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Effects of pre-exposure and co-administration of the cannabinoid receptor agonist CP 55,940 on behavioral sensitization to cocaine

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Abstract

Rats given cocaine (15 mg/kg, i.p.) every second day over a 2-week period displayed a progressively greater locomotor response to the drug over days indicating behavioral sensitization. When the cannabinoid receptor agonist CP 55,940 ((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]–*trans*-4-(3-hydroxypropyl)cyclohexanol) (10, 25 or 50 μg/kg) was administered under a similar regime, no such sensitization was observed. Rather, the two highest doses of CP 55,940 (25 and 50 μg/kg) caused locomotor suppression that lasted throughout administration. When rats pre-exposed 10 times to CP 55,940 were challenged with cocaine (15 mg/kg), no exaggerated locomotor response to cocaine was evident relative to non pre-exposed rats. When these rats were subsequently re-tested with CP 55,940, the cannabinoid continued to produce a dose-dependent suppression of locomotor activity. Finally, when CP 55,940 (50 μg/kg) was co-administered with cocaine, it significantly reduced the locomotor hyperactivity produced by the drug but did not block the development of behavioral sensitization. These results show that CP 55,940 does not sensitize locomotor activity with repeated administration in the same way as cocaine, and that pre-exposure or concurrent exposure to CP 55,940 does not enhance sensitivity to the subsequent behavioral effects of cocaine.

Keywords: Cannabinoid; Cocaine; Sensitization; Locomotor activity; CP 55,940; Polydrug abuse

1. Introduction

Recent years have seen increased debate surrounding the possible health dangers resulting from cannabis use. One particular focus has been the possibility that cannabis may provide a 'gateway' leading users to harder drugs such as heroin and cocaine. There is evidence that cocaine, heroin, tobacco and alcohol use is more prevalent among cannabis users than non-users (Hall et al., 1991; Miller et al., 1990; Swift et al., 1997) lending credence to the hypothesis that cannabis use may lead to enhanced vulnerability to the addictive effects of other drugs. However, the conclusions drawn from these human studies are based on correlation, rather than causation, suggesting the need for laboratory studies to allow a closer focus on possible gateway mechanisms.

Sensitization of mesolimbic dopamine efflux during repeated intermittent exposure to drugs of abuse has been recently hypothesized to be a key neural adaptation underlying the development of pathological drug craving (Robinson and Berridge, 1993). The most widely used behavioral index of such sensitization is the progressively greater locomotor activity response elicited in rats and mice to repeated administration of drugs. Such sensitization of locomotor activity has been documented to a wide range of drugs including nicotine, amphetamine, morphine, heroin, cocaine and phencyclidine (Robinson and Berridge, 1993; Stewart and Badiani, 1993). However, to our knowledge, no study has yet looked at whether similar sensitization may be obtained with cannabinoids. Thus it was an initial aim of the present study to test this hypothesis using the synthetic cannabinoid receptor agonist CP 55,940, a drug which is very similar to the main psychoactive constituent of cannabis (Δ^9-tetrahydrocannabinol) in its behavioral and discriminative stimulus effects (Wiley et al., 1995) although it is approximately 30 times more potent (Gold et al., 1992; Little et al., 1988).

A further interesting phenomenon is cross-sensitization, whereby pre-exposure to one drug increases the sensitivity

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to the activating or rewarding effects of another (Cunningham et al., 1997; Lett, 1989; Stewart and Badiani, 1993). Using locomotor activity as a dependent variable, cross-sensitization is found between many drugs of abuse, for example, cocaine and amphetamine (Bonate et al., 1997), amphetamine and morphine (Cunningham et al., 1997; Vezina et al., 1989) or amphetamine and phencyclidine (Greenberg and Segal, 1985). Cross-sensitization constitutes one of the ways in which the gateway hypothesis might be tested with respect to cannabinoids. If pre-exposure to a cannabinoid rendered an animal hypersensitive to the activating effects of cocaine, then this would provide presumptive evidence in favor of the 'gateway' claim. This hypothesis was tested in the present study by pre-exposing rats to CP 55,940 and testing their subsequent locomotor response to cocaine.

