ORIGINAL INVESTIGATION

Differential involvement of the endocannabinoid system in short- and long-term expression of incentive learning supported by nicotine in rats

**Benoît Forget · Sandrine Barthélémy ·
Françoise Saurini · Michel Hamon ·
Marie-Hélène Thiébot**

Abstract

Rationale We previously reported that the CB1 cannabinoid receptor antagonist, rimonabant, impaired the acquisition and the short-term (24 h), but not long-term (3 weeks), expression of conditioned place preference (CPP) induced by nicotine in rats.

Objective To assess the time interval of efficacy of a single pretest injection of rimonabant to abolish nicotine–CPP, and the effects of chronic CB1 receptor blockade on long-term expression of nicotine–CPP.

Materials and methods Wistar rats were conditioned to nicotine (0.06 mg/kg, subcutaneous) using an unbiased one-compartment procedure. Two test sessions were conducted 24 h (without injection) and 1, 2, or 3 weeks later. Rimonabant (3 mg/kg, intraperitoneal) or vehicle was administered daily between the two test sessions. In addition, the CB1-stimulated $[^{35}S]$GTP-γ-S binding was assessed in rats from the 3-week experiment.

Results The capacity of a single injection of rimonabant (3 mg/kg, 30 min pretest) to block the expression of nicotine–CPP disappeared within 1 week after conditioning. Daily administrations of rimonabant for 6, 13, or 20 days postacquisition did not impair nicotine–CPP but allowed an additional pretest injection of rimonabant to retain its capacity to abolish long-term expression of nicotine–CPP. The CB1 receptor-mediated G-protein signaling was not altered in various brain areas of rats given rimonabant for 3 weeks.

Conclusions The endocannabinoid system is essential to the expression of nicotine–CPP during less than 1 week after conditioning but not later. However, endocannabinoid-dependent mechanisms are critically involved in the development of the neuroadaptive changes responsible for the shift from CB1-dependent to CB1-independent expression of nicotine incentive learning.

Keywords Nicotine · CB1 cannabinoid receptor ·
Rimonabant · Conditioned place preference ·
Neural plasticity · Rat

B. Forget · S. Barthélémy · F. Saurini · M. Hamon · M.-H. Thiébot
INSERM, U677,
Paris F-75013, France

B. Forget · S. Barthélémy · F. Saurini · M. Hamon · M.-H. Thiébot
University Pierre et Marie Curie,
IFR 70 Neurosciences,
Paris F-75013, France

B. Forget · S. Barthélémy · F. Saurini · M. Hamon ·
M.-H. Thiébot (✉)
UMR 677 INSERM/UPMC,
Faculty of Medicine Pierre et Marie Curie, Site Pitié-Salpêtrière,
91 Boulevard de l'Hôpital,
75634 Paris Cedex 13, France
e-mail: thiebot@ext.jussieu.fr

Introduction

Tobacco and cannabis are among the most widely consumed drugs of abuse in humans. Nicotine is the principal component of tobacco smoke that leads to addiction, and Δ^9-tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of cannabis. They exert their primary effect at specific receptors, which are especially abundant in the central nervous system. Nicotine activates several subtypes of neuronal nicotinic acetylcholine (nACh)-gated ion channel receptors, formed by the combination of five α and β subunits. Heterooligomeric nACh receptors containing the β2 and α4 subunits have been convincingly involved in the reinforcing effects of nicotine whereas this

role is still debated for $\alpha7$ homooligomeric receptors (Pidoplichko et al. 2004; Tapper et al. 2004). On the other hand, Δ^9-THC activates CB1 and CB2 cannabinoid Gi/o-protein-coupled receptors (Pertwee 1999), at which anandamide and 2-arachidonoyl glycerol (2-AG) act as endogenous agonists (van der Stelt and Di Marzo 2003). Central effects of cannabinoids are thought to be mediated by the CB1 receptor because most of them are abolished by the potent and selective CB1 receptor antagonist, rimonabant (Rinaldi-Carmona et al. 1995), and are not observed in CB1 receptor knockout (KO) mice (Ledent et al. 1999; Zimmer et al. 1999).

