Pre-exposure to the cannabinoid receptor agonist CP 55,940 enhances morphine behavioral sensitization and alters morphine self-administration in Lewis rats

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Abstract

Three experiments examined the influence of pre-exposure to the cannabinoid receptor agonist CP 55,940 ((−)-cis-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-trans-4-(3-hydroxypropyl)cyclohexanol) on the sensitization of morphine-induced locomotor hyperactivity and self-administration in Lewis rats. In Experiment 1, rats received daily injections of vehicle or CP 55,940 (0.1 mg/kg for 7 days then 0.2 mg/kg for a further 7 days). Four weeks later, the locomotor response to morphine (10 mg/kg s.c.) was tested once per day over a 3-h period for 14 consecutive days. Rats given morphine showed hypoactivity during the first hour following morphine but hyperactivity during the second and third hours. A progressive increase in hyperactivity to morphine was seen over the 14 days of administration, which was significantly greater in rats pre-treated with CP 55,940. In Experiment 2, rats were given morphine (10 mg/kg) once a day for 14 days in combination with either vehicle, CP 55,940 (0.1 mg/kg) or the cannabinoid CB_1 receptor antagonist SR 141716 (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride) (3 mg/kg). Both CP 55,940 and SR 141716 initially inhibited the hyperactive response to morphine, but these effects gradually wore off and by the end of 14 days, hyperactivity was similar in all morphine-treated groups. When tested 3 weeks later for their response to morphine (10 mg/kg) given alone, rats previously given the morphine/CP 55,940 combination, but not the SR 141716/morphine combination, showed a greater locomotor stimulation than those previously exposed to morphine only. In Experiment 3, rats were pre-exposed to CP 55,940 or vehicle for 14 days and were subsequently trained to self-administer morphine intravenously (1 mg/kg per lever press) for 14 days. Rats pre-exposed to CP 55,940 self-administered a significantly greater number of morphine infusions than vehicle pre-exposed rats. However, both active and inactive ('dummy') lever presses were increased by cannabinoid pre-treatment. Overall, these results suggest that cannabinoid pre-exposure can lead to an exaggeration of morphine-induced hyperactivity and may alter the reinforcing effects of morphine in Lewis rats. The implications for 'gateway' theories of cannabinoid effects in humans are discussed.
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1. Introduction

Throughout recorded history, cannabinoids and opioids have been among the most widely used recreational and medicinal drugs. However, it is only recently that the similarities, differences and interaction between these two drug classes have been intensively studied. This research has highlighted a mutual interdependency between the cannabinoid and opioid systems of the brain (Manzanares et al., 1999), which has been particularly manifest with respect to drug reward. Opioid antagonists block the reinforcing properties of cannabinoids in the self-stimulation, conditioned place preference and self-administration paradigms (Braida et al., 2001a,b; Gardner and Vorel, 1998; Navarro et al., 2001). Conversely, the cannabinoid receptor antagonist SR 141716 (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride) reduces the self-administration of opioids in rats and mice (Navarro et al., 2001) and prevents conditioned place preference to opioids in rats (Chaperon et al., 1998). Further, self-administration of

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morphine is reduced in CB_1 receptor knockout mice (Ledent et al., 1999). The neurochemical basis of such effects may involve cannabinoid–opioid interactions on dopamine release in reward relevant pathways (Melis et al., 2000; Tanda et al., 1997).

Prolonged cannabinoid treatment alters opioid receptor binding, opioid gene expression and levels of endogenous opioids and may alter sensitivity to opioid ligands (Manzanares et al., 1999). Previous studies have demonstrated cross-tolerance between chronic cannabinoid and opioid treatment. For example, rats that are pre-exposed to cannabinoids may show a blunted analgesic response to morphine and vice versa (Massi et al., 2001; Smith et al., 1994). However, more recently, the converse phenomenon of cross-sensitization has also been demonstrated. For example, rats pre-exposed to Δ^9-tetrahydrocannabinol or the synthetic cannabinoid receptor agonist WIN 55,212-2 showed a heightened locomotor response to morphine or heroin (Cadoni et al., 2001; Lamarque et al., 2001; Pontieri et al., 2001a). Similarly, rats pre-exposed to morphine showed a heightened locomotor response to WIN 55,212-2 (Pontieri et al., 2001b). These cross-sensitization phenomena are of some significance because they reflect on the enduring controversy surrounding the so-called 'gateway hypothesis' (Fergusson and Horwood, 2000). The claim that cannabis use sensitizes humans to the addictive properties of 'harder drugs' such as heroin gains some credence with the demonstration of such cannabinoid–opioid cross-sensitization effects.

In the present study, we further examined the cross-sensitization between cannabinoids and opioids. In the first experiment, rats were chronically exposed to the synthetic cannabinoid receptor agonist CP 55,940 and the locomotor response to morphine was subsequently examined. CP 55,940 has very similar properties to the prototypical cannabinoid agonist Δ^9-tetrahydrocannabinol but is more potent (Gold et al., 1992). It was predicted that CP 55,950 would lead to an increase in the locomotor response to morphine.

Combinations of opioids and cannabinoids have been sometimes found to have synergistic effects with respect to analgesia and appetite (Kirkham and Williams, 2001; Rowland et al., 2001; Welch and Eads, 1999). It was of interest to determine whether they might produce a synergistic sensitization effect. Therefore, in a second experiment, the behavioral sensitization resulting from the co-administration of CP 55,940 and morphine was examined.

The ability of the cannabinoid antagonist SR 141716 to delay the progression of behavioral sensitization to morphine was also assessed. This would indicate a major role for the cannabinoid system in the development of behavioral sensitization to opioids. Such a role has been suggested by the observation that cannabinoid CB_1 knockout mice show normal morphine-stimulated locomotor activity but no sensitization of these locomotor effects with repeated morphine treatment (Martin et al., 2000).

In the third and final experiment, the impact of CP 55,940 pre-exposure on morphine self-administration was examined. It was predicted that prior exposure to CP 55,940 would facilitate the acquisition of morphine self-administration in rats.