Cross-sensitization between cannabinoids and cocaine might be predicted given that the two drugs share certain key neurochemical effects. Firstly, both are found to enhance dopamine efflux in the nucleus accumbens (Gardner and Lowinson, 1991; Tanda et al., 1997), and this is a key neurochemical mechanism underlying sensitization and cross-sensitization processes (Robinson and Berridge, 1993). Secondly, a recent investigation has shown that the endogenous cannabinoid system may be intrinsically linked to cocaine reward circuitry. Thus the cannabinoid receptor antagonist SR 141716 was shown to block the acquisition of conditioned place preference to cocaine in rats (Chaperon et al., 1998).

Rather than produce cross-sensitization, some drugs may actually hinder the development of sensitization to another drug. For instance the following drugs all block the development of behavioral sensitization to cocaine; the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Kalivas and Alesdatter, 1993; Wolf and Jeziorski, 1993), the dopamine receptor antagonist haloperidol (Mattingly et al., 1996b) and, the inhibitor of neuronal nitric oxide synthase, 7-nitroindazole (Haracz et al., 1997; Itzhak, 1997). The possibility that cannabinoids may prevent the development of behavioral sensitization to cocaine was also tested in the present study by examining whether co-administration of CP 55,940 with cocaine might inhibit behavioral sensitization.

2. Materials and methods

2.1. Animals

All experiments involved male Lewis rats (ARC, Perth, Australia), aged between 75 and 90 days at the time of testing. The rats were group housed (eight animals per box) and were maintained on a 12-h reversed light–dark cycle (light off at 0830 h) 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 4 days prior to the start of experiments. All experiments were approved by the University of Sydney Animal Care and Ethics Committee.

2.2. Apparatus

Locomotor activity was measured in eight Coulbourn operant cages (31 cm (L) × 25 cm (W) × 49 cm (H)) as previously described (McGregor et al., 1996). The cages had a clear perspex front wall and aluminium back wall and sides. The cage floors consisted of 16 metal bars (0.5 cm diameter, spaced 1 cm apart) connected to a high impedance amplifier. When the rat moved on the grid so that contact or breaking of contact between any four bars and the other 12 occurred, an activity count was recorded by a Macintosh computer running WorkbenchMac data acquisition software (McGregor, 1996). Each cage was encased in a wooden sound attenuation chamber which was equipped with a fan which provided masking noise during testing.

2.3. Drugs

Cocaine hydrochloride (Australian Pharmaceutical Industries, Sydney) was dissolved in 0.9% saline and injected intraperitoneally at a dose of 15 mg/kg in a volume of 1 ml/kg. CP 55,940 ((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl] – *trans*-4-(3-hydroxypropyl)cyclohexanol) (Pfizer) was first dissolved in absolute ethanol and then diluted in Tween 80 and saline to make a final vehicle solution of 2.5% ethanol, 2.5% Tween 80 and 95% saline. All drugs were injected intraperitoneally in an injection volume of 1 ml/kg.

2.4. Procedure

2.4.1. Behavioral sensitization to cocaine

The first experiment aimed to establish behavioral sensitization to the repeated intermittent administration of cocaine. The procedure involved three different phases. Phase 1 was a 'habituation' phase in which the rats ($n = 16$) were placed in the testing chambers in the drug-free state for 1 h on 2 consecutive days. This allowed the dissipation of exploratory behavior in the novel context prior to drug testing. In the second 'sensitization' phase, starting 24 h after the final habituation session, the rats were injected with either cocaine (15 mg/kg) or saline and 5 min later placed in the activity cages for 60 min. This procedure was repeated every 48 h to give a total of seven sensitization sessions. Eight rats received cocaine during this phase while the other eight 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, often thought to be very long lasting (Robinson and Berridge, 1993).

2.4.2. CP 55,940 pre-exposure and behavioral sensitization to cocaine

The second experiment aimed to establish behavioral sensitization to various doses of intermittently administered CP 55,940 and to test for cross-sensitization between CP 55,940 and cocaine. Rats were randomly allocated to four different treatment groups ($n = 8$ per group). These groups received 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, while the lowest dose had the possibility of producing locomotor stimulation (McGregor et al., 1996). Rats were tested on alternate days in two separate replicates of 16 rats with drug conditions 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 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 and 5 min later 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 saline 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 drugs of abuse (Stewart and 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 CP 55,940 could modulate the induction of behavioral 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 induced by 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.

