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Locomotor effects of morphine or alcohol in mice after a repeated treatment with the cannabinoid agonist HU 210

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ABSTRACT

The consequences of the consumption of cannabinoids with other drugs of abuse are of particular medical relevance. Several studies investigated the ability of cannabinoids to induce a locomotor cross-sensitization to other addictive drugs, but results remain inconsistent. Therefore, we investigated in mice the consequences of a repeated treatment with the cannabinoid agonist HU 210 on motor effects of morphine or alcohol. In mice receiving a daily injection of HU 210 (12.5 to 200 μg/kg) during 7 days, no hetero-sensitization to the stimulation induced by either morphine (7.5 mg/kg) or alcohol (1 or 1.5 g/kg) emerged, from 1 day up to 35 days after the end of the sub-chronic treatment with HU 210. Even a chronic treatment with a high dose of HU 210 (14 days, 200 μg/kg) induced no subsequent enhancement of the stimulant effects of morphine or alcohol. In fact, the motor stimulant effect of morphine or alcohol in chronically HU 210 pre-treated mice was even abolished until the 3rd day of abstinence. This reduction was presumably due to residual HU 210 since this effect was prevented by the cannabinoid antagonist rimonabant. Afterwards, chronically cannabinoid pre-treated mice remained less active than vehicle pre-treated mice from the 7th day up to the 35th day after the end of the 14-day treatment with HU 210. In conclusion, we failed to detect any hetero-sensitization whatever the pre-treatment regimen. However, only after the 14-day regimen, HU 210 pre-treated mice displayed a long-lasting decrease in activity, suggesting that some neuronal adaptive changes may have occurred.

1. Introduction

Cannabis derivatives, such as marijuana and hashish, are among the most consumed illicit drugs of abuse over the world within adolescents and young adults. Moreover, many cannabis consumers simultaneously use other drugs of abuse. Cannabis consumption is often associated with alcohol ingestion (Degenhardt et al., 2001). Furthermore, the use of cannabis often precedes the consumption of other illicit drugs of abuse including opiates and therefore, cannabis may provide a gateway leading its users to consume other drugs of abuse (Fergusson et al., 2006). Consequently, health issues surrounding the consumption of cannabis derivatives and especially multiple drug abuse remain of major interest.

The main psychoactive constituent in cannabis preparations, Δ-9 tetrahydrocannabinol (Δ-9 THC), is responsible for the rewarding properties of marijuana. Major central effects of cannabinoid agonists are mainly mediated through cannabinoid CB_1 type receptors, which are abundant in key regions of the reward system activated by most drugs of abuse (Mailleux and Vanderhaeghen, 1992).

A growing body of evidence supports the existence of functional interactions between cannabinoid and opioid systems (Manzanares et al., 1999; Corchero et al., 2004; Vigano et al., 2005 for reviews). Recent data have suggested a permissive role of the brain endocannabinoid system in the mediation of both rewarding/reinforcing properties and motor activating effects of opiates. (Fattore et al., 2005, Solinas et al., 2007 for recent reviews). For instance, the invalidation of the gene encoding the cannabinoid CB_1 receptor results in a lack of sensitization to the motor stimulant effect of morphine (Martin et al., 2000). These changes can be, at least in part, linked to the fact that the morphine-induced dopamine release is abolished in the nucleus accumbens of cannabinoid CB_1 receptor knockout mice (Mascia et al., 1999).

The endocannabinoid system has also been involved in the reinforcing effects of alcohol as well as in alcohol-seeking behaviours (Colombo et al., 2005; Manzanares et al., 2005; Basavarajappa, 2007 for reviews). It has also been reported that the alcohol-induced locomotor activation is abolished in cannabinoid CB_1 receptor knock-out mice (Naassila et al., 2004). In contrast, we found that the cannabinoid agonist HU 210 antagonizes the acute stimulant effect of alcohol while rimonabant enhances the stimulation induced by low doses of alcohol (Hagues et al., 2007).

Repeated administration of addictive substances in rodents produces a progressive and enduring increase in the motor stimulant effects induced by a subsequent drug challenge, the so-called "behavioural sensitization". This process has been proposed to underlie certain aspects of drug addiction and adaptive changes in the mesocorticolimbic dopaminergic systems have consistently been associated with behavioural sensitization (Kalivas and Stewart, 1991;

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Robinson and Berridge, 1993; De Vries et al., 1998; Vanderschuren and Kalivas, 2000). Cannabinoids share with other drugs of abuse the ability to stimulate the mesolimbic dopaminergic transmission leading to an enhancement of the dopamine extracellular level in the nucleus accumbens (Gardner, 2005 for review). This common action on dopaminergic pathways may at least partly underlie a possible cannabis gateway effect.

Whether or not repeated exposure to cannabinoid agonists result in behavioural sensitization to their own effects is still a matter of debate (Arnold et al., 1998; Cadoni et al., 2001; Rubino et al., 2001; Kolb et al., 2006; Varvel et al., 2007). Furthermore, although a key role of the endocannabinoid pathway in mediating the effects of numerous drugs of abuse has been clearly established (Maldonado et al., 2006 for review), data remain rather controversial about a long-term modulation of the reinforcing properties or the motor activating effects of these latter by cannabinoid agonists. For example, a chronic treatment with Δ-9 THC fails to enhance the motor-activating properties of morphine (Valverde et al., 2001; Jardinaud et al., 2006). In contrast, previous studies have reported the development of a locomotor cross-sensitization to opiates after a chronic exposure to cannabinoid agonists (Cadoni et al., 2001; Lamarque et al., 2001; Pontieri et al., 2001; Norwood et al., 2003; Singh et al., 2005). Furthermore, a chronic combination of morphine with CP 55,940 increases the subsequent stimulant effect of morphine, when compared with a chronic treatment with morphine alone (Norwood et al., 2003).

As these available results are not conclusive, the first goal of this study was to further investigate in mice to what extent a repeated treatment with a cannabinoid agonist could modulate the subsequent motor activating effect of morphine. The second goal was to evaluate, for the first time, whether a sensitization to the stimulant effect of alcohol could be established after a repeated cannabinoid treatment. For these purposes, we assessed the consequences of repeated treatments with HU 210, a potent cannabinoid agonist (Little et al., 1989; Ottani and Giuliani, 2001) on the motor activating effects of alcohol or morphine. As sensitization usually becomes more pronounced after a period of abstinence (Robinson and Berridge, 1993, Vanderschuren and Kalivas, 2000), we took care to measure morphine- or alcohol-induced motor activity at different latencies after the cessation of the cannabinoid treatment. In addition, to better characterize the impact of a cannabinoid pre-exposure on morphine and alcohol effects, we used two different treatment regimens, a sub-chronic (7 days) treatment using a wide range of doses of HU 210 and a chronic treatment (14 days) with a high dose of the cannabinoid.