In all experiments, Lewis rats were used as this strain may be particularly sensitive to the reinforcing effects of cannabinoids and other drugs (Arnold et al., 2001; Gardner and Vorel, 1998; Lepore et al., 1996).

2. Method

2.1. Subjects

Male Lewis rats aged 55–56 days (Animal Resource Centre, Perth) were used in Experiments 1 and 2 (32 per experiment). Rats weighed approximately 210 g at the beginning of each experiment and were housed in groups of eight in large polypropylene tubs lined with woodchips. Rats were maintained on a 12-h reverse light–dark cycle (lights off at 0900 h). Sixteen 104- to 126-day-old male albino Lewis rats weighing approximately 350 g were used in Experiment 3. These rats were individually housed and maintained on a 12-h conventional light–dark cycle (lights off at 1700 h). Behavioral testing was conducted during the dark phase and rats were given ad libitum access to food and water. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996. All efforts were made to minimize the number of animals used and their suffering. Ethics approval for all experiments was obtained from the Sydney University Animal Ethics Committee.

2.2. Apparatus

Eleven standard operant chambers (250 mm × 310 mm × 500 mm high) were used to detect locomotor activity. The chambers had aluminum sides and tops while the front and back walls were made of Plexiglas. The floor was constructed of 16 metal rods (6 mm diameter) spaced 15 mm apart. In Experiments 1 and 2, the chambers were placed on shelves, which were enclosed by black curtains hanging from the ceiling to the floor. Passive infrared detectors (Quantum passive infrared motion sensor, part no. 890-087-2, NESS Security Products, Australia) were positioned in the center of each side wall approximately 10 mm above the floor. The passive infrared detectors were capable of detecting relatively small movements of the rats' head and body. Passive infrared detector activity counts were recorded by a Macintosh computer running WorkbenchMac software for data acquisition (McGregor, 1996). The room was kept dark throughout the experiment.

In Experiment 3, the operant chambers were housed in sound-attenuating boxes (600 mm × 580 mm × 670 mm

high) equipped with a fan which provided ventilation and masking noise. Each chamber was equipped with two 50-mm-wide retractable levers (Med Associates, part ENV112-BM) on the right hand wall, spaced 110 mm apart and situated 60 mm above the floor. Depression of one of the levers (the active lever) resulted in a 0.05-ml infusion of morphine over 2.5 s followed by the illumination of a cue light (situated 50 mm above the lever) indicating a 20-s time-out period. During the time-out period, depression of either lever had no scheduled consequences. Depression of the other lever (the dummy lever) had no consequences at any time.

The drug infusion system consisted of an infusion pump (Med-PC, VT, USA), 10 ml syringe and 23-gauge cut off needle connected to Tygon tubing (Daigger, IL, USA). The tubing was connected to a fluid swivel assembly (Instech, PA, USA) and PE50 tubing (Plastics One, VA, USA) threaded through a spring connector (CG313, Plastics One). At 20 mm from the base of the spring connector, the spring was separated and the tubing exited to insert into an intravenous catheter with 23-gauge hypodermic tubing connector (10 mm long), while the spring connector was attached to a head mount.

2.3. Drugs

CP 55,940 ((−)-*cis*-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-*trans*-4-(3-hydroxypropyl)cyclohexanol, Tocris, UK) and SR 141716 (Sanofi Recherche) were dissolved in ethanol. Tween 80 and saline were then added to produce a final solution of 5% ethanol, 5% Tween 80 and 90% saline. Control rats were given equivalent injections of vehicle. CP 55,940 and SR 141716 were both administered intraperitoneally (i.p.) at a dose of 0.1 or 0.2 mg/kg (CP 55,940) or 3 mg/kg (SR 141716).

Morphine hydrochloride (Australian Pharmaceutical Industries, Sydney) was dissolved in 0.9% saline and was administered subcutaneously (s.c.) at a dose of 10 mg/kg in a volume of 1 ml/kg body weight in Experiments 1 and 2. In Experiment 3, the morphine solution was filtered through Whatman filter paper (Whatman, 90 mm, Qualitative 1, Maidstone, Kent) and was delivered in a dose of 1 mg/kg body weight per 0.05 ml infusion.

2.4. Procedure

2.4.1. Experiment 1: CP 55,940 pre-exposure and behavioral sensitization to morphine

There were three phases in this experiment; cannabinoid pre-exposure (14 days), a drug-free interval (28 days) and a morphine cross-sensitization test (14 days).

2.4.1.1. Cannabinoid pre-exposure. Each day, rats were given injections 20 min before being placed in the locomotor test chambers for 60 min. The first 2 days of this phase were habituation days in which all of the rats were injected

with saline. This allowed the rats to become familiar with both the apparatus and procedures thus reducing novelty-induced activity on the drug test days. Rats were allocated into either the CP 55,940 or the vehicle condition so that body weights and activity levels during the habituation days were matched. Individual rats were placed in the same activity box throughout all phases of the experiment to avoid any interference from contextual changes. Injections in this and subsequent experiments were given in a different room to the behavioral testing 10 min before the rats were placed in the test chambers.

CP 55,940 was given at a dose of 0.1 mg/kg for the first week of testing and 0.2 mg/kg for the second week. The doubling of the dose was used to overcome well-documented tolerance to the effects of this drug with repeated exposure (Rubino et al., 1994).

2.4.1.2. Drug-free interval. Rats remained in their home cages and no drugs were administered for 28 days after the pre-exposure phase. This drug-free period ensured complete drug washout and is also thought to facilitate subsequent detection of cross-sensitization effects (Vanderschuren et al., 1997).

2.4.1.3. Morphine cross-sensitization test. Rats in each of the CP 55,940 (CP) and vehicle (V) conditions were subdivided into Morphine (M) or Vehicle (V) groups resulting in four groups ($n = 8$ per group); CP 55,940 pre-exposed and tested with morphine (CP-M), CP 55,940 pre-exposed and tested with vehicle (CP-V), vehicle pre-exposed tested with morphine (V-M) and vehicle pre-exposed tested with vehicle (V-V). Group allocations were made such that both activity levels on day 14 of the pre-exposure phase and body weights were matched across groups.

