Chronic treatment with Δ⁹-tetrahydrocannabinol enhances the locomotor response to amphetamine and heroin. Implications for vulnerability to drug addiction

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Abstract

Cannabis sativa preparations are some of the most widely used illicit recreational drugs. In addition to their direct addictive potential, cannabinoids may influence the sensitivity to other drugs. The aim of the present study was to determine if a cross-sensitization between Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and other drugs (amphetamine and heroin) could be demonstrated. We examined the effects of a chronic treatment with Δ⁹-THC (0.6, 3 and 15 mg/kg, ip) on the locomotor response to amphetamine (1 mg/kg, ip) and heroin (1 mg/kg, ip). Chronic treatment with Δ⁹-THC resulted in tolerance to the initial hypothermic and anorexic effects. Pre-treatment with Δ⁹-THC increased the locomotor responses to amphetamine and heroin. This cross-sensitization was time-dependent as it was observed three days after the last injection of Δ⁹-THC for amphetamine, and a relatively long time after the end of chronic treatment (41 days) for heroin. Moreover, the enhanced response to amphetamine or heroin was noted in some individuals only: the high-responder rats (HR). These animals have previously been shown to be vulnerable to drug taking behaviors. It is hypothesised that repeated use of *Cannabis* derivates may facilitate progression to the consumption of other illicit drugs in vulnerable individuals. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Cannabis sativa preparations such as hashish or marijuana are the most widely abused illicit products in the world. The major psychoactive ingredient in *C. sativa*, Δ⁹-tetrahydrocannabinol (Δ⁹-THC), was isolated by Gaoni and Mechoulam (1964). Since then, a number of natural and synthetic compounds with cannabinominetic activity has been identified. However, the addictive properties of these cannabinoids are the subject of considerable debate.

In general, drugs abused by humans yield rewarding effects in animals as assessed by self-administration behaviors, place conditioning paradigms and intracranial self-stimulation. Cannabinoid agonists failed to induce self-administration in monkeys (Harris et al., 1974; Carney et al., 1977; Mansbach et al., 1994) and rats (Corcoran and Amit, 1974; Leite and Carlini, 1974), although they have been reported to be self-administered in mice (Martellota et al., 1998) and rats under certain conditions (Van Ree and De Wied, 1978; Takahashi and Singer, 1979; Singer et al., 1982; Pertwee, 1991).

Where one study (Lepore et al., 1995) reported both the conditioned place preference and place avoidance according to the dose of Δ⁹-THC used, others showed conditioned place aversion with cannabinoids (Parker and Gillies, 1995; McGregor et al., 1996; Sañudo-Peña et al., 1997; Chaperon et al., 1998; Mallet and Beninger, 1998). However, by avoiding the dysphoric consequences of Δ⁹-THC exposure, a clear place preference was demonstrated in mice (Valjent and Maldonado, 2000). Results with intracranial self-stimulation have also been contradictory. Gardner et al. (1988) found an

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enhancement of intracranial self-stimulation with Δ^9-THC, but the other authors did not (Wayner et al., 1973; Stark and Dews, 1980).

Drug dependence is characterized by physical and psychological changes when drug consumption is stopped. There is no clear-cut abstinence syndrome with cannabinoids in man, possibly because of the long half-lives of these compounds. However, use of a competitive cannabinoid receptor antagonist, SR141716A (Rinaldi-Carmona et al., 1994) evidenced a withdrawal syndrome in rats chronically treated with cannabinoids (Aceto et al., 1995; Tsou et al., 1995; Rodriguez de Foncesca et al., 1997; Hutcheson et al., 1998; Rubino et al., 1998).

A neurobiological effect shared by many drugs of abuse or reinforcing substances is their ability to stimulate dopaminergic activity in the nucleus accumbens (Wise and Bozarth, 1987; Koob and Bloom, 1988; Di Chiara, 1995). With the exception of the work of Castañeda et al. (1991) and Gifford et al. (1997), a number of studies have shown that cannabinoids enhance the electrical activity of dopamine neurons (French, 1997; French et al., 1997; Diana et al., 1998a) and dopaminergic transmission (Bloom et al., 1978; Bowers and Hoffman, 1986; Taylor et al., 1988; Chen et al., 1990a,b; Tanda et al., 1997) in the ventral tegmental area. Moreover, as observed on the withdrawal of addictive drugs (Diana et al., 1993, 1995), reduced dopaminergic neuronal activity and dopamine extracellular concentrations in the nucleus accumbens have been reported after the blockade of central cannabinoid receptors in rats chronically treated with Δ^9-THC (Diana et al., 1998b; Tanda et al., 1999).

In addition to their direct addictive potential, cannabinoids may thus influence the sensitivity to other drugs. An important issue is whether prior use of *Cannabis* derivatives facilitates progression to consumption of other illicit drugs (Kandel, 1975; Yamaguchi and Kandel, 1984), at least in certain vulnerable individuals presenting particular psychobiological characteristics. Although this has not been addressed in animal experiments, at least three lines of evidence warrant such an enquiry. (1) In common with drugs of abuse, cannabinoids act on dopaminergic systems (see above). (2) Cannabinoids interact with opioid systems (for review see Manzanares et al., 1999a). Cannabinoids have been found to activate mesolimbic dopamine release mediated by μ-opioid receptors (Chen et al., 1990a; Tanda et al., 1997), increasing the synthesis and/or the release of endogenous opioids (Kumar et al., 1990; Corchero et al., 1997; Manzanares et al., 1998), decreasing naloxone-induced opiate withdrawal in morphine-dependent animals (Vela et al., 1995) and potentiating opioid-induced analgesia (Welch and Stevens, 1992; Smith et al., 1998). It has also been shown that CB_1 cannabinoid receptors are involved in the motivational properties of opiates and in physical dependence (Ledent et al., 1999). (3) Canna-