A large body of congruent data support the idea that the endogenous cannabinoid system plays a key role in brain mechanisms underlying the motivational effects of drugs of abuse and drug-related stimuli. Thus, CB1 receptor blockade decreased nicotine, ethanol, methamphetamine, morphine, and heroin self-administration in rodents (Arnone et al. 1997; Navarro et al. 2001; Cohen et al. 2002; Vinklerová et al. 2002; De Vries et al. 2003), though it is generally ineffective in reducing cocaine self-administration (Tanda et al. 2000; De Vries et al. 2001). Rimonabant was also shown to prevent the establishment of conditioned place preference (CPP) supported by morphine, cocaine, and nicotine (Chaperon et al. 1998; Forget et al. 2005) and to block cue-induced reinstatement of cocaine, heroin, ethanol, and nicotine-seeking behavior (see, e.g., De Vries and Schoffelmeer 2005 and Le Foll and Goldberg 2005 for reviews). Furthermore, no rewarding effects of morphine and nicotine could be detected in CB1 receptor KO mice (Martin et al. 2000; Castañé et al. 2002).

When administered acutely, rimonabant could also impair the expression of nicotine-induced CPP (Le Foll and Goldberg 2004; Forget et al. 2005). However, inhibition of this behavior was effective when rimonabant was administered 24 h after the last conditioning session but not when the CB1 receptor antagonist was given 3 or 12 weeks after conditioning (Forget et al. 2005). These results suggest a differential involvement of the endocannabinoid system in the short- and long-term expression of incentive learning supported by nicotine. The present studies were designed to (1) determine the time interval after the conditioning phase during which a single pretest injection of rimonabant remains efficient to abolish the expression of CPP to nicotine, (2) examine the effects of a chronic blockade of CB1 receptors during the period between the conditioning phase and the test session (1, 2, or 3 weeks) on long-term expression of nicotine-induced CPP, and (3) investigate the consequences of nicotine conditioning followed by a 3-week chronic treatment with rimonabant on the functional G-protein coupling of CB1 receptors in brain areas potentially involved in incentive learning.

Materials and methods

Animals

The experiments were carried out on drug- and test-naïve male Wistar AF rats (CERJ, Le Genest, France). They were 5-week-old (150–160 g) upon their arrival in the laboratory and weighed 225–245 g at the beginning of the experiments 2 weeks later. The rats were housed eight per cage ($40\times40\times18$ cm) under standard conditions (12 h light–dark cycle with light on at 0730 hours; room temperature $21\pm1°C$) with free access to water in their home cage. One week before the beginning of the experiments, the rats were brought daily from the animal housing facility to the laboratory; they were handled, weighed, and given a subcutaneous (s.c.) injection of nicotine (0.06 mg/kg) on Monday, Wednesday, and Friday, or saline on alternate days, immediately before being returned to their home cage. From this first week onward, they were placed on a daily schedule of mild food restriction (150 g of standard chow per day for eight rats, given as a single meal in the evening), which was maintained until the end of the experiment, as food restriction has been reported to enhance the rewarding effects of drugs of abuse (Bell et al. 1997; Carr 2002). Experiments were performed in agreement with the institutional guidelines for use of animals and their care, in compliance with national and international laws and policies (Council directive no. 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Véteacute;rinaire de la Santé et de la Protection Animale, permissions no. 75-116 to MH and 75-118 to MHT).

Place conditioning paradigm

Apparatus

The experiments were conducted in a one-compartment apparatus, using an unbiased experimental design, as previously described (Forget et al. 2005). Briefly, the rats were trained and tested in four black wooden open-fields ($76\times76\times50$ cm) located in a room dimly lit with four 15 W red light bulbs, positioned 150 cm above each open-field (1 lx at floor level), and supplied with continuous masking noise (60 dB). The floor of each open-field was covered with removable quadrants made of one of two textures, wire mesh, or rough Plexiglas. These textures were chosen on the basis of previous studies indicating that naïve rats exhibited no unconditioned preference for one of them. Video cameras, mounted above each open-field, were connected with controlling equipment located in the adjacent room.

