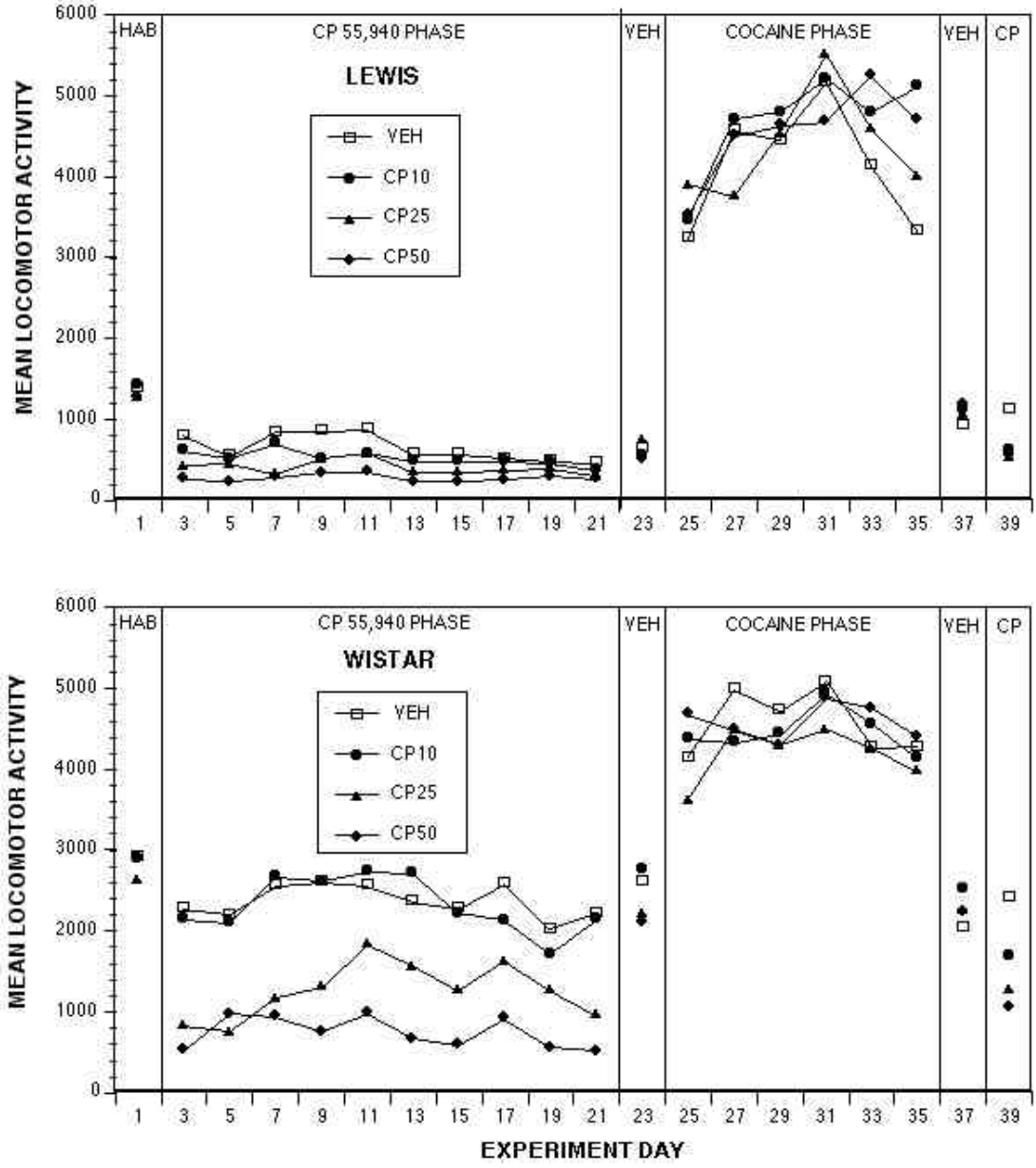

Figure 5.2. Effects of CP 55,940 pre-exposure on the response to intermittent administration of 15 mg/kg of cocaine in Lewis (top) and Wistar (bottom) rats. Mean locomotor activity scores are shown across the six experimental phases for the four groups (n = 8) administered either vehicle (VEH) or 10, 25 or 50 µg/kg of CP 55,940 (CP10, CP25 and CP50 respectively). The six phases from left to right are "HAB" = phase 1 (habituation), "CP 55,940 PHASE" = phase 2 (CP 55,940 phase), "VEH" = phase 3 (conditioning test 1), "COCAINE PHASE" = phase 4 (intermittent cocaine phase), "VEH" = phase 5 (conditioning test 2), and "CP" = phase 6 (CP 55,940 probe). Error bars are omitted for clarity.

5.3.2.7. CP 55,940 probe. When the effects of CP 55,940 on activity were compared with vehicle from the final day of phase 2 to the final CP 55,940 probe test in the Lewis strain, there was a significant overall suppression of activity present in the 10 µg/kg [$F(1,27) = 8.60$, $p < 0.01$], 25 µg/kg [$F(1,27) = 14.51$, $p < 0.001$] and 50 µg/kg [$F(1,27) = 13.96$, $p < 0.001$] groups. There was also a significant group by day interaction in the Lewis rats with the vehicle group showing a greater increase in activity from the first to the second test than the 10 µg/kg [$F(1,27) = 12.46$, $p < 0.01$], 25 µg/kg [$F(1,27) = 14.15$, $p < 0.001$] or 50 µg/kg [$F(1,27) = 9.52$, $p < 0.01$] groups. This reflects the continuation of conditioned hyperactivity in the vehicle-treated rats, an effect that appeared to be blocked by the administration of any of the CP 55,940 doses.

There was also a significant overall suppression of locomotor activity relative to the vehicle group across the two tests in the Wistar rats given 10 µg/kg [$F(1,28) = 8.76$, $p < 0.01$], 25 µg/kg [$F(1,28) = 52.05$, $p < 0.001$] and 50 µg/kg [$F(1,28) = 92.65$, $p < 0.001$] of CP 55,940. However, there were no significant group by day interaction effects (Fs < 1), showing that suppression of activity by drug relative to vehicle was consistent across the two tests.