binoids are potent activators of the hypothalamic–pituitary adrenal axis (HPA) (Jacobs et al., 1979; Dewey, 1986; Elridge et al., 1991; Rodriguez de Foncesca et al., 1995; Corchero et al., 1999; Manzanares et al., 1999b) and we have repeatedly found that enhanced corticosteroid secretion increases the sensitivity to addictive drugs (Piazza et al., 1991a; Deroche et al. 1992, 1993).

Cross-sensitization between cannabinoids and other addictive drugs remains to be demonstrated. The sole existing data pertaining to a possible interaction between cannabinoids and behavioral response to psychostimulants are contradictory. While Gorriti et al. (1999) reported an enhanced effect of amphetamine on locomotor and stereotyped responses following treatment with Δ^9-THC, Arnold et al. (1998) and Ferrari et al. (1999) did not observe any cross-sensitization between cannabinoids and cocaine.

In the present study carried out in rats, we have examined whether chronic treatment with Δ^9-THC led to a behavioral locomotor cross-sensitivity to the two addictive drugs, amphetamine and heroin. It is difficult to generalize the results obtained with cannabinoids for humans in view of the relatively high doses used and the short duration of treatment. This prompted us to employ low doses of Δ^9-THC (0.6 mg/kg) and chronic treatment (up to 4 weeks). In addition, we tested whether there were interindividual differences in the action of Δ^9-THC. Two populations of animals were studied: we have previously shown that high-responder (HR) and low-responder (LR) rats differ in various behavioral, neurochemical and neuroendocrinological characteristics (Piazza et al., 1989, 1991a,b, 1993) and more specifically in their vulnerability to addictive behaviors.

2. Materials and methods

2.1. Animals

One hundred and eighty male Sprague Dawley rats (Iffa Credo, St. Germain sur Arbresle, France), weighing between 180 and 200 g at the beginning of the experiments were used. They were housed four per cage under standard conditions (12 h light–dark cycle; room temperature 22°C; humidity 60%) with food and water available ad libitum. All testing occurred during the light phase.

2.2. Apparatus

It consisted of a circular corridor (10 cm wide, with walls 50 cm high and 55 cm in diameter). Four photoelectric cells placed at the perpendicular axes of this apparatus automatically recorded locomotion. A single locomotor count was recorded by a microprocessor when each beam was broken (Piazza et al., 1989).

2.3. Drugs

Δ^9-THC (Sigma–Aldrich) dissolved in 100% ethanol (200 mg/ml) was diluted in Tween 80 and saline (NaCl 0.9%) to make a final vehicle solution of ethanol/Tween 80/saline (1:1:18). Heroin (Sanofi, Paris, France) and d-amphetamine sulfate (Calaire chimie, Calais, France) were dissolved in isotonic NaCl (0.9%). The drugs doses were expressed as the weight of the salt. All drugs were injected intraperitoneally in an injection volume of 1 ml/kg.

2.4. Procedure

2.4.1. Selection of animals according to their locomotor reactivity

The animals (n=180) were selected according to their level of locomotor reactivity to novelty in the circular corridor. The locomotor response was recorded for 10 min intervals over a period of 2 h between 9:00 and 11:00 h. The score of each animal (number of photocell counts) cumulated over this period was used as an index of individual reactivity to the novel environment (Piazza et al., 1989; Taghzouti et al., 1999). Two groups of animals were formed: (1) A low-responder group (LR) with scores more than 5 standard deviations below the group's mean; and (2) a high-responder group (HR) with scores more than 5 standard deviations above the mean. Each group was then subdivided into four subgroups (control, 0.6, 3 and 15 mg/kg Δ^9-THC) with equal mean levels of locomotor activity.

2.4.2. Experimental design (Fig. 1)

The chronic cannabinoid treatment lasted three weeks during which time the rats received 20 injections. These injections were not regularly delivered throughout this period; the rats receiving each day: one, two or no injection at all. This protocol was chosen as it is closer to the most common type of irregular smoking practices in man. The effects of Δ^9-THC on body temperature and on eating and drinking behaviors were measured at the

beginning (day 1), about the middle (day 13) and at the end (day 20) of this chronic treatment period.

Three days after the 20th day of chronic treatment with Δ^9-THC, all the groups were tested for their loco-motor response to heroin (1 mg/kg, ip) (day 23). From the following day, rats received a further series of seven injections of Δ^9-THC. Three days after the last day (day 29) of chronic treatment (i.e. 27th injection), they were tested for their locomotor response to amphetamine sulf-ate (1 mg/kg, ip) (day 32).

Five weeks later, rats were tested a second time for their locomotor response to heroin (1 mg/kg, ip) (day 70) and then two weeks later for their locomotor response to amphetamine sulfate (1 mg/kg, ip) (day 84).

Eating and drinking behaviors were also evaluated after the chronic treatment, on days 29 and 64.