Rats received either 10 mg/kg morphine or vehicle (s.c.) 20 min before being placed in the activity chambers for 180 min. This session duration was chosen because morphine initially has locomotor depressant effects followed by locomotor stimulatory effects that can be clearly observed 2–3 h post-injection (Babbini and Davis, 1972). Daily tests were given for a total of 14 consecutive days with activity tested on each day.

2.4.2. Experiment 2: cannabinoid and morphine co-administration

There were three phases in this experiment; drug co-administration (14 days), a drug-free interval (14 days) and morphine probes (5 days).

2.4.2.1. Drug co-administration. The first 2 days were habituation sessions where all rats were given two saline injections (one i.p. and the other s.c.) and then placed in the locomotor activity testing chambers 20 min later for 180 min. Rats were then allocated to one of four different groups on the basis of body weight and locomotor activity during

the two habituation sessions. Groups were as follows: CP 55,940 and morphine combined (CP-M), SR 141716 and morphine combined (SR-M), vehicle and morphine combined (V-M), and vehicle and vehicle combined (V-V). The CP 55,940 or SR 141716 (or their vehicle) was injected first followed 10 min later by the morphine (or vehicle) injection. A further 10 min later, the rats were placed in the locomotor activity test cages for 180 min.

2.4.2.2. Drug-free interval. Rats remained in their home cages and no drugs were administered for 14 days after the co-administration phase.

2.4.2.3. Morphine probes. All rats were given an injection of morphine (10 mg/kg s.c.) and placed in the activity chambers for 180 min. This was repeated twice more with an intervening day between treatments. Thus, a total of three morphine injections was administered over a 5-day period.

2.4.3. Experiment 3: Cannabinoid pre-exposure and morphine self-administration

There were two phases in this experiment: cannabinoid pre-exposure (16 days) and morphine self-administration (14 days). Surgery to implant jugular catheters occurred in between these two phases.

2.4.3.1. Cannabinoid pre-exposure. The group allocation and procedure was identical to the pre-exposure phase of Experiment 1 except that the test chambers were placed inside sound attenuation boxes. Although levers were installed before the start of this experiment, they remained retracted during this phase of the experiment.

2.4.3.2. Surgery. At the conclusion of the cannabinoid pre-exposure phase, jugular catheters were surgically implanted. Rats were anaesthetized with a mixture of Ketamine (Troy Laboratories, NSW, Australia, 100 mg/kg i.p.) and Xylazine (Troy Laboratories, 12 mg/kg i.p.) and implanted with an intravenous catheter into the right external jugular vein. Catheters were constructed from 140 mm Tygon Micro Bore tubing (ID 0.06 in. OD 0.02 in., Small Parts, FL, USA) and passed through the center of a 15-mm^2 polypropylene mesh square (1000, Small Parts) attached by cranioplastic cement 25 mm from the distal end of the catheter. Catheters were externalized at the back and secured with a polypropylene mesh assembly and sutures.

Catheters were filled with 10 IU/ml heparinized saline and occluded with a 23-gauge pin. Following insertion of the intravenous catheter, head mounts for the spring connector were implanted into the skull using a stereotaxic apparatus (Stoelting, IL, USA). Head mounts (CG313 bent at 100°, Plastics One) were secured in place with cranioplastic cement (Vertex, Dentimex Zeist, Holland) and four stainless steel screws (Small Parts) tapped into the skull.

Rats were allowed 5–7 days recovery from surgery before the self-administration phase. On the day of surgery

and for two subsequent days, rats were treated with the analgesic Flunixin (Troy Laboratories, 2.5 mg/kg s.c.). Catheter patency was maintained by the daily intravenous flush of 0.2 ml of antibiotic (Cephazolin Sodium, David Bull Laboratories, VIC, Australia, 100 g/ml) in 100 IU/ml of heparinized saline (David Bull Laboratories). Body weight and general health were monitored daily.

2.4.3.3. Self-administration. Self-administration sessions began 8 days after the cannabinoid pre-exposure phase and continued for 14 daily sessions. Rats were placed in the chamber, the intravenous catheter was flushed with 0.1 ml of heparinized saline (10 IU/ml) and the connector to the infusion line was inserted. Self-administration sessions lasted 120 min during which the number of active lever presses, dummy lever presses, drug infusions and locomotor activity were recorded. At the end of each session, the infusion line was disconnected, the intravenous catheter was flushed with 0.2 ml of the antibiotic solution (see above) and the catheter was closed with the pin.

2.5. Data analysis

2.5.1. Experiment 1

Analysis of the differences in activity counts between groups was performed using planned contrasts [repeated measures analysis of variance (ANOVA)]. For each of the 14 days of the pre-exposure phase, a contrast compared activity counts in CP 55,940 and vehicle-treated rats. For the 14 days of the morphine cross-sensitization test, the following specific comparisons of activity counts across groups were performed for each of the 3 h of testing as well as for the complete 3-h test: (1) all rats given morphine with all rats given vehicle, (2) rats pre-exposed to CP 55,940 and given morphine (CP-M) with rats pre-exposed to vehicle and given morphine (V-M), and (3) rats pre-exposed to CP 55,940 and given vehicle (CP-V) with rats pre-exposed to vehicle and given vehicle (V-V).

An additional analysis examined activity across the 180 min of the first day of morphine administration to determine whether there was an immediate cross-sensitization effect evident in cannabinoid pre-exposed rats. This analysis involved comparing groups CP-M and V-M on their locomotor activity counts across the 3 h of testing on that day. For this analysis, linear trend analysis was used to determine differences in the pattern of locomotor activity in groups across the 3 h of testing.

2.5.2. Experiment 2

The data for Experiment 2 were also analyzed using planned contrasts (repeated measures ANOVA). The following specific contrasts were conducted for the 14-day drug co-administration phase and for the 3-day morphine probe phase: (1) rats given morphine only (V-M) with rats only given vehicle (V-V), (2) rats given morphine only (V-M) with rats given CP 55,940 and morphine combined (CP-M)

and (3) rats given morphine only (M-V) with rats given morphine and SR 141716 combined (SR-M). Again separate analyses were performed on activity data for each of the 3 h of testing as well as for the entire 3 h testing period.