2. Materials and methods

2.1. Animals

Male Swiss albino CD1 mice, weighing 20–22 g upon arrival (Iffa Credo, Charles River, St Germain sur l'Arbresle, France) were housed in groups of 20 in transparent Makrolon cages (38 cm × 24 cm × 18 cm) with food and water ad libitum. They were maintained under an artificial 12-h light:12-h dark cycle (lights on at 07:00 a.m.) and in controlled conditions of temperature (21 °C) and humidity (60%), at least 1 week before the beginning of the experiments. These latter were carried out between 09:00 a.m. and 05:00 p.m. in ventilated and sound-attenuated rooms. This study was performed in accordance with the guidelines for the use of animals in research of the European Community Council Directive 86/609/EEC and of the regional ethical committee for animal experimentation (Normandy).

2.2. Drugs

The cannabinoid agonist HU 210 ((−)-11-hydroxy-Δ-8-tetrahydrocannabinol-dimethylheptyl) provided by Tocris (Bristol, United Kingdom) was dissolved in dimethylsulfoxide and cremophor EL (final concentration of 5 and 0.5% respectively) and then diluted in saline (0.9% NaCl). HU 210 was injected at doses ranging from 12.5 to 200 μg/kg. Morphine hydrochloride (Coopération Pharmaceutique Française, Melun, France) was dissolved in saline and injected at the dose of 7.5 mg/kg (free base). The cannabinoid CB_1 receptor antagonist rimonabant (SR 141716, a generous gift from Sanofi Research Laboratories) was diluted in dimethylsulfoxide and cremophor EL (final concentration of 10 and 1% respectively) and then diluted in saline (0.9% NaCl). Rimonabant was injected at the dose of 10 mg/kg. These drugs were injected intraperitoneally in a volume of 10 ml/kg of body weight. Ethanol (99%, v/v, Sigma, USA) was diluted in saline and injected intraperitoneally at doses of 1 and 1.5 g/kg, by adjusting the volume of injection of a 15% (w/v) ethanol solution.

2.3. Locomotor activity

Locomotor activity was measured in a Digiscan actimeter (Omnitech Electronics, Columbus, Ohio, USA) situated in a dimly lit and quiet room. Immediately after the injection of either alcohol or morphine, animals were placed individually in Plexiglas boxes (20 cm × 20 cm × 30 cm). Infrared cells located at the periphery at the height of 2 cm measured the horizontal activity of mice. Horizontal locomotor activity was expressed as the distance travelled (in m) during the test session. As previously described (Hagues et al., 2007), the locomotor activity was measured during 45 min for mice tested with morphine. Because of the rapid onset but the short-lasting duration of alcohol effects, the duration of the test session for alcohol-treated mice was limited to the first 15 min after the i.p. administration (Hagues et al., 2007).

2.4. Experimental procedure

2.4.1. Influence of a sub-chronic HU 210 pre-exposure on subsequent locomotor effects of morphine or alcohol

We assessed whether a sub-chronic cannabinoid treatment could induce a behavioural sensitization to the subsequent locomotor stimulant effects of morphine or alcohol. For this purpose, animals were injected once daily with HU 210 (12.5 to 200 μg/kg) or vehicle over 7 days. Then, they were challenged with saline or with morphine at an intermediate dose (7.5 mg/kg) or with alcohol at a low and an intermediate dose (1 or 1.5 g/kg) on the 1st, 3rd, 7th, 14th, 21st and 35th day after the last HU 210 injection.

The tested doses of HU 210 ranged from low doses (12.5 and 25 μg/kg) consistently devoid of proper effect on locomotion up to doses consistently inducing hypolocomotion (100 and 200 μg/kg) (Hagues et al., 2007).

2.4.2. Locomotor effects of morphine or alcohol after a chronic administration of HU 210

We investigated the consequences of a chronic 14-day treatment with HU 210 on subsequent motor effects of morphine or alcohol. Mice received an injection of HU 210 (200 μg/kg) once daily during 14 days. On the 1st, 3rd, 7th, 14th, 21st and 35th day after the end of the chronic treatment, the locomotion of each animal was measured after administration of either morphine (7.5 mg/kg) or alcohol (1.5 g/kg). Before each test injection, animals were isolated in individual cages in order to observe possible somatic manifestations of a withdrawal syndrome (Cook et al., 1998; Hutcheson et al., 1998).

2.4.3. Influence of rimonabant on the consequences of a HU 210 pre-exposure

The effect of the cannabinoid CB_1 receptor antagonist rimonabant on the locomotor response to morphine was assessed in chronically HU 210 pre-treated animals. Animals were treated once daily with HU 210 (200 μg/kg) or its vehicle during 14 days. The effect of the cannabinoid CB_1 receptor antagonist was assessed in separate groups

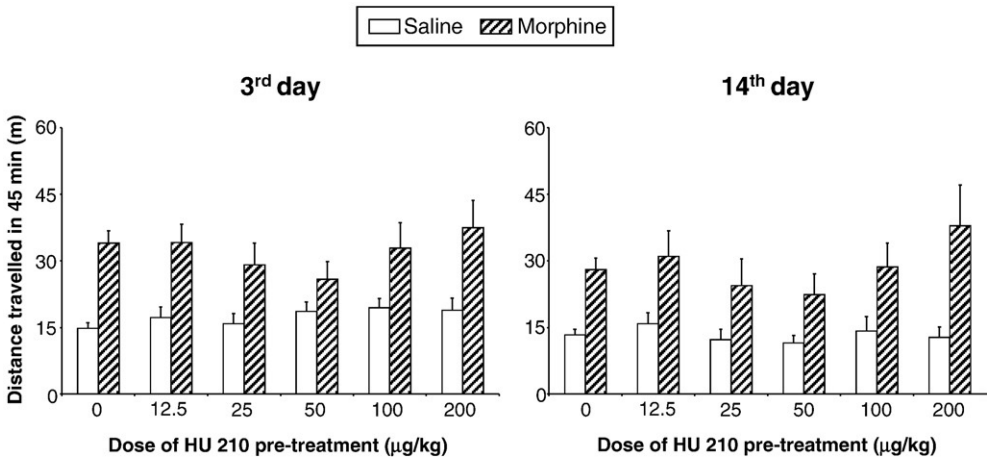

Fig. 1. Acute motor stimulant effect of morphine after the cessation of a 7-day treatment with HU 210 (12.5–200 µg/kg). The distance travelled (m) during 45 min was measured in mice tested with saline or morphine (7.5 mg/kg) on the 3rd and on the 14th day after the last HU 210 injection. No difference between HU 210 pre-treated animals vs. respective vehicle pre-treated animals was observed. A main stimulant effect of morphine was observed ($F(1,162)=35.7$ and 27.3 respectively on the 3rd and 14th days; $P<0.001$). $n=12$–14 animals per group.

tested on the 3rd or on the 14th day after the last HU 210 injection. Within each condition of cannabinoid pre-treatment, mice were divided into two subgroups, receiving either rimonabant (10 mg/kg) or the respective solvent. This rather high dose of rimonabant was chosen in an attempt to block a large amount of cannabinoid CB_1 receptors. Thirty minutes later, animals were injected with morphine (7.5 mg/kg) or saline and placed into the actimeter.