2.4.3. Locomotor response to drugs

Animals were placed in the circular corridor at 9 a.m. After a 2 h period of habituation, all animals were injected intraperitoneally with saline. One hour and thirty minutes later, the animals were injected with her-oin or amphetamine sulfate and immediately replaced in the apparatus. Locomotor activity was recorded for 3 H over 10 min intervals. This experimental procedure was used for the two locomotor responses to heroin (at differ-ent times after Δ^9-THC treatment: days 23 and 70) and the two locomotor responses to amphetamine (days 32 and 84).

2.4.4. Body temperature, eating and drinking

The body temperature of each rat was monitored by a thermistor rectal probe. Rats were first habituated to the rectal probe. The temperature was measured 15 min before drug treatment and at several times (30, 60, 120 min) following administration of the drug or vehicle.

The eating and drinking behaviors were measured in cages containing four rats. Food and bottles of water were weighed at fixed times. Food spillage was collected on a paper placed under the grid floor of each cage and was weighted to calculate the food actually consumed.
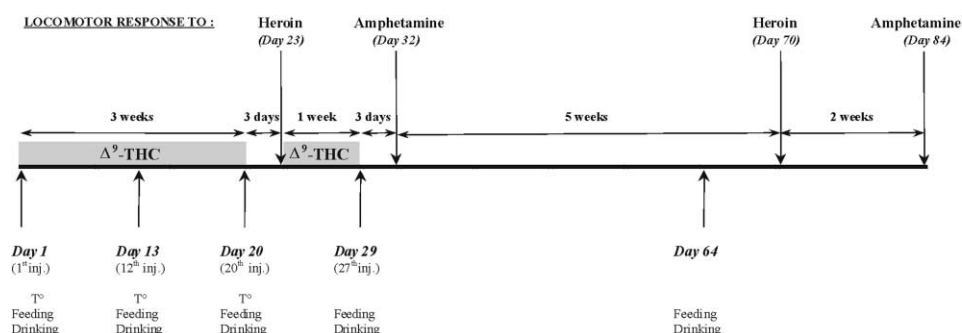

Fig. 1. Experimental design: description of the chronic treatment with Δ^9-THC. Body temperature ($T°$), eating and drinking behaviors were measured at different times. Locomotor response to heroin and amphetamine were tested at different intervals after the chronic cannabinoid treatment.

Loss of water when our animals drank was judged negligible.

2.5. Data analysis

2.5.1. Body temperature and eating behavior

Statistical analysis for each experiment involved one-way and/or repeated measures analysis of variance (ANOVA). Simple main effect was used for comparisons between the groups at different times. Dunnett's test was used for post hoc analysis when ANOVA was significant.

2.5.2. Locomotor activity

The normality of the 2 groups (LR and HR rats) was verified using Shapiro–Wilk's test. In each group (intragroup), locomotor response to drugs was analyzed using the Kruskal–Wallis nonparametric rank test with Δ^9-THC dose as the independent variable. Post hoc comparisons were based on nonparametric pairwise comparison versus controls. Mann–Whitney's test was used for comparison (intergroup) between LR and HR rats treated with the same dose of Δ^9-THC.

3. Results

3.1. Individual locomotor reactivity and selection of animals

The animals were selected according to their locomotor reactivity to novelty. Among our rats ($n=180$), marked interindividual variability was observed with scores ranging from 297 to 1569 counts in 2 h (mean±sem: 737±18). According to their scores of locomotor activity, eight groups of rats were formed: LR (controls), 477±25; LR (Δ^9-THC, 0.6 mg/kg), 464±19; LR (Δ^9-THC, 3 mg/kg), 476±17; LR (Δ^9-THC, 15 mg/kg), 510±15; HR (controls), 1124±65; HR (Δ^9-THC, 0.6 mg/kg), 1081±57; HR (Δ^9-THC, 3 mg/kg), 1085±65; HR (Δ^9-THC, 15 mg/kg), 1051±55. The normality of the 2 groups was verified: LR, $w=0.93$, $P<0.024$; and HR, $w=0.91$, $P<0.006$.

3.2. Body temperature

The time course of body temperature following administration of Δ^9-THC at different times during the chronic Δ^9-THC treatment (days 1, 3 and 20) is shown in Fig. 2.

At the beginning of the chronic treatment (i.e. after the first injection), Δ^9-THC had a dose-dependent effect on the body temperature in both LR and HR rats (ANOVA, LR: $F(3,38)=9.605$, $P<0.001$; HR: $F(3,38)=24.75$, $P<0.001$). The change in body temperature depended on the dose of Δ^9-THC used (ANOVA,

LR: $F(9,114)=11.06$, $P<0.001$; HR: $F(9,114)=12.52$, $P<0.001$) was significantly modified to 30, 60 and 120 min after Δ^9-THC injection (LR and HR, Simple Main Effect, $P<0.001$).

The highest dose of Δ^9-THC (15 mg/kg) produced a pronounced hypothermia on the first day for the two subgroups (LR and HR 15 mg/kg). Two hours after administration of Δ^9-THC, the hypothermia was always statistically significant (at T120, LR 15 mg/kg, ANOVA: $F(3,38)=6.64$, $P<0.001$; Dunnett, $P<0.05$ and HR 15 mg/kg, ANOVA: $F(3,38)=15.12$, $P<0.001$; Dunnett, $P<0.01$).

The dose of 3 mg/kg Δ^9-THC induced less hypothermia in the HR subgroup. This decrease was statistically significant 60 min following administration (ANOVA: $F(3,38)=42.78$, $P<0.001$; Dunnett, $P<0.01$). Two hours after the injection, the temperature had returned to that of the controls (ANOVA: $F(3,38)=15.12$, $P<0.01$; Dunnett, n.s).