2.5.3. Experiment 3

Planned contrasts (repeated measures ANOVA) were used to compare the number of infusions received and locomotor activity between groups across the 14 days of the experiment. The number of active lever presses versus the number of dummy lever presses was also compared across groups across days.

A significance level of 0.05 was employed for all analyses.

3. Results

3.1. Experiment 1: CP 55,940 pre-exposure and behavioral sensitization to morphine

As can be seen in Fig. 1, rats given CP 55,940 were significantly less active than those given vehicle across the 14 days of drug pre-exposure ($F(1,30) = 7.79$, $P < 0.01$). Data for day 1 of the morphine co-administration phase are shown in Fig. 2. Rats pre-exposed to CP 55,940 and given morphine (CP-M) did not differ significantly in activity from rats pre-treated with vehicle and given morphine (V-M) ($F(1,14) = 2.21$, $P = 0.15$). However, there was a significant group by linear trend effect for this day ($F(1,14) = 5.71$, $P < 0.05$). This linear trend reflects the CP-M group increasing in activity over the second and third hour at a significantly faster rate than the V-M group (see Fig. 2). Rats pre-treated with CP 55,940 and then tested with vehicle (CP-V) showed similar locomotor activity to the rats that were pre-treated with vehicle and then given
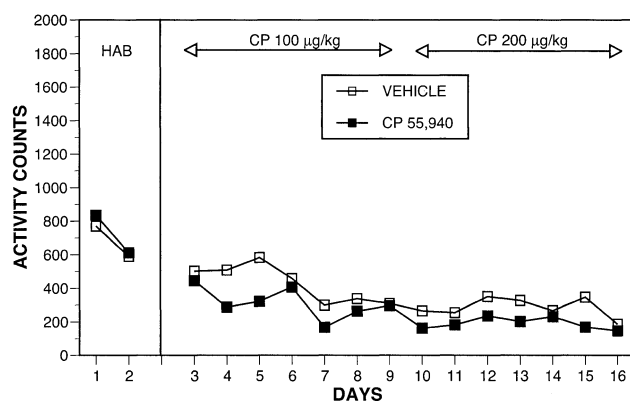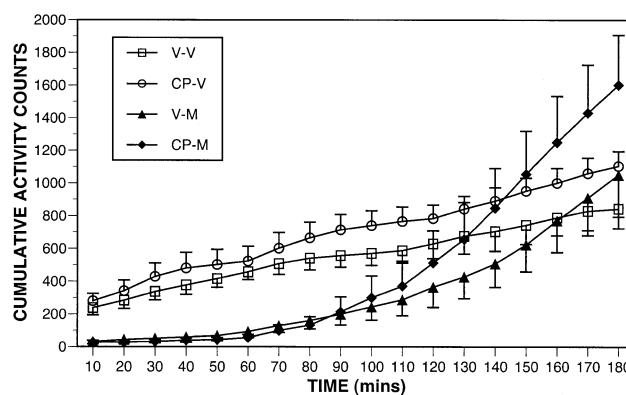

Fig. 2. Cumulative locomotor activity counts ($\pm$ S.E.M.) over the 180 min of testing on the first day of the cross-sensitization phase of Experiment 1. Abbreviations: V-V, pre-exposed to vehicle and tested with vehicle; CP-V, pre-exposed to CP 55,940 and tested with vehicle; V-M, pre-exposed to vehicle and tested with morphine; CP-M, pre-exposed to CP 55,940 and tested with morphine.

vehicle (V-V) ($F < 1$). There was no difference in linear trend between these two groups ($F < 1$).

Results from the 14-day morphine cross-sensitization test in Experiment 1 are shown in Fig. 3. A comparison between all rats given morphine and all rats given vehicle revealed that morphine-treated rats were significantly less active than the vehicle rats in the first hour ($F(1,30) = 41.57$, $P < 0.001$). However, morphine-treated rats were significantly more active in the second hour ($F(1,30) = 169.74$, $P < 0.001$), third hour ($F(1,30) = 122.61$, $P < 0.001$) and overall across the 3 h of testing ($F(1,30) = 166.72$, $P < 0.001$). Rats pre-treated with CP 55,940 and given vehicle injections (CP-V) did not differ from rats that were only given vehicle injections (V-V) in any of the hours assessed ($F < 1$).

The activity of the cannabinoid pre-exposed rats given morphine (CP-M), and the vehicle pre-exposed rats given morphine (V-M) did not differ in the first hour of testing across the 14 days of treatment ($F(1,14) = 2.21$, $P = 0.15$). However, rats in the CP-M group were significantly more active than the V-M group in the second hour of treatment across these 14 days ($F(1,14) = 16.74$, $P < 0.001$). In the third hour, the differences between these groups were again non-significant ($F(1,14) = 2.14$, $P = 0.16$). Comparison of overall activity levels for the 3 h of testing showed that the CP-M rats were significantly more active than the V-M rats ($F(1,14) = 7.77$, $P < 0.01$).

3.2. Experiment 2: cannabinoid and morphine co-administration

Data for Experiment 2 are shown in Fig. 4. On the first day of the co-administration phase, rats given the combination of vehicle and morphine (V-M) were significantly more active overall than rats given either CP 55,940 or SR 141716 in conjunction with morphine (CP-M and SR-M) ($F(1,14) = 45.66$, $P < 0.001$, and $F(1,14) = 16.52$, $P < 0.001$, respectively). The rats given morphine alone (V-M) were