It is noteworthy that rimonabant, administered at a short time (usually 4 h) after the last injection of chronic cannabinoid treatments, is widely used to precipitate a withdrawal syndrome (Gonzalez et al., 2005 for review). We observed somatic signs of withdrawal in HU 210 pre-treated mice receiving rimonabant 4 h after the end of pre-treatment (unpublished data), as found for other cannabinoids in mice (Cook et al., 1998; Hutcheson et al., 1998). Therefore, we tested whether rimonabant, injected on the 3rd or the 14th day after the end of HU 210 chronic treatment, retained its ability to induce a significant abstinence syndrome. For this purpose, during the latency between the injection of rimonabant and that of morphine, animals were observed in individual cages in order to evaluate possible withdrawal behaviours. Here, we mainly looked for the most prominent sign, i.e. paw tremors.

A similar experiment was performed to evaluate the consequences of rimonabant on the alcohol-induced stimulation (1.5 g/kg) in cannabinoid pre-treated mice 3 or 14 days after the end of the chronic treatment with HU 210.

2.5. Statistical analysis

Statistics were performed using the SigmaStat software (SPSS, USA). Results were expressed as means ± S.E.M. (standard error of the mean) of the distance travelled. Data were tested for conformity to normality and homogeneity of variances before parametric analysis. To overpass the lack of equality of variances, all locomotor activity data were analysed after a logarithmic transform. Statistical comparisons between groups were performed by using two-way analysis of variance (ANOVAs) with pre-treatments and treatments as factors at each day of testing. Furthermore, two-way ANOVA for repeated measures, with group and day of testing as factors, were used to study how the effects of the 14-day pre-treatment develop with time. Post hoc comparisons were performed using a Student–Newman–Keuls test.

3. Results

3.1. Influence of a sub-chronic HU 210 pre-exposure on subsequent locomotor effects of morphine or alcohol

The motor effects of morphine or alcohol were measured at different time intervals after the end of the seven-day treatment with HU 210. Main data obtained on the 3rd (left panel) and the 14th days (right panel) following the cessation of the treatment are shown on Fig. 1 for morphine and on Fig. 2 for alcohol.

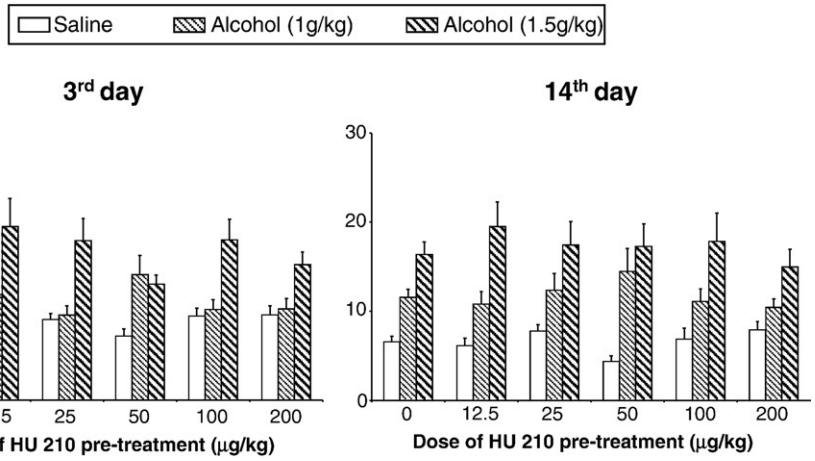

Fig. 2. Acute motor stimulant effect of alcohol after the cessation of a 7-day treatment with HU 210 (12.5–200 µg/kg). The distance travelled (m) during 15 min was measured in mice tested with saline or alcohol (1 g/kg or 1.5 g/kg) on the 3rd and on the 14th day after the last HU 210 injection. No difference between HU 210 pre-treated animals vs. respective vehicle pre-treated animals was observed. A main stimulant effect of alcohol was observed ($F(2,177)=45$ and 70 on the 3rd and 14th days, $P<0.001$). $n=9$–12 animals per group.

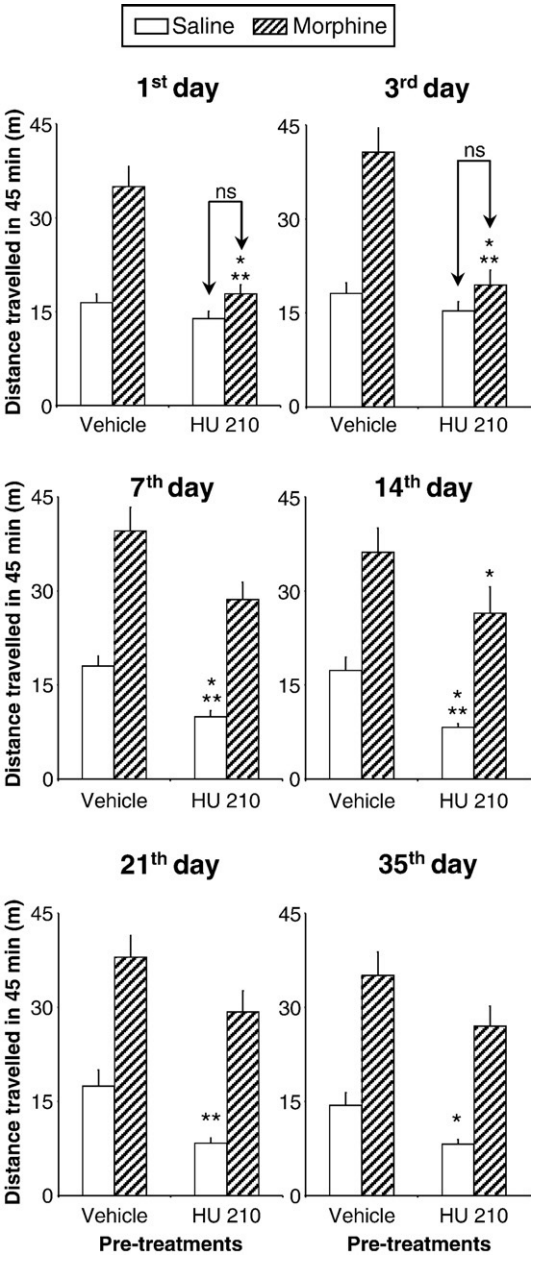

Fig. 3. Acute motor stimulant effect of morphine after the end of a 14-day treatment with HU 210 (200 µg/kg. The distance travelled (m) during 45 min was measured in mice tested with saline or morphine (7.5 mg/kg) at various time after the last HU 210 injection. *P<0.05; **P<0.01; ***P<0.001: animals pre-treated with HU 210 vs. respective vehicle pre-treated animals within saline-tested or morphine-tested mice. "ns": non significant difference between saline-tested and morphine-tested mice. n=12 animals per group.