The lowest dose of Δ^9-THC (0.6 mg/kg) had no effect on the body temperature in the LR subgroup. In contrast, in the HR subgroup, this dose induced a small but significant hyperthermia compared with controls, 30 min (ANOVA: $F(3,38)=21.47$, $P<0.001$; Dunnett, $P<0.05$) and 60 min after drug administration (ANOVA: $F(3,38)=42.78$, $P<0.001$; Dunnett, $P<0.05$).

On day 13 of the chronic treatment, there was no significant effect of Δ^9-THC treatment on the body temperature in either LR or HR groups (LR group, ANOVA: $F(3,38)=1.99$, n.s; and HR group, ANOVA: $F(3,38)=1.83$, n.s), indicative of tolerance to the Δ^9-THC-induced hypothermia and hyperthermia.

On day 20 of the chronic treatment, there was no effect of Δ^9-THC on the body temperature in LR rats irrespective of the dose (ANOVA: $F(3,38)=1.25$, n.s). However, in HR rats, ANOVA showed a significant group effect (ANOVA: $F(3,38)=4.15$, $P<0.01$). The time course of body temperature differed according to the Δ^9-THC treatment (ANOVA: $F(9,114)=3.37$, $P<0.001$). The dose of 3 mg/kg Δ^9-THC caused a significant increase in body temperature compared with the control rats at 30 min (ANOVA: $F(3,38)=3.62$, $P<0.05$; Dunnett, $P<0.05$) and 60 min (ANOVA: $F(3,38)=3.71$, $P<0.05$; Dunnett, $P<0.01$) following administration.

3.3. Eating and drinking

During the chronic Δ^9-THC treatment, food intake depended on the dose injected in both LR and HR groups (LR, ANOVA: $F(15,20)=3.85$, $P<0.01$; and HR, ANOVA: $F(15,20)=2.92$, $P<0.01$) (Fig. 3).

At the beginning of the chronic treatment (i.e. after the first injection), the highest dose of Δ^9-THC (15 mg/kg) induced a marked decrease in food intake in the two subgroups (LR, ANOVA: $F(3,4)=37.01$, $P<0.01$; Dunnett, $P<0.01$; and HR, ANOVA: $F(3,4)=11.94$, $P<0.05$;
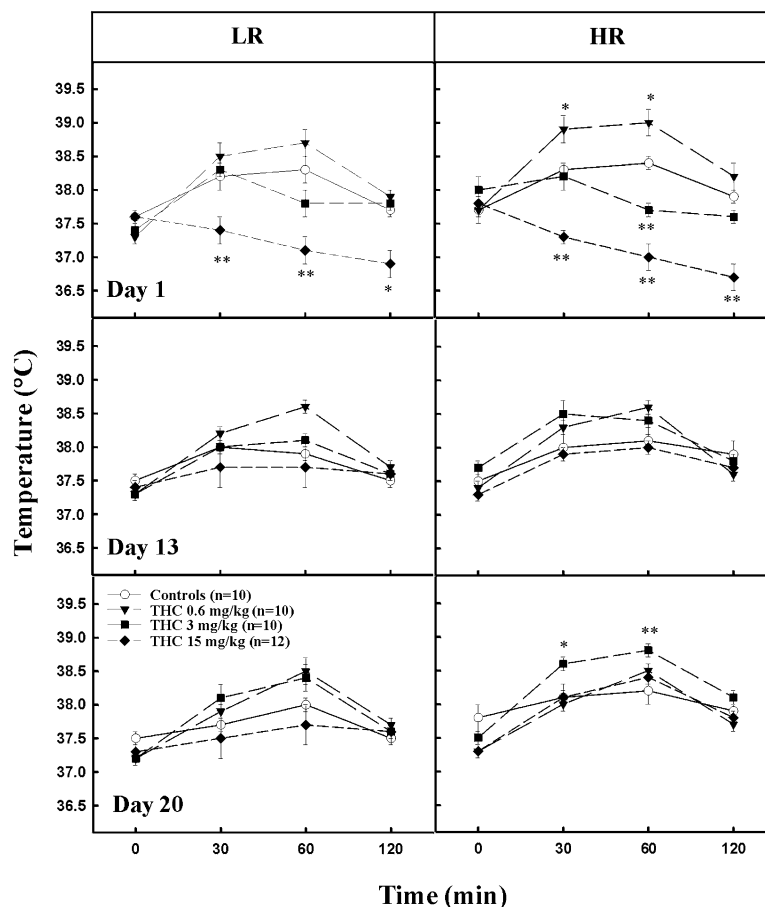
Fig. 2.   Effect of Δ^9-THC (0.6, 3 and 15 mg/kg) on the body temperature at different times during the chronic treatment in LR and HR groups. An acute administration induces hypothermia or hyperthermia depending on the dose of Δ^9-THC. A development of tolerance was observed by day 13 of the chronic treatment with Δ^9-THC. Dunnett, $*P<0.05$, $**P<0.01$.

Dunnett, $P<0.05$). The intermediate dose of Δ^9-THC (3 mg/kg) only led to a significant decrease in food intake in the LR subgroup ($F(3,4)=37.01$, $P<0.01$; Dunnett, $P<0.05$). The lowest dose of Δ^9-THC (0.6 mg/kg) had no effect (LR, ANOVA: $F(3,4)=37.01$, $P<0.01$; Dunnett, n.s; and HR, ANOVA: $F(3,4)=11.94$, $P<0.05$; Dunnett, n.s).