Fig. 1. Locomotor activity of rats ($n = 16$ per group) injected with CP 55,940 or vehicle on the two habituation days (HAB) and through the 14-day drug pre-exposure phase of Experiment 1. CP 55,940 (0.1 mg/kg) was given in the first 7 days of drug pre-exposure and 0.2 mg/kg in the final 7 days.
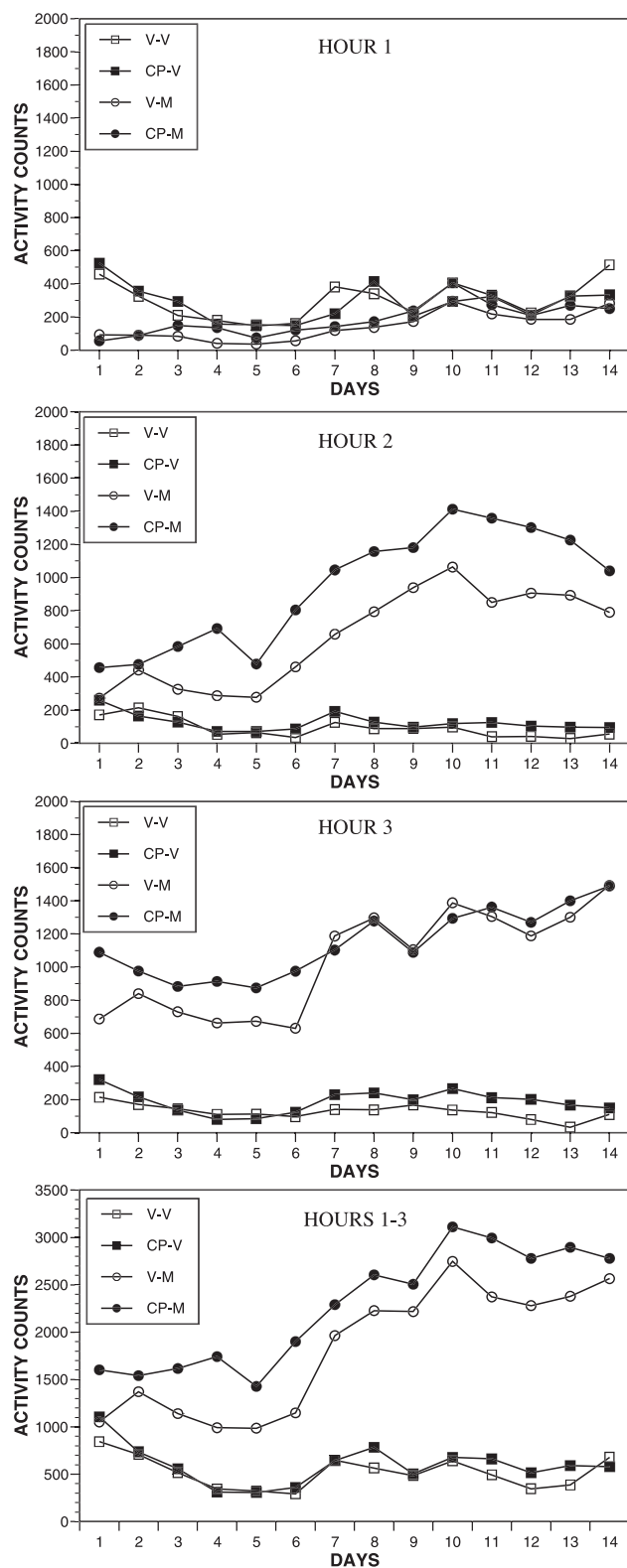
Fig. 3. Locomotor activity of rats over each of the 3 h of testing (top graph and two middle graphs) and for the entire 3 h of testing (bottom) through the 14-day cross-sensitization test phase of Experiment 1. Abbreviations: V-V, pre-exposed to vehicle and tested with vehicle; CP-V, pre-exposed to CP 55,940 and tested with vehicle; V-M, pre-exposed to vehicle and tested with morphine; CP-M, pre-exposed to CP 55,940 and tested with morphine.
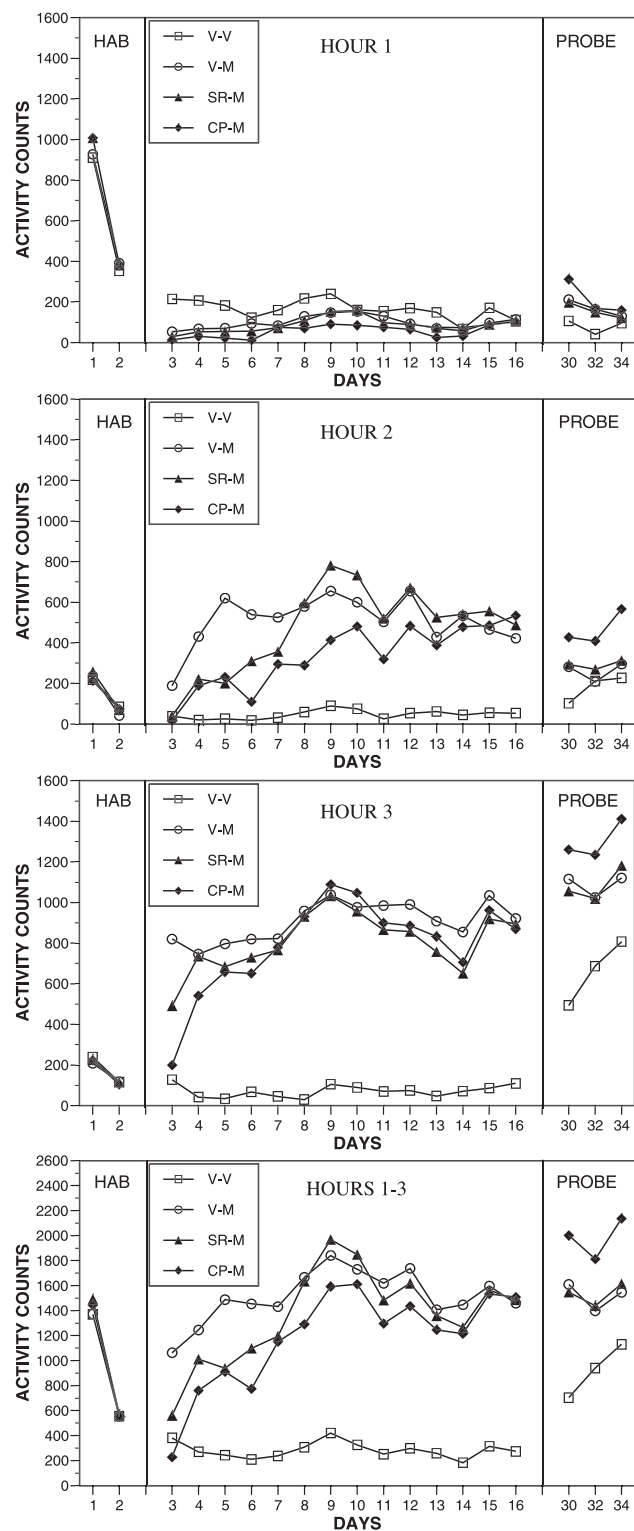
Fig. 4. Locomotor activity of the rats on the two habituation days, during the 14-day drug co-administration phase and during the 3-day morphine probe phase of Experiment 2. Graphs show data for each of the 3 h of testing (top graph and two middle graphs) and for the entire 3 h of testing (bottom). Abbreviations: V-V, rats co-administered vehicle and vehicle; V-M, rats co-administered vehicle and morphine; SR-M, rats co-administered SR 141716 and morphine; CP-M, rats co-administered CP 55,940. HAB = habituation phase. PROBE = morphine probe phase. Note all rats were given morphine on the last 3 days (morphine probe) of the experiment on days 30, 32 and 34.
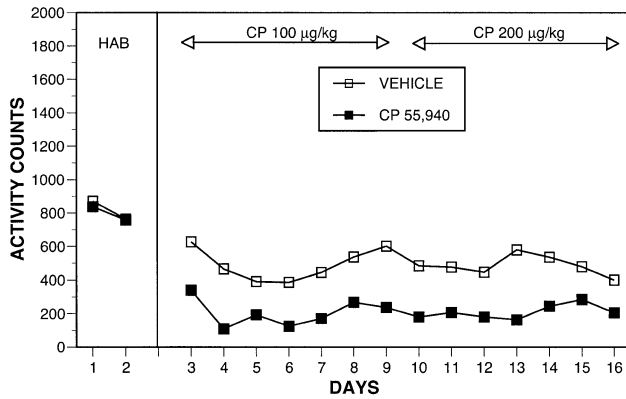
Fig. 5. Locomotor activity of rats ($n = 8$ per group) injected with CP 55,940 or vehicle on the two habituation days (HAB) and through the 14-day drug pre-exposure phase of Experiment 3. CP 55,940 (0.1 mg/kg) was given in the first 7 days of drug pre-exposure and 0.2 mg/kg in the final 7 days.