5.4. Experiment 5C. CP 55,940 and cocaine coadministration in Lewis rats

5.4.1. Methods

5.4.1.1. Subjects. The subjects were 32 male Lewis rats (ARC, Perth, Australia), aged between 75 and 90 days at the time of testing. Rats were housed under identical conditions to Experiment 5A and each rat was handled for at least 2 min daily for five days prior to the start of the experiment. Experiment 5B indicated that cross-sensitization between CP 55,940 and cocaine is unlikely to occur in Lewis or Wistar rats. Thus, in Experiment 5C it was of interest to observe whether CP 55,940 could actually inhibit the development of behavioural sensitization to cocaine. As shown in Experiment 5A, Lewis rats were the only strain to show behavioural sensitization to cocaine. Therefore, Wistar rats could not be used to test the hypothesis that cannabinoids might inhibit the development of behavioural sensitization to cocaine in Experiment 5C.

5.4.1.2. Apparatus. The same apparatus was used as in Experiment 5A and 5B.

5.4.1.3. Procedure. Experiment 5C aimed to test whether the intermittent coadministration of CP 55,940 with cocaine could block the development of behavioural sensitization to cocaine. The experiment consisted of three phases: Phase 1 ("habituation"), phase 2 ("coadministration") and phase 3 ("cocaine probe"). Phase 1 was run over two consecutive days. On these days rats received two saline injections 5 min before being placed in the activity cages for 60 min. Phase 2 ("coadministration") started 24 h later. This phase consisted of seven treatment days spaced 48 h apart in which rats were given either 50 μg/kg of CP 55,940, 15 mg/kg of cocaine, a combination of the two or no drug. Four experimental groups (n=8 per group) were thus employed: saline + vehicle (SAL+VEH), cocaine + vehicle (COC+VEH), saline + CP 55,940 (SAL+CP) and finally cocaine + CP 55,940