On day 13 of the chronic treatment, only the LR subgroups treated with the two highest doses of Δ^9-THC (3 and 15 mg/kg) ate less than the controls (ANOVA: $F(3,4)=594.8$, $P<0.001$; Dunnett, LR 3 mg/kg $P<0.01$, LR 15 mg/kg $P<0.01$).

On day 20 of the chronic treatment with Δ^9-THC, food intake of the LR and HR did differ from that of the controls (ANOVA, LR, $F(3,4)=1.88$, n.s; HR, $F(3,4)=1.48$, n.s).

After the end of the chronic treatment (days 29 and 64), food intake was not significantly different from controls in either LR or HR groups for all doses of Δ^9-THC (ANOVA, LR, day 29, $F(3,4)=0.88$, n.s; day 64, $F(3,4)=0.57$, n.s; HR, day 29, $F(3,4)=1.26$, n.s; day 64, $F(3,4)=0.21$, n.s).

None of the doses of Δ^9-THC had any effect on the water intake (data not shown) at any time.

3.4. Locomotor response to drugs

3.4.1. Locomotor response to heroin (Fig. 4)
The locomotor response to heroin (1 mg/kg, ip) was evaluated at two different times after chronic treatment with Δ^9-THC: 3 days after day 20 (20th injection); and 41 days after day 29 (27th and last injection).

At a short time (3 days) after Δ^9-THC treatment, analysis of heroin-induced total locomotor activity did not show any effect of the Δ^9-THC treatment in either LR or HR groups, irrespective of the dose considered (LR and HR, Kruskal–Wallis, n.s) (Fig. 4A).

At a longer interval (41 days) after Δ^9-THC treatment, no difference in the total locomotor response (2 h) to heroin was observed in LR rats for all doses of Δ^9-THC (Kruskal–Wallis, n.s). On the contrary, in HR rats, the total locomotor response to heroin was Δ^9-THC dose dependent (Kruskal–Wallis, $H(3)=10.84$, $P<0.01$). Rats chronically treated with 0.6 and 3 mg/kg of Δ^9-THC
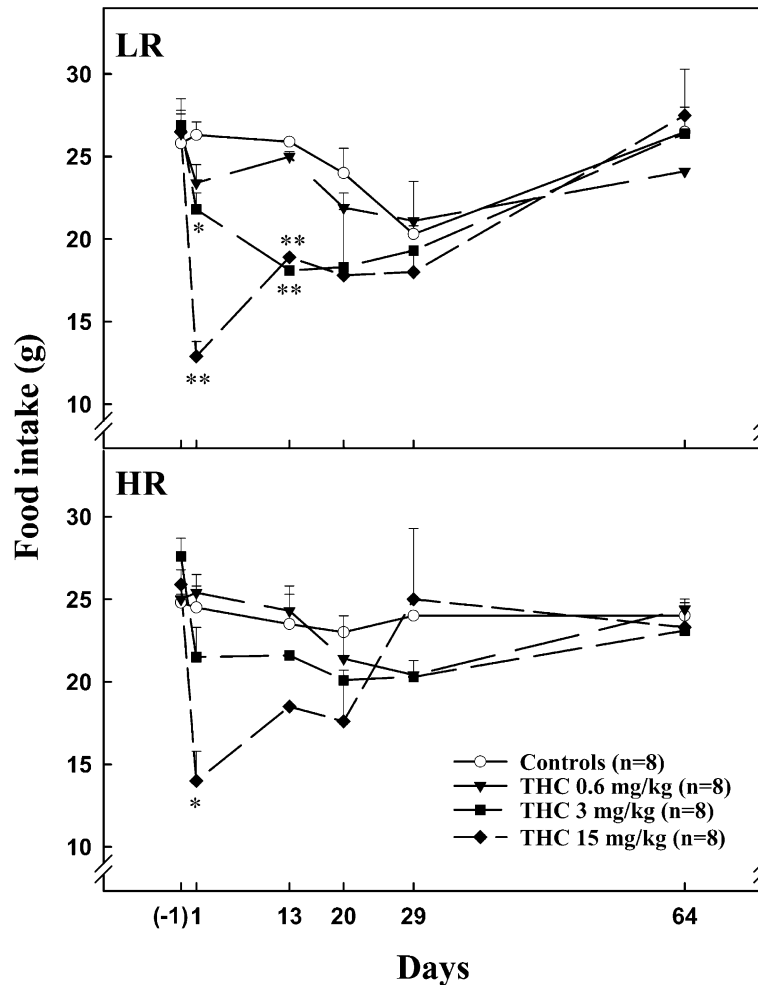
Fig. 3.   Effect of Δ^9-THC (0.6, 3 and 15 mg/kg) on eating behavior at different times during and after the chronic treatment. Δ^9-THC reduced food intake in both LR and HR rats. A development of tolerance to this effect was seen, at the latest, by day 20 of the chronic treatment. Dunnett, *$P<0.05$, **$P<0.01$.

exhibited an enhanced total locomotor response to heroin (HR 0.6 mg/kg: 1651±150; HR 3 mg/kg: 1698±375) compared with the vehicle pre-treated group (HR: 792±125) (nonparametric pairwise comparisons Δ^9-THC dose vs controls: HR 0.6 mg/kg vs HR controls, $P<0.001$ and HR 3 mg/kg vs HR controls $P<0.01$) (Fig. 4B). For each Δ^9-THC dose, the between comparison LR vs HR, showed that the heroin-induced total locomotor activity was greater in HR rats (Mann–Whitney, LR 0.6 mg/kg vs HR 0.6 mg/kg, $U=5.0$, $P<0.001$; and LR 3 mg/kg vs HR 3 mg/kg, $U=15.0$, $P<0.05$). These results showed that pre-exposure to Δ^9-THC increased the sensitivity to the subsequent behavioral effects of heroin in the HR rats only.