Experimental procedure

The general procedure consisted of two phases: conditioning and testing. Each rat was subjected to eight 30-min conditioning sessions (one session per day) in an open-field (always the same for each rat) whose four floor quadrants were of the same texture. Nicotine (0.06 mg/kg, s.c.), or its vehicle for the control group, was administered immediately before sessions 1, 3, 5, and 7, paired with one floor texture. Saline was injected (same administration schedule) before sessions 2, 4, 6, and 8, paired with the other floor texture. Nicotine–texture pairings were counterbalanced so that within each treatment group, nicotine would be associated with the wire mesh floor for half of the rats and with the Plexiglas floor for the other half. For each experiment, two 20-min test sessions were conducted in the same open-field whose floor was covered with two quadrants of the saline-paired texture and two quadrants of the nicotine-paired texture. Quadrants of the same texture were positioned diagonally opposite to each other. The first test session, conducted on day 9, i.e., 24 h after the last conditioning session, aimed at assessing the establishment of CPP to nicotine. Rats received no injection before this "probe test". The second test session, preceded by repeated administrations of rimonabant (see below), was conducted either 7, 14, or 21 days later. The time (in seconds) spent on each texture and the distance traveled (in centimeters) were automatically recorded by means of the video system and analyzed by appropriate software (SuperG Software, Hans C. Neijt; Novartis Pharma, Basel, Switzerland). Briefly, the cameras were connected to a frame grabber (type DT3155; Data Translation, Marlboro, MA, USA). Every second, the digitized frame was compared with the previously stored frame, whereby the pixels with altered intensity were identified and used to compute the position of the rat and the distance traveled. Half of the time spent on the dividing lines was added to the total time spent on the nicotine- and the saline-paired textures.

Treatment schedules

Three independent experiments were performed to assess the effects of postacquisition repeated intraperitoneal (i.p.) administrations of rimonabant [3 mg/kg per day, a dose chosen on the basis of previous study (Forget et al. 2005)] on the long-term expression of nicotine-induced CPP. These experiments were conducted according to the same treatment schedule and differed by only the number of days elapsing between the "probe test" and the second test session, i.e., 7, 14, or 21 days.

The rats were subjected to the acquisition phase of place conditioning to nicotine (0.06 mg/kg, s.c.) and to the probe test (without injection) as described above. In each experiment, the rats were divided into four treatment groups matched according to the time spent on the nicotine-paired texture during the probe test. Chronic treatments were then administered intraperitoneally daily between 1600 and 1700 hours, from day 10 (i.e., the day after the probe test) until the day before the second test session, and animals were given an additional injection 30 min before the test session. The rats of group 1 received vehicle daily and 30 min before the second test session ("Veh/Veh" group). The rats of group 2 were given vehicle daily and rimonabant (3 mg/kg) 30 min before the test session ("Veh/R3" group). The rats of group 3 received rimonabant (3 mg/kg) daily and vehicle 30 min pretest ("R3/Veh" group). The rats of group 4 received rimonabant (3 mg/kg) daily and an additional dose 30 min before the test session ("R3/R3" group).

In "Experiment 1", the rats were given six chronic plus one acute injections, and the second test session was performed 7 days after the probe test. In "Experiment 2", there were 13 chronic plus one acute injections, and the two test sessions were conducted 14 days apart. In "Experiment 3", the rats received 20 daily plus one acute injections, and 21 days elapsed between the two test sessions. "Experiments 1 and 3" included an additional "Saline control" group of animals which never received nicotine (i.e., given saline only during the conditioning phase) and which were subjected to the schedule of chronic plus acute intraperitoneal injections of the vehicle of rimonabant.

Quantitative autoradiography of CB1 receptor-mediated [^{35}S]GTP-γ-S binding

This study was conducted in rats used for "Experiment 3" (chronic rimonabant for 3 weeks). Twenty-four hours after the second test session, five randomly selected rats from each of the "Saline control", the "Veh/Veh", and the "R3/R3" nicotine groups were decapitated; their brains were rapidly removed and frozen in isopentane chilled at −30°C with dry ice. Coronal sections (20 μm thick) were cut in a cryostat at −20°C, thaw-mounted onto superfrost slides, and then stored at −80°C until use.

Autoradiography of agonist-stimulated [^{35}S]GTP-γ-S binding was performed according to published procedures with slight modifications (Breivogel et al. 1999; Fabre et al. 2000). Briefly, brain sections were (1) preincubated at 25°C for 10 min in 50 mM of Trizma base (Tris), pH 7.4, supplemented with NaCl (100 mM), $MgCl_2$ (3 mM), EGTA (0.2 mM) and bovine serum albumin (0.5% *w/v*), then (2) incubated for 15 min in the same buffer with 2 mM guanosine-5′-diphosphate sodium salt and 10 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, an A1 adenosine