Morphine (7.5 mg/kg) increased the distance travelled from the 1st to the 35th day after the end of the repeated treatment with HU 210 (main effect of morphine: P<0.001 during each test day). The sub-chronic treatment with HU 210 had no remaining effect on locomotor activity from the 1st day up to the 35th following the last cannabinoid injection (no main effect of HU 210 at each test session). Neither enhancement nor decrease of the morphine stimulant effect occurred 3 days or 14 days or at any time after the end of the repeated treatment (no HU 210×morphine interaction) (Fig. 1).

As for morphine, the HU 210 pre-treatment, devoid of remaining effect, did not modify the alcohol-induced motor stimulation at any time after the end of the sub-chronic HU 210 treatment (no HU 210×alcohol interaction) (Fig. 2).

3.2. Locomotor effects of morphine or alcohol after a chronic administration of HU 210

Mice were tested with morphine or alcohol at various time intervals after the end of a 14-day treatment with HU 210 (200 µg/kg) (Figs. 3 and 4). It is noteworthy that no sign of spontaneous withdrawal was observed at any day of testing after the last injection of HU 210.

In both sets of experiments (morphine or alcohol), two-way ANOVA for repeated measures showed that scores in various groups were differentially affected by the day of testing (group×day interaction: F(15,220)=4.7; P<0.001 and F(15,220)=6.3; P<0.001, respectively for morphine and alcohol experiments). Multiple

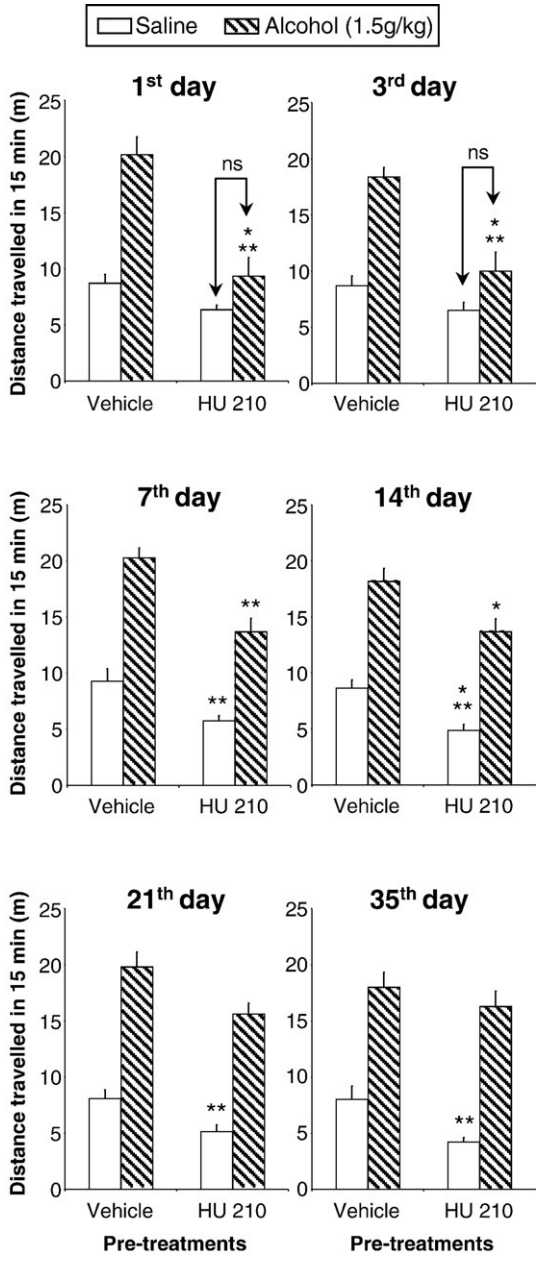

Fig. 4. Acute motor stimulant effect of alcohol after the end of a 14-day treatment with HU 210 (200 µg/kg). The distance travelled (m) during 15 min was measured in mice tested with saline or alcohol (1.5 g/kg) at various time after the last HU 210 injection. *P<0.05; **P<0.01; ***P<0.001: animals pre-treated with HU 210 alone vs. respective vehicle pre-treated animals within saline-tested or alcohol-tested mice. "ns": non significant difference between saline-tested and alcohol-tested mice. n=12 animals per group.

comparisons revealed that the 1st and 3rd days of testing differed from the other ones in groups pre-treated with HU 210. Data were then analysed, for each day of testing, using a two-way ANOVA, with HU 210 pre-treatment and treatment (morphine or alcohol) as factors.

On the first and the 3rd days after the cessation of pre-treatments, the effect of morphine differed according the pre-treatment condition (HU 210×morphine interaction: $F(1,44)=7.0$; $P=0.011$ and $F(1,44)=6.8$; $P=0.012$ respectively for the 1st and the 3rd days). Multiple comparisons showed that morphine-induced locomotor activity was lower in mice pre-treated with HU 210 than in vehicle pre-treated ones. This effect led to a lack of morphine-induced stimulation in HU 210 pre-treated mice relatively to the respective saline-tested mice (Fig. 3, 1st and 3rd days).

The loss of the morphine stimulant effect observed on the 1st and the 3rd day in mice pre-treated by HU 210 disappeared from the 7th day following cessation of pre-treatments (no more HU 210×morphine interaction from the 7th day). Yet, the locomotor activity in all mice pre-treated with HU 210 remained lower than that observed in vehicle pre-treated mice, from the 7th day after abstinence up to the 35th day (main HU 210 effect: $F(1,44)=17.4$; $P<0.001$ and $F(1,44)=6.2$; $P=0.016$ respectively on the 7th and 35th days). Post hoc analysis revealed that the spontaneous activity of saline-tested mice was significantly lowered by the pre-treatment with HU 210 from the 7th day up to the 35th day after the last injection of HU 210. Mice pre-treated with HU 210 were stimulated by morphine to a lower extent in comparison with those chronically injected with vehicle, although in a significant manner only on the 14th day (Fig. 3).