3.4.2. Locomotor response to amphetamine (Fig. 5)

Locomotor response to amphetamine (1 mg/kg, ip) was characterized at two different times after chronic treatment with Δ^9-THC: 3 and 55 days after day 29 (27th injection).

Three days after the Δ^9-THC treatment, no significant difference in the total locomotor response to amphetamine was found between the LR groups pre-treated with Δ^9-THC and vehicle (Kruskal–Wallis, n.s).

On the other hand, HR rats pre-exposed to 0.6 and 15 mg/kg of Δ^9-THC had a higher total locomotor response to amphetamine (HR 0.6 mg/kg: 1896±241 and HR 15 mg/kg: 1911±168) than rats pre-exposed to vehicle (HR: 1135±212) (Kruskal–Wallis, $H(3)=7.27$, $P<0.05$; nonparametric pairwise comparisons of Δ^9-THC dose vs controls: HR 0.6 mg/kg vs HR controls, $P<0.05$ and HR 15 mg/kg vs HR controls, $P<0.01$) (Fig. 5A). The comparison between LR and HR revealed that, only HR rats treated with 15 mg/kg of Δ^9-THC had a higher locomotor response to amphetamine than the LR rats treated with the same dose (Mann–Whitney, LR 0.6 mg/kg vs HR 0.6 mg/kg, $U=25$, n.s and LR 15 mg/kg vs HR 15 mg/kg, $U=21.5$, $P<0.01$). These results showed that pre-exposure to Δ^9-THC increased the locomotor response induced by amphetamine only in the HR rats.
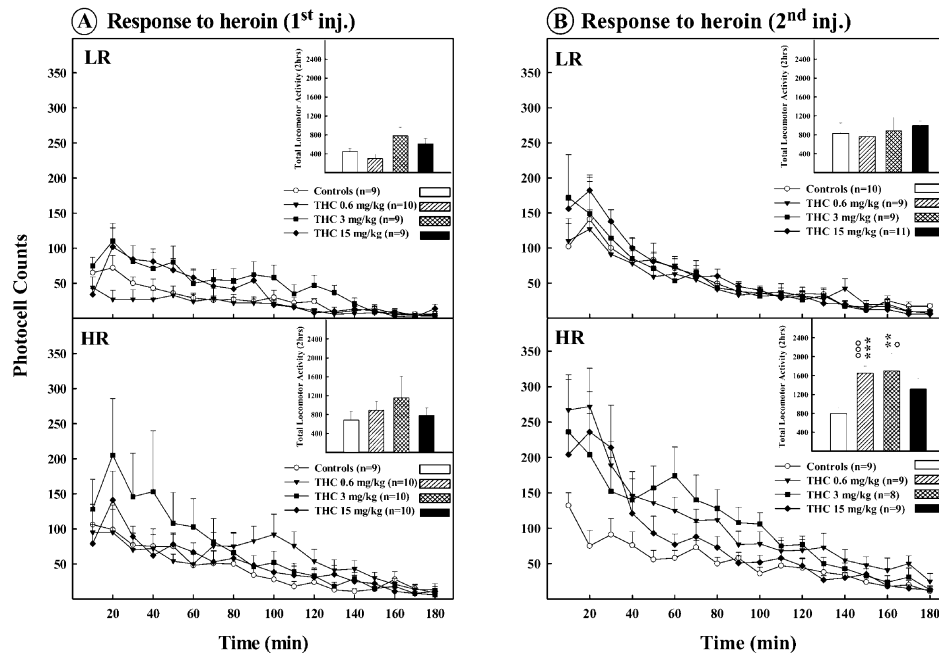
Fig. 4. Effect of pre-treatment with Δ^9-THC (0.6, 3 and 15 mg/kg) on the locomotor response to heroin measured at a short time (A) or at a long time (B) after Δ^9-THC treatment. Locomotor activity was recorded 3 h over 10 min intervals following heroin administration (1 mg/kg, ip). Total locomotor activity during the first 2 h is shown in the inset. For the first locomotor response to heroin, the LR and HR subgroups pre-treated with Δ^9-THC did not differ from control rats. In contrast, for the second locomotor response to heroin, pre-treatment with Δ^9-THC (0.6 and 3 mg/kg) induced a significant increase in locomotor activity compared with the vehicle pre-treated group in HR rats. Nonparametric pairwise comparisons Δ^9-THC dose vs controls, $**P<0.01$, $***P<0.001$. Between comparisons LR vs HR for each dose, Mann–Whitney, $^\circ P<0.05$, $^{\circ\circ\circ}P<0.001$.