2.4.3. CP 55,940 and cocaine co-administration

The final experiment aimed to test whether the co-administration of CP 55,940 with cocaine could block the development of behavioral sensitization. The experiment consisted of three phases: phase 1 ('habituation'), phase 2 ('co-administration') 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 ('co-administration') 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 ($n = 8$) 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 1 week after the final co-administration 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.

2.5. Data analysis

In all experiments, 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-way or repeated measures analysis of variance (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).

3. Results

3.1. Behavioral sensitization to cocaine

3.1.1. Habituation phase

The locomotor activity data for Experiment 1 are shown in Fig. 1. No significant differences between experimental groups (cocaine vs. saline) were found in activity over habituation days 1 and 2 (Fs < 1.60).

3.1.2. Sensitization phase

Rats given cocaine demonstrated a clear increment in locomotor activity over days relative to saline-treated rats. A significant group by day interaction was seen when comparing activity on the first to seventh day of drug

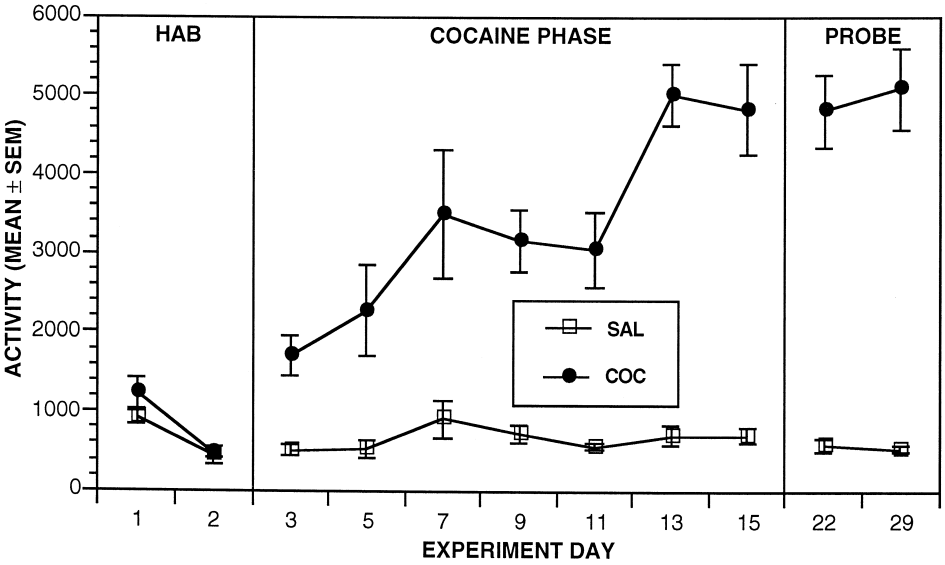

Fig. 1. Behavioral sensitization to 15 mg/kg of cocaine. Mean locomotor activity scores are shown across the three experimental phases for the groups administered either cocaine or saline ($n = 8$).

treatment across the two groups ($F(1,14) = 36.17$, $P < 0.001$).

3.1.3. Weekly probes

The sensitization of locomotor activity in cocaine groups was still present at both 1 and 2 weeks following the final day of the sensitization phase. This is highlighted by a significant group by day interaction when comparing activity on the first day of the cocaine phase with activity on the first weekly probe test ($F(1,14) = 41.71$, $P < 0.001$) or the second probe test ($F(1,14) = 63.18$, $P < 0.001$).

3.2. CP 55,940 pre-exposure and behavioral sensitization to cocaine

During this experiment one rat from the 50 μg/kg CP 55,940 group died from unknown causes (on the last day of phase 2). Data from this rat were removed from the experiment.

3.2.1. Habituation phase

The results are shown in Fig. 2. In the baseline phase, prior to drug administration, there were no significant differences between any of the experimental groups in locomotor activity (Fs < 1.00).