Fig. 4 shows that on the 1st and on the 3rd days after the end of pre-treatments, the responsiveness to alcohol depended on the pre-treatment condition (HU 210×alcohol interaction: $F(1,44)=11.9$; $P=0.001$ and $F(1,44)=7.9$; $P=0.007$ respectively for the 1st and the 3rd days). Alcohol-induced locomotor activity was lower in mice pre-treated with HU 210 than in those pre-treated with vehicle. In HU 210-pretreated mice, locomotor activity in alcohol-tested mice did not differ from that measured in saline-tested mice (Fig. 4, 1st and 3rd days).

From the 7th day after abstinence, the interaction between alcohol-induced stimulation and HU 210 pre-treatment disappeared (no HU 210×alcohol interaction). Nevertheless, the locomotor activity in mice pre-treated with HU 210 remained lower than that observed in mice chronically injected with vehicle up to the 35th day (main HU 210 effect: $F(1,44)=19.1$; $P<0,001$ and $F(1,44)=7.9$; $P=0,007$ respectively on the 7th and 35th days). In saline-tested mice, the spontaneous activity in mice pre-treated by HU 210 remained significantly lower than that observed in vehicle pre-treated mice from the 7th day up to the 35th day after cessation of pre-treatment. Alcohol-induced stimulation in mice pre-treated by HU 210 was significantly lower than that observed in mice chronically injected with vehicle on the 7th day and the 14th day after abstinence (Fig. 4).

3.3. Influence of rimonabant on the consequences of a chronic HU 210 pre-exposure

The consequences of the 14-day HU 210 treatment were actually biphasic. An initial tolerance to drug-stimulant effects lasting up to the 3rd day after withdrawal was followed by a phase of general hyporeactivity in all mice pre-exposed to HU 210. Because of its long half-life, a significant level of HU 210 may persist for a long time after the last injection and that might, at least partially, explain the data obtained. In an attempt to counteract a possible role of residual HU 210, locomotor trials were performed on the 3rd and the 14th day after discontinuation of the pre-treatment in mice acutely treated with the cannabinoid CB_1 receptor antagonist rimonabant.

The effect of rimonabant on the locomotor effect of morphine or alcohol on the 3rd or the 14th day after the end of a 14-day treatment with HU 210 is presented in Figs. 5 and 6. No sign of precipitated withdrawal was observed when rimonabant was injected 3 or 14 days after the last exposure to HU 210.

On the 3rd day after withdrawal, the morphine-induced stimulation was lowered after a cannabinoid pre-treatment in mice non-injected with rimonabant, confirming our previous data shown in Fig. 3 (HU 210×morphine interaction: $F(1,28)=4.2$; $P=0.05$). This antagonism led to the loss of the morphine-induced stimulation in mice pre-treated with HU 210. This antagonism disappeared in mice injected with the cannabinoid CB_1 receptor antagonist before locomotor tests inducing the reappearance of morphine stimulant effect (no more HU 210×morphine interaction) (Fig. 5, 3rd day).

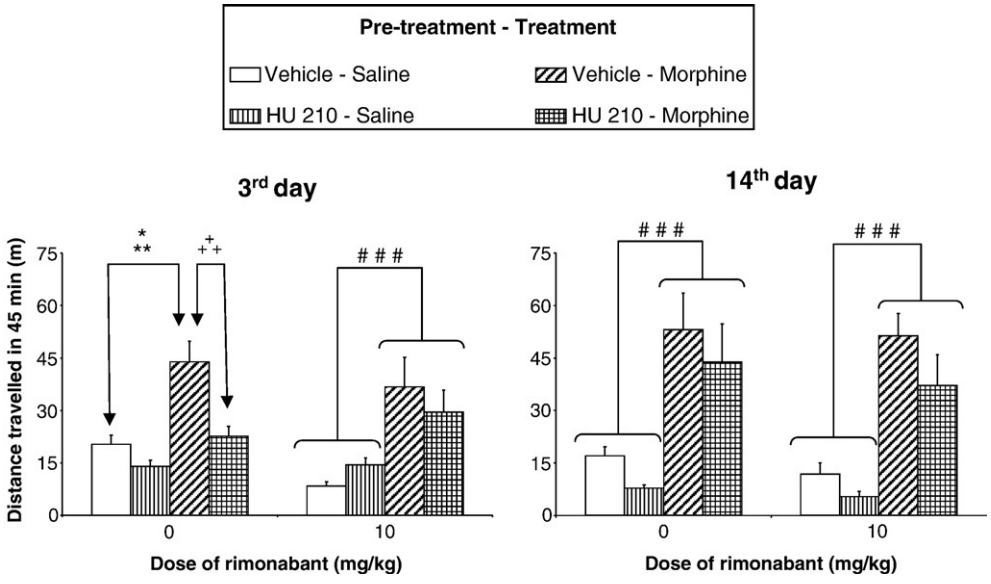

Fig. 5. Modulation by rimonabant of the motor stimulant effect of morphine in mice chronically pre-treated with HU 210. Animals were pre-treated once daily with HU 210 (200 µg/kg) or its vehicle during 14 days. On the 3rd or on the 14th day after the last HU 210 injection, the distance travelled (m) during 45 min was measured in mice tested with saline or morphine (7.5 mg/kg), after a single rimonabant injection (10 mg/kg). ***$P<0.001$: morphine-tested mice vs. controls. +++$P<0.001$: morphine effect in animals pre-treated by HU 210 vs. respective vehicle pre-treated animals tested under morphine. # # #$P<0.001$: main effect of morphine of ANOVA (on the 3rd day with rimonabant: ($F(1,28)=16.6$; on the 14th day: $F(1,42)=75.8$ and 44.3 respectively without or with rimonabant). $n=8$–12 animals per group.