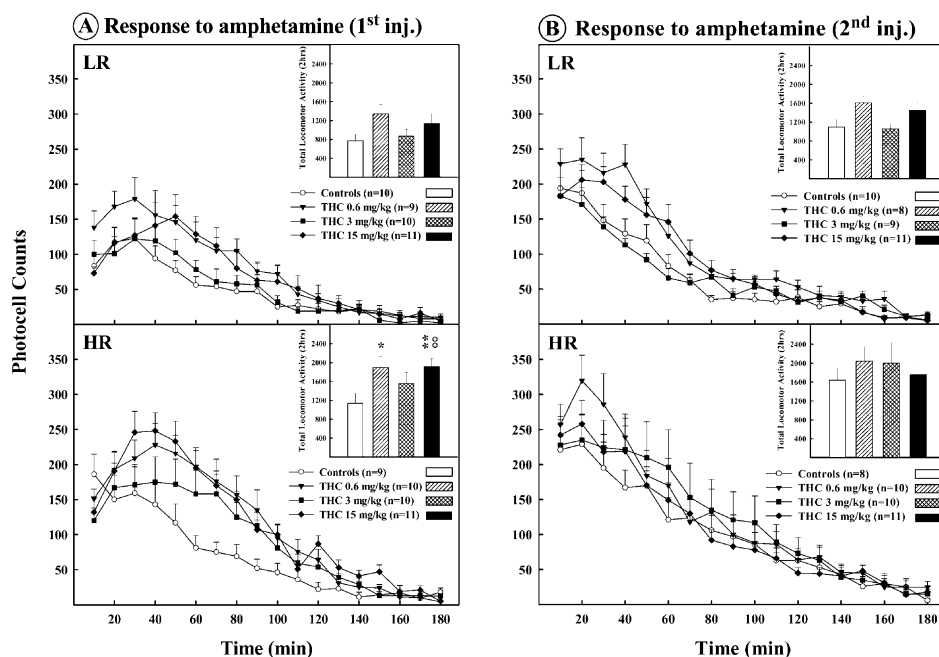
Fig. 5. Effect of pre-treatment with Δ^9-THC (0.6, 3 and 15 mg/kg) on the locomotor response to amphetamine sulfate measured at a short time (A) or at a long time (B) after Δ^9-THC treatment. Locomotor activity was recorded for 3 h over 10 min intervals after amphetamine injection (1 mg/kg, ip). Total locomotor activity during the first 2 h is shown in the inset. For the first locomotor response to amphetamine, only the HR rats pre-exposed to 0.6 and 15 mg/kg Δ^9-THC had an enhanced locomotor response to amphetamine compared with the control rats. For the second locomotor response to amphetamine, there were no differences between the subgroups pre-treated with Δ^9-THC and those pre-exposed to vehicle. Non parametric pairwise comparisons Δ^9-THC dose vs controls, $*P<0.05$, $**P<0.01$. Between comparisons LR vs HR for each dose, Mann–Whitney, $^{\circ\circ}P<0.01$.

At a longer time (55 days) after Δ^9-THC treatment, no significant effect of amphetamine on the total locomotor activity was observed irrespective of the dose (LR and HR, Kruskal–Wallis, n.s) (Fig. 5B).

4. Discussion

The present results showed that chronic treatment with Δ^9-THC enhanced the locomotor response to heroin and amphetamine. This cross-sensitization was associated with the development of a tolerance to the initial hypothermic and anorexic effects.

Hypothermia is commonly observed after an acute administration of Δ^9-THC (Holtzmann et al., 1969; Fennessy and Taylor, 1977; Davies and Graham, 1980). However, we noted an hyperthermic response after the lowest dose of Δ^9-THC (0.6 mg/kg, ip). This biphasic effect is consistent with the observations of Sofia (1972) and Taylor and Fennessy (1977), who found that low doses (0.5 and 1 mg/kg, ip, or 0.05 and 0.1 mg/kg, iv) caused hyperthermia, while higher doses (4 and 8 mg/kg, ip, or 1 to 5 mg/kg, iv) produced a profound and dose-dependent hypothermic effect.

In agreement with the other authors (Lomax, 1971; Ten Ham and De Jong, 1974; Anderson et al., 1975; Taylor and Fennessy, 1978), we observed a tolerance to the hypothermic action of Δ^9-THC after repeated injections.

After the development of tolerance for both the hypothermic and hyperthermic effects of Δ^9-THC, an hyperthermic effect reappeared in the HR rats treated with 3 mg/kg. Although the physiological mechanism involved is unclear, this effect is consistent with the results obtained in rats made tolerant to the hypothermic action of Δ^9-THC (Lomax, 1971; Taylor and Fennessy, 1978).

In agreement with the other authors (Manning et al., 1971; Elsmore and Fletcher, 1972; Järbe and Henriksson, 1973; Sjödén et al., 1973; Sofia and Barry, 1974), we observed a marked reduction in food intake after an acute administration of Δ^9-THC, which disappeared progressively. After the 20th injection, food consumption of rats treated with Δ^9-THC did not differ from that of the controls. The development of tolerance to the anorexogenic effect of Δ^9-THC was not found by Sofia and Barry (1974). This can perhaps be explained by the fact that these authors used a shorter chronic treatment (9 days). It can be noted that the tolerance to the anorexogenic effect of Δ^9-THC developed more slowly than did the tolerance to the hypothermic effects.

The major finding of this study is that pre-treatment with Δ^9-THC enhanced the locomotor response to heroin and amphetamine. This cross-sensitization was time-dependent as it was observed early with amphetamine (3 days) but later for heroin (41 days) after chronic treatment with Δ^9-THC. Moreover, this cross-sensitization differed between individuals, as it was observed only in the HR group.