3.2.2. CP 55,940 phase

Intermittent exposure of rats to any dose of CP-55,940 did not induce behavioral sensitization. This can be seen in Fig. 2 which shows consistent locomotor depressant effects of higher CP 55,940 doses when compared to vehicle over days. Planned contrasts based on a repeated measures ANOVA (comparing the first vs. last day of CP 55,940 administration) revealed no significant differences between the 10 μg/kg group and vehicle group but a significant main effect when comparing the 25 μg/kg ($F(1,27) = 8.94$, $P < 0.01$) or the 50 μg/kg group ($F(1,27) = 15.60$, $P < 0.001$) with the vehicle group. This reflects a global inhibition of activity caused by the 25 and 50 μg/kg doses. There was also a significant group by day interaction effect when comparing the 50 μg/kg dose with vehicle group ($F(1,27) = 5.54$, $P < 0.016$). However, consideration of Fig. 2 suggests that this effect is due to the activity in the vehicle group decreasing over time as opposed to activity in the 50 μg/kg group increasing.

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 ($F < 1.40$).

3.2.4. First day of cocaine phase

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 (Fs < 1.05). This suggests that intermittent pre-exposure to a cannabinoid does not alter the locomotor activation produced by a moderate dose of cocaine.

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 (Fs < 1.40). This suggests that intermittent pre-exposure to a cannabinoid does not alter the locomotor effects produced by repeated intermittent exposure to cocaine.

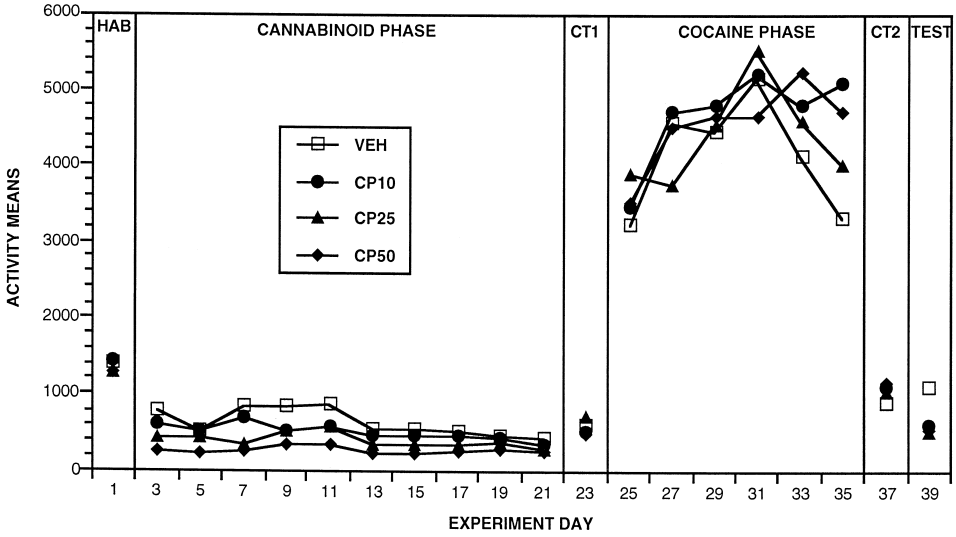

Fig. 2. Effects of cannabinoid pre-exposure on behavioral sensitization to 15 mg/kg of cocaine. Mean locomotor activity scores are shown across the six experimental phases for the four groups ($n = 8$) administered either vehicle (VEH) or 10, 25 or 50 μg/kg of CP 55,940 (CP10, CP25 and CP50, respectively). The six phases from left to right are 'HAB' = phase 1 (habituation), 'CANNABINOID PHASE' = phase 2, 'CT1' = phase 3 (environmental conditioning test 1), 'COCAINE PHASE' = phase 4, 'CT2' = phase 5 (environmental conditioning test 2), and 'TEST' = phase 6 (CP 55,940 probe). Error bars are omitted for clarity.

3.2.6. Conditioning test 2

The CP 55,940 groups did not differ in activity from the vehicle group on the second conditioning test (Fs < 1.00). Comparison of activity during the conditioning test 2 with conditioning test 1, indicated an overall increase in activity ($F(1,30) = 60.23$, $P < 0.001$). This indicates that cocaine caused conditioned hyperactivity to the test environment in all four groups.