(COC+CP). On treatment days rats were injected twice consecutively, according to the experimental conditions above and 5 min later were placed in the activity cages for a 60 min test. Phase 3 ("cocaine probe") began one week after the final coadministration treatment. In this phase, all rats received one injection of 15 mg/kg cocaine before being placed in the activity cages for a 60 min test. This phase assessed the expression of a sensitized response to cocaine that might have occurred as a result of phase 2 treatments.

5.4.1.4. Drugs. The same drugs were prepared as described in Experiment 5B.

5.4.1.5. Data Analysis. As in Experiments 5A and 5B, activity counts were totaled within each 60 min test to give a single activity count for each rat for each test day. Statistical analysis for each experiment involved either one-factor or repeated measures ANOVA with planned contrasts comparing locomotor activity across groups for particular phases of the given experiment. Where appropriate, Bonferroni corrections were used to control the familywise error rate (Keppel, 1991).

5.4.2. Results

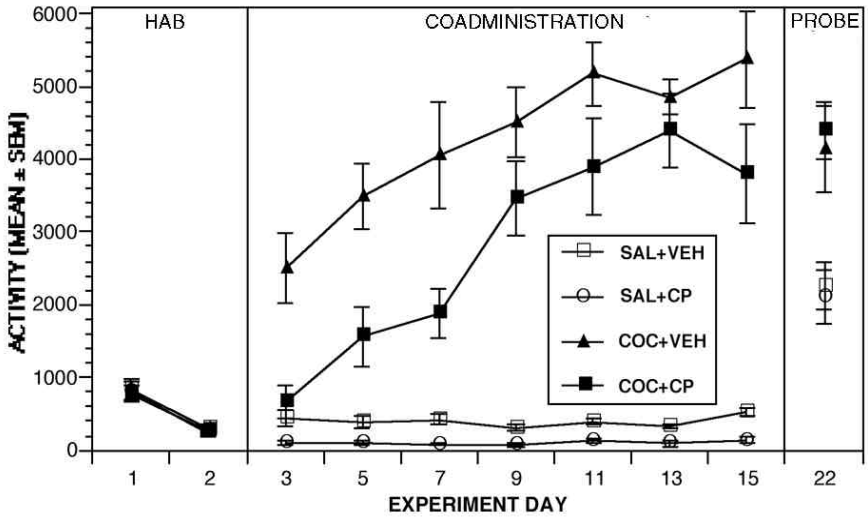

Figure 5.3. Effects of the coadministration of 50 µg/kg of CP 55,940 on behavioural sensitization to 15 mg/kg of cocaine in Lewis rats. Mean locomotor activity scores are shown across the three experimental phases for the four groups administered either saline+vehicle (SAL+VEH), saline+CP 55,940 (SAL+CP), cocaine+vehicle (COC+VEH) and, cocaine+CP 55,940 (COC+CP) (n = 8 per group). The three phases from left to right are "HAB" = phase 1 (habituation phase), "COADMINISTRATION" = phase 2 (coadministration phase), "PROBE" = phase 3 (cocaine probe phase).

5.4.2.1. Habituation phase. The results are displayed in Figure. 6.3. When each of the drug treatment groups (COC+VEH, COC+CP, SAL+CP) were compared with the control group (SAL+VEH) on each of the habituation days, no significant differences were seen (Fs < 1.0).