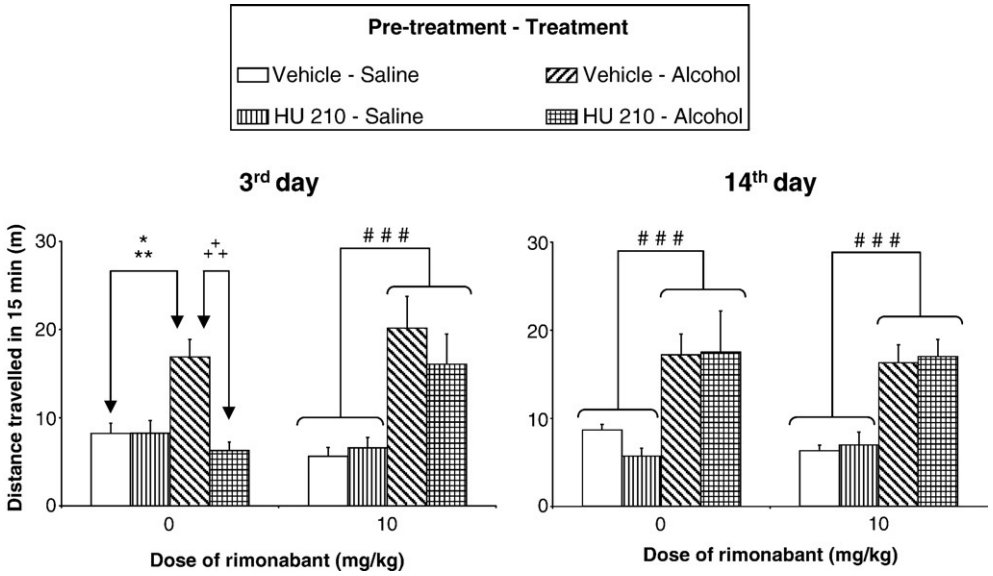

Fig. 6. Modulation by rimonabant of the motor stimulant effect of alcohol in mice chronically pre-treated with HU 210. Animals were pre-treated once daily with HU 210 (200 µg/kg) or its vehicle during 14 days. On the 3rd or on the 14th day after the last HU 210 injection, the distance travelled (m) during 15 min was measured in mice tested with saline or alcohol (1.5 g/kg), after a single rimonabant injection (10 mg/kg). ***$P<0.001$: alcohol-tested animals vs. controls. +++$P<0.001$: alcohol effect in animals pre-treated by HU 210 vs. respective vehicle pre-treated animals tested under alcohol. # # #$P<0.001$: main effect of alcohol of ANOVA (on the 3rd day with rimonabant: ($F(1,28)=27.0$; on the 14th day: $F(1,42)=37.4$ and 30.7 respectively without or with rimonabant). $n=8$–12 animals per group.

Two weeks after the last HU 210 injection, in mice non-injected with rimonabant, the cannabinoid pre-exposed animals remained slightly less active than those chronically injected with vehicle (main HU 210 effect: $F(1,42)=6.1$; $P=0.018$). Moreover, the morphine-induced stimulation was not modified in HU 210-pretreated mice anymore (no HU 210×morphine interaction). A similar profile was obtained in mice receiving rimonabant and the residual hypolocomotor effect of the HU 210 pre-treatment was preserved in the presence of rimonabant (main HU 210 effect: $F(1,42)=7.2$; $P=0.01$; no HU 210×morphine interaction) (Fig. 5, 14th day).

In mice non-injected with rimonabant, the alcohol stimulant effect was lessened in HU 210 pre-treated animals on the 3rd day after the last cannabinoid injection (HU 210×alcohol interaction: $F(1,28)=13.6$; $P<0.001$). This antagonism induced a loss of the alcohol-induced hyperlocomotion in cannabinoid pre-treated animals. In contrast, when the cannabinoid antagonist rimonabant was injected before the locomotor measurement, the antagonism of the alcohol-induced stimulation in HU 210 pre-treated mice was no more apparent (no HU 210×alcohol interaction). Thus, the locomotor stimulant effect of alcohol was fully restored in the presence of rimonabant in HU 210 pre-treated mice (Fig. 6, 3rd day).

On the 14th day of withdrawal, in mice untreated with rimonabant, the alcohol stimulant effect was not affected by the pre-treatment (no HU 210×alcohol interaction). The locomotor activity in HU 210 pre-treated mice did not differ significantly from that observed in mice pre-exposed to vehicle (no main effect of HU 210). Rimonabant influenced neither the stimulant response to alcohol nor the effect of HU 210 pre-treatment (alcohol effect: $P<0.001$; no interaction and main HU 210 effect) (Fig. 6, 14th day).

4. Discussion

Existing data about the long-term modulation of the motor stimulant effects of drugs of abuse by cannabinoid agonists remain controversial to date. The major findings in the present study are: First, we failed to observe any hetero-sensitization to the morphine- and alcohol-induced locomotor stimulation whatever the cannabinoid treatment regimen. Second, only after the chronic treatment regimen, the morphine- and alcohol-induced activation was even blunted after a short drug-free period. Third, after a protracted abstinence, we observed a decrease in motor activity in mice chronically exposed to HU 210.

After a 7-day HU 210 treatment with doses ranging from 12.5 to 200 µg/kg, mice were not sensitized to the morphine- or alcohol-induced stimulant effects, even after a long drug-free period. A similar lack of sensitization was observed after a longer treatment (14 days) with a high dose of the cannabinoid agonist (200 µg/kg) in order to mimic a heavy use. No previous experiment measured the long-term consequences of a chronic cannabinoid treatment on the alcohol stimulant effect. Earlier results regarding the locomotor stimulant effects of opiates in animals pre-exposed to cannabinoid CB_1 agonists remain inconsistent. In agreement with us, the locomotor activating properties of morphine were not facilitated by pre-treatment with Δ-9 THC (Valverde et al., 2001; Jardinaud et al., 2006). In contrast, some previous studies have shown the development of a cross-sensitization to the motor effects of opiates after a chronic cannabinoid exposure (Cadoni et al., 2001; Lamarque et al., 2001; Pontieri et al., 2001; Norwood et al., 2003; Singh et al., 2005).

Differences in the treatment regimen as well as pharmacodynamic and pharmacokinetic properties of different agonists might have influenced the extent to which cross-sensitization to opiates could develop. Among the most widely used cannabinoid agonists, CP 55,940, WIN 55,212-2 and HU 210 have all been characterized as highly efficient agonists. In contrast, Δ-9 THC has lower CB_1 and CB_2 affinities and relative intrinsic activities than these other cannabinoids, behaving as a partial agonist (Howlett et al., 2002 for review). Here, we used HU 210, a classical cannabinoid that displays pharmacokinetic features resembling those of Δ-9 THC, having a slightly longer duration of action than Δ-9 THC (Little et al., 1989; Ottani and Giuliani, 2001). Some previous studies were performed with short half-life agonists such as CP 55,940 (Norwood et al., 2003) or WIN 55,212-2 (Pontieri et al., 2001) that, in contrast to Δ-9 THC or HU 210, lead to successive peak effects which may favour the development of sensitization to behavioural effects of morphine. Moreover, using a pattern of increasing doses produces a strong and rapid desensitization of cannabinoid receptors (Cadoni et al., 2001; Pontieri et al., 2001). Besides, a long-term sensitization to opiates after chronic cannabinoid treatments was observed in rats displaying a high locomotor response to novelty (Lamarque et al., 2001) or in Lewis rats (Norwood et al., 2003), all rats described as highly sensitive to drugs of

abuse, suggesting that individual differences in susceptibility to drug addiction may strongly determinate the consequences of a cannabinoid exposure.