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, planned contrasts revealed 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

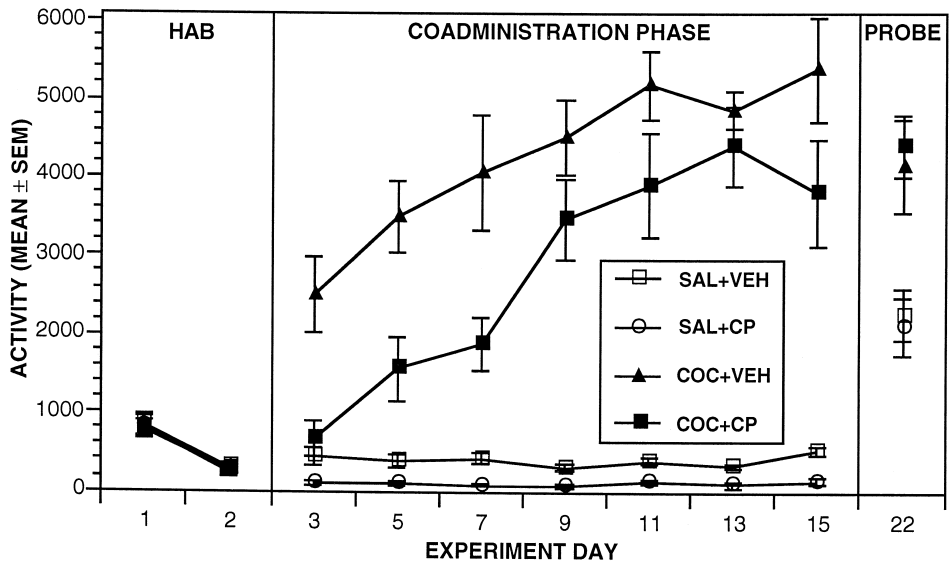

Fig. 3. Effects of the co-administration of 50 μg/kg CP 55,940 on behavioral sensitization to 15 mg/kg of cocaine. Mean locomotor activity scores are shown across the three experimental phases for the four groups administered either saline + vehicle (SAL + VEH), saline + CP 55,940 (SAL + CP), cocaine + vehicle (COC + VEH) and, cocaine + CP 55,940 (COC + CP) ($n = 8$). The three phases from left to right are 'HAB' = phase 1 (habituation), 'COADMINISTRATION PHASE' = phase 2, 'PROBE' = phase 3 (cocaine probe).

group by day interaction 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 cocaine-induced 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.

3.3. CP 55,940 and cocaine co-administration

3.3.1. Habituation phase

The results can be seen in Fig. 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).

3.3.2. Co-administration phase

Planned contrasts (first vs. last day of this phase) showed that rats in the COC + CP group ($F(1,28) = 43.22$, $P < 0.001$) or COC + VEH group ($F(1,28) = 168.29$, $P < 0.001$) 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 ($F(1,28) = 40.94$, $P < 0.001$). 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 indicated that the COC + CP group ($F(1,28) = 10.21$, $P < 0.001$) and the COC + VEH group ($F(1,28) = 8.50$, $P < 0.001$) showed a significant increase in activity from the first to the last day of this phase relative to the SAL + VEH group. Thus behavioral sensitization was induced by these treatments. When comparing the COC + VEH group to the COC + CP group no significant interaction effect was found ($F < 1$). Finally there was no significant interaction effect when the SAL + CP group was compared with the SAL + VEH group ($F < 1$).

3.3.3. Cocaine probe

One-way ANOVA with planned contrasts revealed rats pretreated 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. This confirms the presence of behavioural sensitization to cocaine in these rats. 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 the previous experiment, that pre-exposure to CP 55,940 does not sensitize rats to the subsequent effects of cocaine.

4. Discussion

The first important finding of the present study 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 confirms previous findings (Camp et al., 1994; Kosten et al., 1994; Ortiz et al., 1995) and appears to be relatively long lasting since it remained at asymptotic levels even 2 weeks following the final day of the sensitization phase. In addition, the 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 in Experiment 2 (Jodogne et al., 1994).

One possible concern with the present results is the failure of CP 55,940 to produce an acute increase in locomotor activity at any of the doses tested. Indeed, both the 25 and 50 μg/kg dose of CP 55,940 caused pronounced locomotor suppression that lasted throughout their administration (Fig. 2). The failure of the 10 μg/kg dose to produce increased activity contrasts with a previous report from our laboratory which showed a moderate hyperactive response to a single 10 μg/kg dose of CP 55,940 (McGregor et al., 1996). 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 generally found to be necessary for behavioral sensitization since 7-OH-DPAT, quinpirole and bromocriptine can suppress locomotor activity on first administration but are still able, with repeated administration, to produce hyperactivity (Hoffman and Wise, 1992, 1993; Mattingly et al., 1996a; Rowlett et al., 1995).