also significantly more active than rats given only vehicle injections (V-V) ($F(1,14) = 30.33$, $P < 0.001$).

Over the 14 days of co-administration, rats given combined vehicle and morphine injections (V-M) were significantly less active than vehicle only rats (V-V) during the first hour ($F(1,28) = 18.12$, $P < 0.001$), but significantly more active during the second hour ($F(1,28) = 139.56$, $P < 0.001$), third hour ($F(1,28) = 165.15$, $P < 0.001$) and over the entire 3 h of testing ($F(1,28) = 144.24$, $P < 0.001$).

Rats given CP 55,940 with morphine (CP-M) were significantly less active than rats given vehicle and morphine (V-M) over the first hour ($F(1,28) = 6.15$, $P < 0.05$), second hour ($F(1,28) = 19.76$, $P < 0.001$) and overall over the 3 h of testing ($F(1,28) = 10.52$, $P < 0.01$). During the third hour, however, no significant differences in activity were observed between these two groups ($F(1,28) = 3.20$, $P = 0.09$). Rats given the combination of SR 141716 and morphine (SR-M) showed no significant overall difference in activity relative to the rats given only morphine (V-M) during the first hour ($F < 1$), second hour ($F(1,28) = 1.3$, $P = 0.26$), third hour ($F(1,28) = 2.38$, $P = 0.14$) or overall ($F(1,28) = 2.27$, $P = 0.15$) throughout the co-administration phase.

In the morphine probe tests (Fig. 4, far right panels), rats pre-exposed to morphine (V-M) were significantly more active overall than the rats that had been pre-exposed to vehicle (V-V) ($F(1,14) = 15.07$, $P < 0.001$). This difference also held when the first ($F(1,14) = 4.86$, $P < 0.05$) and third ($F(1,14) = 20.46$, $P < 0.001$) hours of testing were analyzed separately but not the second hour ($F(1,14) = 1.6$, $P = 0.23$).

Rats given CP 55,940 in conjunction with morphine during the co-administration phase (CP-M) were more active overall during the three morphine probe tests than the rats pre-exposed to morphine alone (V-M) ($F(1,14) = 9.28$, $P < 0.01$). These two groups did not differ significantly in the first hour of testing of the probes ($F(1,14) = 1.3$, $P = 0.27$) but differed significantly in the second ($F(1,14) = 9.77$, $P < 0.01$) and third hours ($F(1,14) = 5.27$, $P < 0.05$).
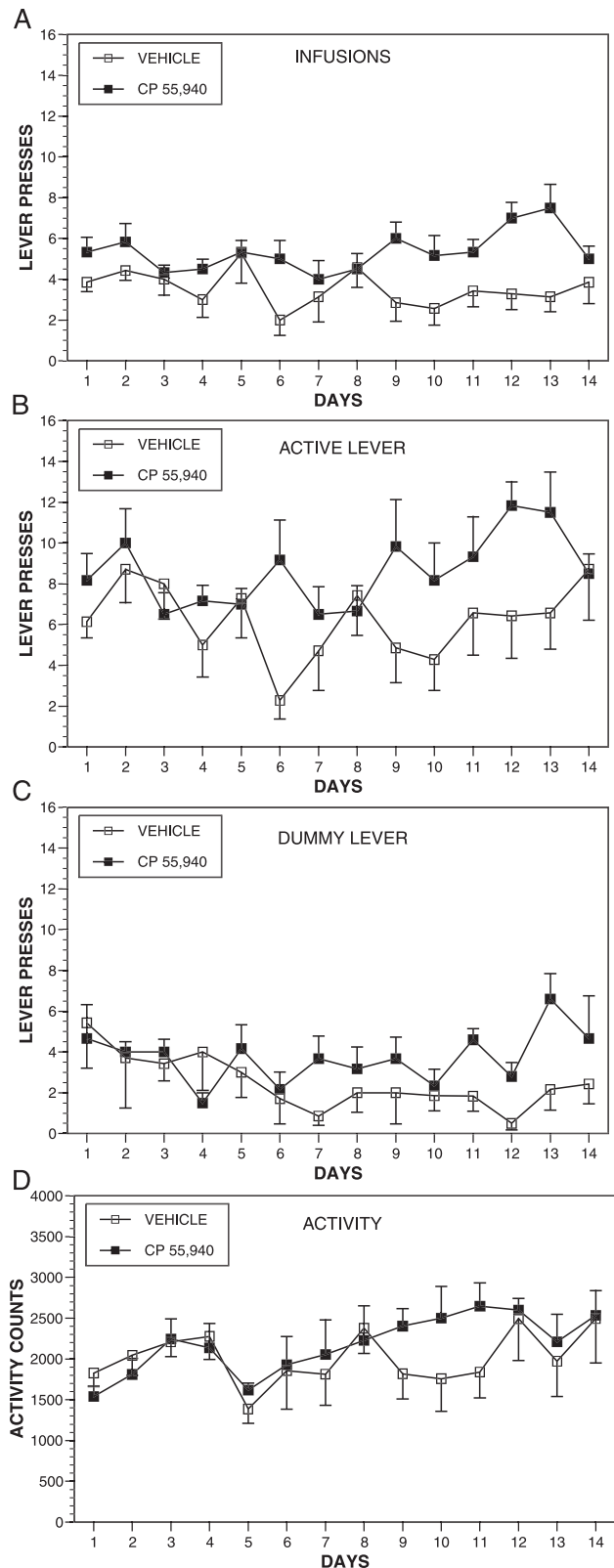