5.4.2.2. Coadministration phase. Using a repeated measures ANOVA (first versus last day of this phase) with planned contrasts it was found that rats in the COC+CP group [$F(1,28) = 43.22$, $p < 0.01$] or COC+VEH group [$F(1,28) = 168.29$, $p < 0.01$] showed greater overall levels of locomotor activity than the SAL+VEH control group. Rats in the COC+VEH group showed greater overall locomotor activity than rats given COC+CP [$F(1,28) = 40.94$, $p < 0.01$]. SAL+CP administration did not significantly suppress activity relative to SAL+VEH [$F(1,28) = 1.87$, $p = 0.183$].

Group by day interaction contrasts of the coadministration phase showed that the COC+CP group [$F(1,28) = 10.21$, $p < 0.01$] or the COC+VEH group [$F(1,28) = 8.50$, $p < 0.01$] displayed a significant increment in activity over days compared to the

SAL+VEH group. Thus behavioural sensitization was induced by these treatments. When comparing the COC+VEH group to the COC+CP group no significant difference was found ($F < 1$). Finally there was no difference between rats administered SAL+CP compared to the SAL+VEH group in terms of locomotor activity over days ($F < 1$).

5.4.2.3. Cocaine probe. One-factor ANOVA with planned contrasts revealed Lewis rats pre-treated with COC+CP [$F(1,28) = 12.18$, $p < 0.01$] or COC+VEH [$F(1,28) = 9.42$, $p < 0.01$] showed significantly greater activity to 15 mg/kg cocaine on the final probe test than rats pre-exposed to SAL+VEH. No significant differences were found between the COC+CP group and the COC+VEH group [$F(1,28) = 1.78$, $p = 0.68$], or between the SAL+CP and the SAL+VEH group ($F < 1$). The latter result confirms the finding of Experiment 5B, that pre-exposure to CP 55,940 does not sensitize Lewis rats to the subsequent effects of cocaine.

5.5. Discussion

The first finding of the present chapter is that the cannabinoid receptor agonist, CP 55,940, does not produce a sensitization of locomotor activity when administered under a regime that produces clear sensitization of the locomotor response to cocaine. The clear sensitization of activity to cocaine seen in the present study, particularly in the Lewis strain of rat, confirms previous findings (Camp et al., 1994; Kosten et al., 1994; Ortiz et al., 1995). This appears to be relatively long-lasting since it remained at asymptotic levels in Lewis rats even two weeks following the final day of the sensitization phase. In addition, Lewis rats showed strong environmental conditioning of this locomotor response to cocaine as indexed by higher post-cocaine activity in the test environment relative to pre-cocaine activity. This finding is consistent with the suggestion that rats more vulnerable to addiction display stronger environmental conditioning of drug-induced hyperactivity (Jodogne et al., 1994).

Behavioural sensitization to cocaine was less impressive in Wistar rats, when compared to Lewis rats, with the effect failing to reach significance in Experiments 5A or 5B in this strain. Notably, in both experiments and in Chapter 4 (see section 4.2.2.3.), Wistar rats showed much greater baseline locomotor activity than Lewis rats, and it is conceivable that clear sensitization of the locomotor response to cocaine was harder to obtain in Wistar rats given this high baseline activity. Lower baseline activity in Lewis rats has been previously reported in comparison with Fischer 344 rats (Chaouloff et al., 1995) and also in Chapter 4 when compared to Wistar rats. It can be noted, however, that despite starting from a much higher baseline, Wistar rats did not reach the asymptotic levels of cocaine hyperactivity achieved by Lewis rats in Experiment 5A. This suggests that despite the differences in baseline activity, Lewis rats are more susceptible to cocaine-induced sensitization as measured by locomotor activity.