The cross-sensitization between Δ^9-THC and heroin was observed some time after the end of Δ^9-THC treatment (41 days), possibly owing to the time lag in the neurobiological mechanisms involved. Nevertheless, alternatives explanations can be proposed: (1) the time interval (3 days) between the last administration of Δ^9-THC and the first administration of heroin was short. It is known that Δ^9-THC has a relatively long half-life and depresses locomotor activity (Pryor et al., 1978; Tulunay et al., 1982), which may thus have masked the behavioral expression of sensitization. However, this explanation is not supported by the results obtained for the locomotor response to amphetamine. (2) There may not have been enough injections of Δ^9-THC to induce sensitization. However, the animals received 20 injections over a three-week period which is a relatively long time in comparison with the experimental design generally used to study behavioral sensitization.

For the locomotor response to amphetamine, we obtained the mirror image to that found with heroin. The cross-sensitization between Δ^9-THC and amphetamine was observed within three days after the end of Δ^9-THC treatment, but had disappeared 55 days later. This disappearance may correspond to the duration of the neurobiological mechanisms involved in the behavioral sensitization, although it can be hypothesised that the sensitization phenomenon had not really disappeared. The fact that Δ^9-THC-treated animals responded no better than the controls might be because of the increased response of the vehicle-treated rats between the first and the second injection of amphetamine. Vehicle animals appeared to exhibit a behavioral sensitization in response to the second injection of amphetamine. The enhanced locomotor response to amphetamine after a chronic treatment with Δ^9-THC is in partial agreement with the results of Gorriti et al. (1999), but contradicts the results of a previous study (Pryor et al., 1978). Some discrepancies may stem from the schedule of Δ^9-THC and amphetamine injections between studies. When the two substances are administered within a short time interval, the locomotor response to amphetamine may be masked by alterations in autonomic functions induced by Δ^9-THC.

It is noteworthy that the enhanced locomotor response to heroin and amphetamine could not be attributed to the anorexogenic effects of Δ^9-THC. While food deprivation is known to enhance the locomotor response to some addictive drugs (Campbell and Fibiger, 1971), we found that Δ^9-THC had no effect on food or water intake when the locomotor responses to drugs were measured.

The cross-sensitization between Δ^9-THC and heroin is consistent with an interaction of cannabinoids with brain opioids systems (see Section 1). Neuroanatomical

studies have shown that CB1 receptors are co-expressed with μ-opioid receptors in the basal ganglia (Navarro et al., 1998). Cannabinoids and opioids may interact at the level of signal-transduction mechanisms as both types of receptors are coupled to similar intracellular transduction systems; a decrease in c-AMP production via activation of Gi proteins (for review see Manzanares et al., 1999a).

Two opposite hypothesis can account for the cross-sensitization observed between Δ^9-THC and amphetamine. First, the endocannabinoid system may act as an inhibitory feedback mechanism counteracting the increase in motor activity by agonists of dopaminergic transmission (Beltramo et al., 2000; Giuffrida et al., 1999). Down-regulation of CB1 cannabinoids receptors resulting from chronic treatment with Δ^9-THC may dampen the ability of endogenous cannabinoids to counteract the activity of dopaminergic neurons thereby enhancing their responsiveness. However, down-regulation of CB1 receptors has not been observed in the limbic regions (Romero et al., 1998), especially the nucleus accumbens, the brain structure implicated in the locomotor response to amphetamine. Moreover, anandamide formation is shown to decrease in the striatum, whereas it is enhanced in the limbic region following chronic treatment with Δ^9-THC (Di Marzo et al., 2000).

Secondly, Δ^9-THC has shown to enhance dopaminergic activity (see Section 1). In addition, anandamide release is increased following the stimulation of dopaminergic D2-like receptors (Giuffrida et al., 1999). Thus, the effect of amphetamine on dopaminergic neurons could be potentiated by Δ^9-THC, resulting in an enhanced locomotor response. Interestingly, it was found that following long-term exposure to Δ^9-THC, dopaminergic neurons of the ventral tegmental area continue to be activated in contrast to those of the substantia nigra (Wu and French, 2000).

The last finding in the present study is that the enhanced response to heroin and amphetamine was observed only in some individuals: the high-responder rats. This differential sensitivity, noted with addictive drugs, was not observed for the other effects of Δ^9-THC such as hypothermia or anorexia. These data support the idea that the enhanced response to heroin and amphetamine is not the result of metabolic alterations induced by Δ^9-THC. The differences in response between HR and LR were not thought to be because of differences in pharmacokinetics or blood–brain barrier penetration of Δ^9-THC.

Interestingly, HR animals were found to be prone to develop addictive behavior (Piazza et al., 1989, 1991a,b) suggesting that Δ^9-THC and addictive drugs act directly or indirectly on a common neurobiological system. The mechanism by which Δ^9-THC leads to a cross-sensitization with heroin and amphetamine remains to be discovered, but it is possible that dopaminergic neurons and/or the pituitary/hypothalamus axis are involved. Indeed,

these biological systems are modified by Δ^9-THC (see Section 1) and their activity differs between HR and LR animals (Piazza et al., 1991a,b).

In conclusion, the present study showed a cross-sensitization between Δ^9-THC and heroin or amphetamine. Moreover, the enhanced effects of these two addictive drugs was evidenced only in HR animals, i.e. individuals vulnerable to drug taking behaviors. These findings suggest that long-term use of *Cannabis* in humans might enhance the vulnerability to addictive drugs in certain individuals.

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