Fig. 6. The mean number of (A) morphine infusions received, (B) presses on active lever, (C) presses on dummy lever, and (D) locomotor activity counts for rats pre-exposed to either CP 55,940 or vehicle over the 14 days of morphine self-administration in Experiment 3. Error bars show + S.E.M. for CP 55,940 group and − S.E.M. for vehicle group.

Rats given SR 141716 in conjunction with morphine (SR-M) did not differ in activity from rats given morphine alone (V-M) when tested across the three morphine probes ($F < 1$). No differences were seen between these groups either when the first, second or third hours were analyzed separately ($F < 1$).

3.3. Experiment 3: CP 55,940 pre-exposure and morphine self-administration

CP 55,940 significantly reduced locomotor activity relative to vehicle controls across all 14 days of the pre-exposure phase of Experiment 3 (see Fig. 5), ($F(1,14) = 56.72$, $P < 0.001$).

One rat in the vehicle condition developed a blocked catheter early in the self-administration phase and had to be removed from the experiment.

On the first day of morphine self-administration, the number of dummy lever presses and the number of activity counts did not differ significantly between groups ($F < 1.3$).

Across the 14 days of the morphine self-administration acquisition phase, the rats pre-exposed to cannabinoids received significantly more morphine infusions than the vehicle pre-exposed rats ($F(1,13) = 7.12$, $P < 0.05$). Locomotor activity did not differ significantly between groups ($F < 1$).

Comparison of active versus dummy lever presses across groups showed that there were significantly more active lever presses than dummy lever presses across the 14 days of the experiment ($F(1,13) = 71.10$, $P < 0.0001$). The cannabinoid pre-exposed rats made significantly more lever presses overall than the vehicle pre-exposed rats ($F(1,13) = 5.31$, $P < 0.05$) but the groups were not differentiated across the active versus dummy lever presses ($F < 1.3$) (Fig. 6).

4. Discussion

These results indicate that pre-exposure to the cannabinoid receptor agonist CP 55,940 enhances subsequent morphine-induced locomotor activity and self-administration. Locomotor results are in general agreement with recent reports showing cross-sensitization between cannabinoids and opioids, although previous studies have used the different cannabinoid receptor agonists Δ^9-tetrahydrocannabinol and WIN 55,212-2 (Cadoni et al., 2001; Lamarque et al., 2001; Pontieri et al., 2001a,b). The present study therefore represents the first report of behavioral cross-sensitization between an opioid receptor agonist and the synthetic cannabinoid receptor agonist CP 55,940.

Results reported here are also unique in that Lewis rats were used, whereas Sprague–Dawley rats were employed in previous reports of cannabinoid–opioid cross-sensitization (Cadoni et al., 2001; Lamarque et al., 2001; Pontieri et al., 2001a,b). Lewis rats are reported to be especially responsive

to the rewarding effects of drugs including cannabinoids and opioids (Gardner and Vorel, 1998; Lepore et al., 1996). The finding that cannabinoid–opioid cross-sensitization can be found in this rat strain is therefore not unexpected, although the rather small magnitude of the cross-sensitization effects obtained is perhaps a little surprising. Nonetheless, the findings with Lewis strain rats here agree with a previous report that "high responder" rats, also noted for their vulnerability to addictive behavior, are prone to cannabinoid–opioid cross-sensitization (Lamarque et al., 2001).

During the pre-exposure phases of Experiments 1 and 3, CP 55,940 inhibited locomotor activity, which is in agreement with previous reports (Arnold et al., 1998, 2001). The magnitude of this effect was not great, particularly in Experiment 1, despite the relatively high doses of CP 55,940 used. This can be partly explained by the low levels of activity seen in vehicle-treated rats, a phenomenon documented in previous studies using the Lewis strain (Arnold et al., 1998, 2001). It remains an open question whether the slightly greater locomotor suppression seen with CP 55,940 in Experiment 3 relative to Experiment 1 is a function of the different ages of rats, the use of sound attenuating chambers in Experiment 3, or some other unknown reason.

In Experiment 1, when rats that had been pre-exposed to CP 55,940 were first given morphine, they showed a different pattern of locomotor activation to vehicle pre-treated rats. While locomotor activity of all morphine-treated rats was depressed in the first hour of administration, locomotor activity of the CP 55,940 pre-treated rats was stimulated to a greater extent than the vehicle pre-treated rats in the second and third hours.

The overall activity levels of the cannabinoid pre-treated rats continued to be higher than the vehicle pre-treated rats throughout most of Experiment 1 (Fig. 3). So, in addition to initial enhancement of morphine locomotor activity, cannabinoid pre-treated rats displayed faster progression of morphine sensitization. Furthermore, morphine-induced sensitization in cannabinoid pre-treated rats reached a higher asymptote, suggesting that cannabinoid pre-treatment can increase the extent to which morphine produces behavioral sensitization.