One possible concern with the present results is the failure of CP 55,940 to produce an acute increase in locomotor activity in either strain at any of the doses tested. Indeed, both the 25 and 50 µg/kg doses of CP 55,940 caused locomotor

suppression that lasted throughout their administration. The failure of the 10 µg/kg dose to produce increased activity contrasts with a previous report which showed a moderate hyperactive response to a single 10 µg/kg dose of CP 55,940 (McGregor et al., 1996c). However, in this earlier study, the rats were not habituated to the testing apparatus prior to drug administration, and this factor could conceivably explain the difference in responses across studies. In any case, the presence of an initial hyperactive response to a drug has not been generally found to be necessary for behavioural sensitization since nicotine, 7-OH-DPAT, quinpirole and bromocriptine can suppress locomotor activity on first administration but are still able, with repeated administration, to produce hyperactivity and behavioural sensitization (Hoffman & Wise, 1992, 1993; Mattingly et al., 1996a; Rowlett, Mattingly, & Bardo, 1995; Shoaib et al., 1994).

Certain treatments, such as intra-VTA injection of amphetamine, or systemic apomorphine may fail to alter locomotor activity with repeated exposure yet after this treatment rats are sensitized to the subsequent effects of peripherally administered cocaine, amphetamine or morphine (Kalivas & Weber, 1988; Vezina & Stewart, 1990). Such a "latent" effect, however, was not observed in the current experiments with CP 55,940, since repeated intermittent treatment did not cause subsequent enhanced sensitivity to the locomotor-activating effects of cocaine. A possible objection here might be that the dose of cocaine used was too high to show relative differences between groups, producing robust hyperactivity in all rats. However, it is clear from the results of Experiment 5A that the activity produced by the first dose of cocaine in Experiment 5B still left ample headroom for a larger hyperactive response to be seen, should it have been present. It is also worth noting that an earlier pilot study, published in abstract form, failed to find any differential hyperactivity to a lower dose of cocaine (5 mg/kg) in rats given multiple intermittent pre-exposures to CP 55,940 or vehicle (McGregor, Bryant, & Arnold, 1995).

An apparent difference was observed in the response to the first injection of cocaine in Lewis rats in Experiment 5B compared to Experiment 5A. In Experiment

5B, when the rats were given cocaine after pre-treatment with ten doses of either vehicle or CP 55,940, there appeared to be a greater locomotor response to the drug than in Experiment 5A, when cocaine was given immediately after habituation. It can only be presumed that the stress of repeated injection may have produced a mild sensitization to the effects of cocaine in all animals, regardless of whether they were pre-treated with CP 55,940 or vehicle.

It is also noteworthy that rats given repeated exposure to cocaine in Experiment 5B, still displayed a dose-dependent suppression of activity when subsequently re-tested with CP 55,940. If cocaine pre-exposure were to sensitize the behavioural response to CP 55,940, then a hyperactive response to CP 55,940 might have been expected. Thus, cross-sensitization does not appear to exist in either the cocaine-cannabinoid or cannabinoid-cocaine direction. These results argue against recent suggestions that cannabis use may promote vulnerability to the effects of drugs such as cocaine or heroin (Rodriguez de Fonseca et al., 1997; Tanda et al., 1997; Wickelgren, 1997). On the basis of the present results reported here with CP 55,940, it appears that cannabinoids may have atypical effects in comparison to other drugs of abuse, where pre-exposure to one drug of abuse can enhance sensitization and vulnerability to the reinforcing effects of another drug of abuse. Obviously, this can not be accepted unequivocally without further research which tests whether other cannabinoid receptor agonists are able to produce sensitization or cross-sensitization to the rewarding effects of other drugs of abuse.

A further point to mention is the apparent lack of clear behavioural sensitization to cocaine in the rats given cocaine after CP 55,940 administration in Experiment 5B. This is in contrast to the clear behavioural sensitization seen with the same dose of cocaine in Experiment 5A. There are at least two points to note here. One is that a significant overall cocaine sensitization effect is evident in Lewis rats when activity data for the fourth rather than the sixth cocaine administration of the cocaine phase are compared with the first day of administration. Thus the problem is more that activity went up over the first few sessions of the cocaine phase but then came down again in

the later sessions. This pattern has been previously noted in Lewis rats given 15 mg/kg of cocaine (Kosten et al., 1994). One other thing to note is that by the time the rats had been given cocaine, they had been exposed to the chamber on twelve previous occasions in the absence of cocaine. To the extent that behavioural sensitization depends upon classical conditioning of hyperactivity to environmental cues (Stewart & Badiani, 1993), it might be expected that repeated exposure to the environment in the absence of cocaine would engage processes of latent inhibition that would make subsequent behavioural sensitization to cocaine more difficult to observe.