We previously found that an acute injection of HU 210, even at doses devoid of intrinsic hypolocomotor effects, antagonized the stimulant effect of morphine or alcohol, implying an involvement of the cannabinoid CB_1 receptors in acute motor effects of these agents. A tolerance to this antagonistic interaction between HU 210 and alcohol or morphine occurred at the end of a 7-day or a 14-day HU 210 treatment (Hagues et al., 2007). However, adaptive changes triggered by the repeated activation of cannabinoid CB_1 receptors do not contribute to a facilitation of subsequent drug-induced locomotion. Given the wide distribution of cannabinoid CB1 receptors, it cannot be excluded that some cellular neuroadaptations elicited throughout the brain by systemically injected HU 210 may counteract the behavioural expression of sensitization.

On the 1st and 3rd days following withdrawal from the HU 210 chronic treatment regimen (14 days, 200 μg/kg), the locomotor stimulant effects of morphine or alcohol were not enhanced, but even alleviated, whereas a shorter pre-exposure to HU 210 (7 days) had no obvious consequences. A similar reduction of the motor stimulant effect of morphine was also observed 3 days after the end of a 14-day pre-exposure to Δ-9 THC (Jardinaud et al., 2006). We found that rimonabant, administered on the 3rd day after cessation of the chronic treatment with HU 210, was able to reverse the decrease in morphine or alcohol stimulant effects, confirming the implication of cannabinoid CB_1 receptors. These data must be put in line with the fact that HU 210, when co-administered with either morphine or alcohol, antagonized their acute stimulant effects (Hagues et al., 2007). It leads us to suggest that the sensitivity of cannabinoid CB_1 receptors might be partially restored on the 3rd day after cannabinoid abstinence, so that they might be stimulated by traces of HU 210, explaining the lessening of morphine or alcohol effects. In agreement, a partial reappearance of the analgesic and hypolocomotor effects of a test dose of Δ-9 THC occurred within 4.5 days in mice having received thirteen injections of this cannabinoid (Bass and Martin, 2000; Gonzalez et al., 2005 for reviews). This hypothesis of residual HU 210 after a 14-day treatment should be verified by determining the cerebral level of this agonist. Alternatively, the chronic treatment with HU 210 could lead to a transient reduction in the ability of morphine or alcohol to elicit a dopamine release. Interestingly, the alcohol-induced dopamine increase in the nucleus accumbens was abolished one day after cessation of a repeated treatment with the cannabinoid agonist WIN 55,212-2 in rats (López-Moreno et al., 2008).

Moreover, the motor activity of animals pre-treated with HU 210 during 14 days remained lower than the activity of controls from the 7th day after withdrawal. This hypolocomotor effect could be induced by a hyperactivity of the endocannabinoidergic pathway due to a withdrawal-induced rebound effect. Furthermore, the very high lipophilicity of HU 210 may lead to an increased storage in fat tissues and to a possible delayed release from these latter (Little et al., 1989) inducing significant levels of residual HU 210. However, the fact that rimonabant had no strong influence on the locomotor activity on the 14th day after withdrawal do not support these assumptions. An alternative explanation for this long-lasting hypolocomotor effect could be a decline in the mesolimbic dopaminergic tone following the cannabinoid withdrawal. Such a decreased mesolimbic dopaminergic transmission was already documented after either a spontaneous or a precipitated cannabinoid withdrawal in rats (Diana et al., 1998; Tanda et al., 1999). In this respect, it is noteworthy that this effect actually appeared only after a 2-week treatment at the highest dose of HU 210 (200 μg/kg) mimicking a heavy use, but not after a shorter treatment.

In conclusion, we failed to observe any hetero-sensitization to morphine or alcohol stimulant effects after both sub-chronic and chronic treatment regimens. These data argue against the hypothesis that repeated exposure to various drugs of abuse result in final common neuroadaptations triggering behavioural sensitization. Sensitization of the mesocorticolimbic dopaminergic pathway is thought to have a prominent role for most drugs of abuse. However, whether a sensitized dopaminergic response develops after a repeated administration of cannabinoid actually remains to be established. Yet, it is of note that behavioural and neurochemical sensitization to a given agent does not necessarily extend to drugs of abuse of different pharmacological classes, as previously proposed (Cadoni and Di Chiara, 1999).

Moreover, a 14-day treatment with a large dose of HU 210 (200 μg/kg), better mimicking a continuous heavy use, induced a long-term decrease of the spontaneous locomotor activity, possibly linked to a long-term decrease in the dopaminergic mesolimbic transmission. Therefore, further investigations are required to test this hypothesis and to evaluate with appropriate models whether a long enough period of high consumption of cannabinoids could induce an enhanced susceptibility to depressive-like state or to subsequent drug-seeking behaviours.

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References

Arnold, J.C., Topple, A.N., Hunt, G.E., McGregor, I.S., 1998. Effects of pre-exposure and co-administration of the cannabinoid receptor agonist CP 55,940 on behavioural sensitization to cocaine. Eur. J. Pharmacol. 354, 9–16.

Basavarajappa, B.S., 2007. The endocannabinoid signalling system: a potential target for next-generation therapeutics for alcoholism. Mini Rev. Med. Chem. 7, 769–779.

Bass, C.E., Martin, B.R., 2000. Time course for the induction and maintenance of tolerance to D-9 tetrahydrocannabinol in mice. Drug Alcohol Depend. 60, 113–119.

Cadoni, C., Di Chiara, G., 1999. Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. Neuroscience 90, 447–455.

Cadoni, C., Pisanu, A., Solinas, M., Acquas, E., Di Chiara, G., 2001. Behavioural sensitization after repeated exposure to Δ-9 tetrahydrocannabinol and cross-sensitization with morphine. Psychopharmacology 158, 259–266.

Colombo, G., Serra, S., Vacca, G., Carai, M.A.M., Gessa, G.L., 2005. Endocannabinoid system and alcohol addiction: pharmacological studies. Pharmacol. Biochem. Behav. 81, 369–380.

Cook, S.A., Lowe, J.A., Martin, B.R., 1998. CB1 receptor antagonist precipitates withdrawal in mice exposed to Δ^9-tetrahydrocannabinol. J. Pharmacol. Exp. Ther. 285, 1150–1156.

Corchero, J., Manzanares, J., Fuentes, J.A., 2004. Cannabinoid/opioid crosstalk in the central nervous system. Crit. Rev. Neurobiol. 16, 159–172.

De Vries, T.J., Shoffelmeer, A.N.M., Binnekade, R., Mulder, A.H., Vandershuren, L.J.M.J., 1998. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. Eur. J. Neurosci. 10, 3565–3571.