Importantly, no differences were found between the cannabinoid and vehicle pre-treated rats given vehicle injections throughout the testing period of Experiment 1. This indicates that the differences between the cannabinoid and the vehicle pre-treated rats given morphine is not due to general hyperactivity resulting from cannabinoid pre-exposure. Instead, the cannabinoid pre-treatment influences the way in which morphine affected locomotor activity and produced behavioral sensitization.

An enhanced behavioral sensitization to morphine following cannabinoid pre-exposure in Experiment 1 was evident despite the fact that there was no development of locomotor hyperactivity to the cannabinoid during the first phase of the experiment. This result agrees with those of

Arnold et al. (1998) in their studies of the effects of cannabinoid pre-exposure on behavioral sensitization to cocaine. More recent studies have suggested that sensitization to cannabinoids can occur with repeated exposure, but that locomotor activation may not be the best measure of this as cannabinoids have a strong inhibitory effect on locomotor activity. Rather, such sensitization may be best observed using measures of stereotypy such as repetitive gnawing, licking and sniffing (Cadoni et al., 2001; Rubino et al., 2001). It would clearly be of interest to take such additional measures in future studies involving repeated administration of CP 55,940.

In Experiment 2, cross-sensitization was again evident between CP 55,940 and morphine, although this time in a design where pre-exposure involved simultaneous administration of the two drugs. Thus, rats that had been pre-exposed to a morphine and CP 55,940 combination showed a greater subsequent locomotor response to morphine than rats pre-exposed to morphine alone. It was interesting to note that in rats given the combination during pre-exposure, CP 55,940 decreased the locomotor hyperactivity seen to morphine, particularly over the first few days of testing. The ability of CP 55,940 to decrease the acute morphine-induced locomotor stimulation agrees with similar reports of its ability to blunt locomotor activation to amphetamine (Gorriti et al., 1999; Pryor et al., 1978) and cocaine (Arnold et al., 1998; Pryor et al., 1978). It is all the more striking then, that when CP 55,940 was removed in the morphine probe phase of Experiment 2, a strong sensitization to the locomotor activating effects of morphine was unmasked that was greater than that seen in rats sensitized to morphine alone.

Another interesting finding from Experiment 2 was the failure of co-administration of the cannabinoid antagonist SR 141716 to affect the acquisition of morphine sensitization. SR 141716, like CP 55,940, tended to decrease the acute locomotor response to morphine particularly over the first 5 days of co-administration. However, when morphine was given alone in the probe phase, rats that had been pre-exposed to morphine and SR 141716 combined showed an equivalent locomotor response to rats that had been pre-exposed to morphine only. This suggests that cannabinoid CB_1 receptors do not play a major role in the acquisition of morphine sensitization in Lewis rats. This conclusion is at odds with the recent findings of Martin et al. (2000) that sensitization to morphine's activating effects may be absent in cannabinoid CB_1 receptor knockout mice. As well as the obvious species differences, this discrepancy might also be related to the fact that the activity of the mice were only tested for 15 min, 10 min after morphine injection. This is in contrast to the 3-h test period used here, with maximal sensitization seen in the third hour of testing.

A major prediction from incentive sensitization theory is that sensitization to drugs, indexed by increasing locomotor activation, plays a key role in compulsive drug self-administration (Robinson and Berridge, 1993). If this were the case, then it would be expected that results obtained in a locomotor sensitization paradigm will transfer across into a related paradigm where drug self-administration is examined. This was the aim of Experiment 3, where rats that had been pre-exposed to cannabinoids in exactly the same fashion as Experiment 1 were tested on drug self-administration. The results of Experiment 1 gave rise to the prediction that cannabinoid pre-exposed rats should self-administer a greater amount of morphine than controls. This prediction was largely confirmed, although some caveats must be noted.

First, as reported in previous experiments (Ambrosio et al., 1995; Martin et al., 1999), levels of morphine self-administration in Lewis rats were relatively low in both pre-exposed and non pre-exposed rats and a clear acquisition curve was not present across self-administration sessions. Thus, the greater number of infusions received by cannabinoid pre-exposed rats must be seen within the context of a low baseline and uncertainty about whether cannabinoid pre-exposure was affecting the acquisition or the maintenance of opiate self-administration. Second, changes in the reinforcing efficacy of morphine in animals pre-exposed to cannabinoids could not be established in the present study due to the use of only one dose level of morphine. Future studies might usefully include dose response curves for opiate self-administration in cannabinoid pre-exposed rats. Third, it must be noted that the increased lever presses seen in CP 55,940 pre-exposed rats were not specific to the active lever; that is, dummy (inactive) lever presses were also increased. Thus, an explanation of self-administration results in terms of greater general behavioral activation to morphine in cannabinoid pre-exposed rats cannot be ruled out. Indeed, the results from Experiments 1 and 2 invite the suggestion that such heightened locomotor activity in cannabinoid pre-exposed rats should exist, although not necessarily at the doses that were self-administered in Experiment 3. The locomotor activity data collected in Experiment 3 did not indicate significantly higher overall locomotor activity in CP 55,940 pre-exposed rats, although there was a suggestion of this, on days 9–11 of the experiment. Of course, measures of locomotor activity may be confounded when lever pressing is also being performed in the same chamber, as lever pressing itself tends to involve fairly minimal activity.

Taken together, results of the three experiments reported here provide support for the phenomenon of cross-sensitization between cannabinoids and opioids in Lewis rats. Observed cross-sensitization effects were relatively small and were admittedly produced by pre-exposure to relatively high doses of cannabinoids, so caution should be used when extending these results to "gateway" theories of human drug abuse. Future experiments may uncover other dose regimes or sensitization protocols that will unmask an even greater cannabinoid–opioid cross-sensitization. It will also be the goal of future research to determine whether cannabinoid pre-exposure affects subsequent self-administration of other opioids such as heroin, and other drugs of abuse such as cocaine and amphetamine.

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