One other speculative hypothesis to explain the results of Experiment 5B invokes the possible inhibitory effects of cannabinoids on glutamatergic function. It is well known that NMDA receptor antagonists block the development of behavioural sensitization to a variety of abused drugs (Kalivas & Alesdatter, 1993; Wolf, 1998; Wolf & Jeziorski, 1993), and evidence also shows that sensitization to psychomotor stimulants is associated with an altered sensitivity to glutamate in mesolimbic neurons (White et al., 1995) as well as enhanced glutamate and aspartate release in the NAS (Pierce et al., 1996; Reid et al., 1996; Robinson et al., 1997). It has been shown that cannabinoids, including CP 55,940, inhibit the release of glutamate in cultured hippocampal neurons via a presynaptic G-protein mediated mechanism (Shen et al., 1996). Taken together, these findings suggest a mechanism whereby CP 55,940 may prevent behavioural sensitization through an inhibition of glutamate release in the NAS and VTA. However, the results of Experiment 5C, do not support this position with behavioural sensitization clearly observed in Lewis rats intermittently injected with CP 55,940 plus cocaine over days. The development of enhanced locomotor activity to cocaine was partially masked in rats treated with cocaine plus CP 55,940 but as soon as the CP 55,940 was removed, a normal sensitized response to cocaine became apparent.

The present data invite speculation as to the possible neural mechanisms that may prevent the response to CP 55,940 being sensitized. One possibility is that the doses of CP 55,940 here failed to increase DA efflux in the NAS. On one hand this

may be true as it has also been demonstrated that CP 55,940 reduced electrically-evoked DA release in rat striatal slices (Cadogan, Alexander, Boyd, & Kendall, 1997). However, this conflicts with other findings using *in vivo* microdialysis studies that showed that doses of peripherally administered Δ^9-THC (0.5 mg/kg) increased DA efflux in the NAS of Lewis rats (Chen et al., 1991; Gardner & Lowinson, 1991) and that systemically injected Δ^9-THC or WIN 55,212-2 promoted the release of DA in the shell of the NAS (Tanda et al., 1997). Given estimates that CP 55,940 is approximately 30 times more potent than Δ^9-THC (Gold et al., 1992; Little et al., 1988; Wiley, Barrett, Lowe, Balster, & Martin, 1995), it would be expected that both the 25 and 50 µg/kg doses of CP 55,940 would be well within the dose range expected to increase DA levels (see section 2.2.1.3.). Thus, at the very least it seems acute cannabinoid administration does modulate DA in the striatum. However this may not be sufficient to produce behavioural sensitization. Future studies could assess whether intermittent administration of CP 55,940 produces neurochemical sensitization as measured by a progressive increase in DA release from the NAS over days.

In Chapters 2, 3 and 4 it was shown that Lewis rats were less vulnerable to the anxiogenic effects of CP 55,940 than Wistar rats. In light of this it was hypothesised that Wistar rats may also be susceptible to CP 55,940-induced behavioural sensitization. That is, anxiety may coincide with the stress response and the subsequent release of adrenal hormones such as corticosterone. Stress and corticosterone release have been shown to have a role in drug-induced sensitization (Deroche et al., 1995; Johnson et al., 1995; Prasad, Sorg, Ulibarri, & Kalivas, 1995; Prasad, Ulibarri, Kalivas, & Sorg, 1996; Rivet et al., 1989). Indeed, cannabinoids, like stress, are well known to increase corticosterone release (Rodriguez de Fonseca et al., 1997; Rodriguez de Fonseca et al., 1991; Rodriguez de Fonseca et al., 1996; Weidenfeld et al., 1994; Wenger et al., 1997). However, it is not known whether the doses of CP 55,940 used here actually increase corticosterone levels, since other studies of cannabinoid-induced corticosterone release have employed different cannabinoid receptor agonists and doses (Rodriguez de Fonseca et al., 1997;