Degenhardt, L., Hall, W., Lynskey, M., 2001. The relationship between cannabis use and other substance use in the general population. Drug Alcohol Depend. 64, 319–327.

Diana, M., Melis, M., Muntoni, A.L., Gessa, G.L., 1998. Mesolimbic dopaminergic decline after cannabinoid withdrawal. Proc. Natl. Acad. Sci. U. S. A. 95, 10269–10273.

Fattore, L., Deiana, S., Spano, S.M., Cossu, G., Fadda, P., Sherma, M., Fratta, W., 2005. Endocannabinoid system and opioid addiction: behavioural aspects. Pharmacol. Biochem. Behav. 81, 343–359.

Fergusson, D.M., Boden, J.M., Horwood, L.J., 2006. Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. Addiction 101, 556–569.

Gardner, E.L., 2005. Endocannabinoid signalling system and brain reward: emphasis on dopamine. Pharmacol. Biochem. Behav. 81, 263–284.

Gonzalez, S., Cebeira, M., Fernandez-Ruiz, J., 2005. Cannabinoid tolerance and dependence: a review of studies in laboratory animals. Pharmacol. Biochem. Behav. 81, 300–318.

Hagues, G., Costentin, J., Duterte-Boucher, D., 2007. Modulation of morphine and alcohol motor stimulant effects by cannabinoid receptors ligands. Behav. Brain Res. 178, 274–282.

Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R., Pertwee, R.G., 2002. International union of pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol. Rev. 54, 161–202.

Hutcheson, D.M., Tzavara, E.T., Smadja, C., Valjent, E., Roques, B.P., Hanoune, J., Maldonado, R., 1998. Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with Δ-9-tetrahydrocannabinol. Brit. J. Pharmacol. 125, 1567–1577.

Jardinaud, F., Roques, B.P., Noble, F., 2006. Tolerance to the reinforcing effects of morphine in delta9-tetrahydrocannabinol treated mice. Behav. Brain Res. 173, 255–261.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223–244.

Kolb, B., Gorny, G., Limebeer, C.L., Parker, L.A., 2006. Chronic treatment with Δ-9 tetrahydrocannabinol alters the structure of neurons in the nucleus accumbens shell and medial prefrontal cortex of rats. Synapse 60, 429–436.

Lamarque, S., Taghzouti, K., Simon, H., 2001. Chronic treatment with Δ-9 tetrahydrocannabinol enhances the locomotor response to amphetamine and heroin. Implications for vulnerability to drug addiction. Neuropharmacology 41, 118–129.

Little, P.J., Compton, D.R., Mechoulam, R., Martin, B.R., 1989. Stereochemical effects of 11-OH-Δ-8-tetrahydrocannabinol-dimethylheptyl in mice and dogs. Pharmacol. Biochem. Behav. 32, 661–666.

López-Moreno, J.A., Scherma, M., Rodríguez de Fonseca, F., González-Cuevas, G., Fratta, W., Navarro, M., 2008. Changed accumbal responsiveness to alcohol in rats pretreated with nicotine or the cannabinoid receptor agonist WIN 55,212-2. Neurosci. Lett. 433 (1), 1–5.

Mailleux, P., Vanderhaeghen, J.-J., 1992. Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience 48, 655–668.

Maldonado, R., Valverde, O., Berrendero, F., 2006. Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. 29, 225–232.

Manzanares, J., Corchero, J., Romero, J., Fernandez-Ruiz, J.J., Ramos, J.A., Fuentes, J.A., 1999. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol. Sci. 20, 287–294.

Manzanares, J., Ortiz, S., Oliva, J.M., Perez-Rial, S., Palomo, T., 2005. Interactions between cannabinoid and opioid receptor systems in the mediation of ethanol effects. Alcohol Alcohol. 40, 25–34.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., Valverde, O., 2000. Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. Eur. J. Neurosci. 12, 4038–4046.

Mascia, M.S., Obinu, M.C., Ledent, C., Parmentier, M., Böhme, G.A., Imperato, A., Fratta, W., 1999. Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB1 receptor knockout mice. Eur. J. Pharmacol. 383, R1–R3.

Naassila, M., Pierrefiche, O., Ledent, C., Daoust, M., 2004. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. Neuropharmacology 46, 243–253.

Norwood, C.S., Cornish, J.L., Mallet, P.E., McGregor, I.S., 2003. Pre-exposure to the cannabinoid receptor agonist CP 55,240 enhances morphine behavioural sensitization and alters morphine self-administration in Lewis rats. Eur. J. Pharmacol. 465, 105–114.

Ottani, A., Giuliani, D., 2001. HU 210: a potent tool for investigations of the cannabinoid system. CNS Drug Rev. 7, 131–145.

Pontieri, F.E., Monnazzi, P., Scontrini, A., Buttarelli, F.R., Patacchiolo, F.R., 2001. Behavioural sensitization to heroin by cannabinoid pre-treatment in the rat. Eur. J. Pharmacol. 421, R1–R3.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18, 247–291.

Rubino, T., Vigano, D., Massi, P., Parolaro, D., 2001. The psychoactive ingredient of marijuana induces behavioural sensitization. Eur. J. Neurosci. 14, 884–886.

Singh, M.E., McGregor, I.S., Mallet, P.E., 2005. Repeated exposure to Δ-9 tetrahydrocannabinol alters heroin-induced locomotor sensitisation and Fos-immunoreactivity. Neuropharmacology 49, 1189–1200.

Solinas, M., Yasar, S., Goldberg, S.R., 2007. Endocannabinoid system involvement in brain reward processes related to drug abuse. Pharmacol. Res. 56, 393–405.

Tanda, G., Loddo, P., Di Chiara, G., 1999. Dependence of mesolimbic dopamine transmission on Δ-9 tetrahydrocannabinol. Eur. J. Pharmacol. 376, 23–26.

Valverde, O., Noble, F., Beslot, F., Daugé, V., Fournié-Zaluski, M.-C., 2001. Δ-9 tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur. J. Neurosci. 13, 1816–1824.

Vanderschuren, L.J.M.J., Kalivas, P.W., 2000. Alrerations in dopaminergic and glutamatergic transmission in the induction and expression of behavioural sensitization: a critical review of preclinical studies. Psychopharmacology 151, 99–120.

Varvel, S.A., Martin, B.R., Lichtman, A.H., 2007. Lack of behavioural sensitization after repeated exposure to THC in mice and comparison to methamphetamine. Psychopharmacology 193, 511–519.

Vigano, D., Rubino, T., Parolaro, D., 2005. Molecular and cellular basis of cannabinoid and opioid interactions. Pharmacol. Biochem. Behav. 81, 360–368.