In addition, certain treatments, such as intra-ventral tegmental area 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 and Weber, 1988; Vezina and Stewart, 1990). Such a 'latent' effect, however, was not evident with CP 55,940 since repeated intermittent treatment did not cause subsequent enhanced sensitivity to the activating effects of cocaine in the present study. It is also worth noting that an earlier study in our laboratory, 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 et al., 1995).

A further point to mention is the apparent lack of clear behavioral sensitization to cocaine in the rats given cocaine after CP 55,940 administration in Experiment 2. This is in contrast to the clear behavioral sensitization seen with the same dose of cocaine in Experiments 1 and 3. There

are at least two points to note here. One is that a significant overall cocaine sensitization effect was evident in Experiment 2 when activity data for the fourth rather than the sixth cocaine administration are compared with 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 12 previous occasions in the absence of cocaine. To the extent that behavioral sensitization depends upon classical conditioning of hyperactivity to environmental cues (Stewart and 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 behavioral sensitization to cocaine more difficult to observe.

Interestingly, after repeated exposure to cocaine in Experiment 2 (Fig. 2), rats still displayed a dose-dependent suppression of activity when subsequently re-tested with CP 55,940. If cocaine pre-exposure were to sensitize the behavioral 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–CP 55,940 or CP 55,940–cocaine direction.

In light of the observation that cross-sensitization between cocaine and CP 55,940 did not occur, it was deemed unlikely that exaggerated behavioral sensitization would be observed when the cannabinoid was co-administered with cocaine. It thus became important to answer the question of whether CP 55,940 could actually attenuate the development of behavioral sensitization to cocaine. The results of the co-administration experiment did not support this position with behavioral sensitization clearly observed in rats intermittently injected with CP 55,940 plus cocaine. 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 cannabinoid was removed, a normal sensitized response to cocaine became apparent (Fig. 3).

One important caveat to discuss is the continuing doubt over whether cannabinoids exert a positively reinforcing effect in rats. Recently, we have found dose-dependent enhancement of anxiety in the predatory-odor avoidance and conditioned ultrasonic vocalization models in Wistar rats given CP 55,940 (Arnold and McGregor, unpublished data). Interestingly, Lewis rats failed to show significant cannabinoid-induced anxiety in either model. This of course does not imply that Lewis rats find cannabinoids rewarding—the evidence for that assertion is more contentious. Thus, while studies have shown lowering of self-stimulation thresholds (Gardner and Lowinson, 1991; Lepore et al., 1996), at least one other study has documented conditioned place aversions to Δ^9 THC in Lewis rats (Parker and Gillies, 1995). However, even if CP 55,940 were anxiogenic we might still expect it to produce sensitization to cocaine, in the same way that stressors such as footshock do (Kalivas and Duffy, 1989).

The present data invite speculation as to the possible neural mechanisms that may prevent the response to cannabinoids being sensitized. One possibility is that the doses of CP 55,940 here failed to increase dopamine efflux in the nucleus accumbens. However, this seems unlikely given previous findings that peripheral Δ^9 THC doses of 0.5 mg/kg increased dopamine efflux in the nucleus accumbens of Lewis rats (Gardner and Lowinson, 1991). Given estimates that CP 55,940 is approximately 30 times more potent than Δ^9-tetrahydrocannabinol (Gold et al., 1992; Little et al., 1988; Wiley et al., 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 dopamine levels.

5. Conclusion

The present study provides presumptive evidence against the notion that cannabinoids act as a 'gateway' towards cocaine. We do not claim that this demonstration is definitive and hope that other studies will be forthcoming on this issue. It may be important to test sensitization and cross-sensitization with other cannabinoid receptor agonists such as Δ^9-tetrahydrocannabinol and anandamide, since different cannabinoids are sometimes found to have different behavioral and neural effects (McGregor et al., 1998). It may also be worthwhile to test cross-sensitization between cannabinoids and drugs of abuse other than cocaine, such as heroin, nicotine and alcohol. In addition, we would welcome the extension of this work to the drug self-administration paradigm, to see whether cannabinoid pre-exposure might affect self-administration of opiates or psychomotor stimulants.

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