Rodriguez de Fonseca et al., 1991; Rodriguez de Fonseca et al., 1996; Weidenfeld et al., 1994; Wenger et al., 1997). One recent study shows that chronic administration of 1 mg/kg of CP 55,940 increases the expression of CRH mRNA levels in the PVN of rats (Corchero et al., 1999). However, this dose is much larger than used in the current study (10, 25 and 50 µg/kg) and those used by humans. Future research could test whether CP 55,940 affects the release of corticosterone or the expression of CRH mRNA in the PVN at ecologically relevant doses like those employed in the present chapter.

The current chapter may provide a useful explanation of why rodents do not display cannabinoid self-administration. In rats, a reliable and valid model of cannabinoid self-administration has never been reported. However, a recent landmark study indicates that this assertion may be short-lived (see section 1.5.5.). This study demonstrated that mice acquire self-administration of the cannabinoid receptor agonist, WIN 55,212-2 (Martellotta et al, 1998). While acquisition of self-administration was demonstrated in this study, the ongoing maintenance of this self-administration behaviour was not tested. Sensitization is thought to be a long-lasting neuroadaptation that occurs with the chronic intake of drugs of abuse and may provide a mechanism for "craving", the persistent nature of drug dependence, and the high rate of drug relapse observed in abstaining addicts (Robinson & Berridge, 1993). While the phenomenon of sensitization and its relationship to the maintenance of drug-seeking behaviour is controversial, the current findings may explain why WIN 55,212-2 may not maintain the self-administration habit over days. Future studies need to attempt to train mice to self-administer cannabinoids voluntarily over days, as this is the most ecologically valid and reliable model of human drug dependence and self-administration.

A recent study indicates that cross-sensitization may occur between cannabinoids and other drugs of abuse. This study showed that chronic pretreatment with Δ^9-THC sensitized the stimulatory effects of amphetamine on locomotor activity and stereotypy (Gorriti et al., 1999). The discrepancy between the study by Gorriti *et al* (1999) and the current study may be based on obvious methodological differences

between these studies. These differences include: 1) Δ^9-THC and amphetamine were administered rather than CP 55,940 and cocaine, 2) a higher dose of cannabinoid was used in the Gorriti study (6.4 mg/kg of Δ^9-THC) than in the current study (10, 25 and 50 µg/kg of CP 55,940), 3) the cannabinoid was administered every 24 hours in the Gorriti study rather than every 48 hours as in the current study, and 4) an amphetamine probe was administered 24 or 48 hours after the final Δ^9-THC injection, whereas a cocaine probe was administered 96 hours after the final injection of CP 55,940 in Experiment 5B of the current study.

To conclude, the present study provides presumptive evidence to challenge the prevalent notion that cannabinoids act as gateway drugs. Obviously, this demonstration is not definitive and many future studies need to be conducted. In light of the recent work of Gorriti and colleagues (1999) (see section 1.7.) it seems particularly important to test other cannabinoids, such as Δ^9-THC, using similar procedures to the current investigation. Also, it would be important to test cross-sensitization between cannabinoids and drugs of abuse other than cocaine, such as amphetamine, heroin, nicotine and alcohol, all of which have a different constellation of neural effects. In addition, this work may be extended to the drug self-administration paradigm, to see whether cannabinoid pre-exposure affects self-administration of opiates or psychomotor stimulants.