receptor antagonist) to decrease background labeling (Laitinen and Jokinen 1998). Thereafter, sections were incubated for 2 h at 25°C in the same (but fresh) buffer into which 0.05 nM [^{35}S]GTP-γ-S (1,000 Ci/mmol) had been added without (basal conditions) or with (stimulated conditions) the CB receptor agonist, WIN 55212-2 (1, 10, and 100 μM). Nonspecific binding was determined with WIN 55212-2 (1, 10, and 100 μM) plus rimonabant (10 μM) to block CB1 receptors. Sections were then washed twice for 2 min each in ice-cold Tris buffer and briefly immersed in ice-cold distilled water. The slides were dried in a stream of cool air and apposed to a Kodak BioMax MR film for 48 h. Optical density (in arbitrary units) on autoradiograms was measured using a Biocom image analyzer (Les Ulis, France). [^{35}S]GTP-γ-S binding was quantified from six to eight sections for each assay condition [basal, stimulated (three agonist concentrations), and nonspecific (vs three agonist concentrations)] tested in each rat.

Drugs and chemicals

Rimonabant (SR 141716 base, micronized, Sanofi-Aventis, Montpellier, France) was suspended with one drop of Tween 80 in saline (0.9% NaCl, w/v) for in vivo studies or dissolved in DMSO (final concentration ≤0.2%, v/v) for in vitro studies. (−)Nicotine bitartrate (Sigma Chemicals, St. Louis, MO, USA) was dissolved in saline, and the pH was adjusted to between 7.3 and 7.5 with a few drops of 0.1 M NaOH. The doses were expressed as the free base. The drugs or their respective vehicle were injected in a volume of 5 ml/kg body weight. Other compounds were [^{35}S]GTP-γ-S (Amersham Pharmacia Biotech, Bucks, UK), WIN 55212-2, DPCPX, and GTP sodium salt (Sigma Chemicals).

Statistical analyses

Place conditioning results were expressed as mean (±SEM) time (in seconds) spent on the nicotine-paired floor texture during the test session. Place preference was assessed by testing whether the time spent on the nicotine-paired texture was longer than the time spent on the unpaired texture, using one-tailed, paired Student's t test. Treatment effects on preference time (i.e., paired minus unpaired time differences) were analyzed by one-way analysis of variance (ANOVA) followed, where appropriate, by Fisher's protected least significant difference (PLSD) test for multiple comparisons. "Experiments 1 and 3" were performed in duplicate; there were no statistically significant differences between the corresponding groups within each experiment, and data were pooled. Preference for the nicotine-paired texture, expressed as percent of the respective nicotine "Veh/Veh" group (percent of preference time), was plotted

against the time elapsed between the probe test and the test session (i.e., the chronic treatment duration), and simple linear regression fitting was calculated. Motor activity during the test session, expressed as mean (±SEM) distance (in centimeters) traveled, was analyzed by one-way ANOVA followed by pairwise comparisons using Fisher's PLSD test. Behavioral data were analyzed using StatView 5.0 software (SAS Institute, Cary, NC, USA).

The WIN 55212-2-stimulated [^{35}S]GTP-γ-S binding was plotted as percent augmentation above nonspecific binding (mean±SEM) and analyzed by two-way (WIN 55212-2 concentrations and treatment groups) ANOVAs. Nonlinear regression fitting was carried out using Prism 4.02 (Graph-Pad software, San Diego, CA, USA) software for the calculation of EC_{50} values and maximal effects (B_{max} value) of WIN 55212-2.

Results

Effects of postacquisition chronic administration of rimonabant on long-term expression of nicotine-induced conditioned place preference

Experiment 1

The rats of the "Saline control" group ($n=11$), never given nicotine during the conditioning phase, spent 614±55 s on the vehicle-paired floor texture during the first test session (probe test) and 589±60 s during the second test session conducted 1 week later. These values, not significantly different from those for the unpaired texture ($t_{10}=0.87$ and 0.19, respectively), indicated that there was no unconditioned preference for either floor texture.

Eighty rats were given nicotine (0.06 mg/kg, s.c.) during the conditioning phase. During the probe test conducted 24 h after the last conditioning session, they spent 787±23 s on the floor texture previously paired with nicotine (vs time spent on the unpaired texture: $t_{79}=8.22$; $p<0.0001$). Thus, the rats developed the expected preference for the nicotine-associated texture.

The results of the test session conducted 7 days after the probe test are reported in Fig. 1a. The rats given seven injections of vehicle ("Veh/Veh" group) spent 796±37 s on the texture previously paired with nicotine; this time was significantly above that spent on the unpaired texture ($t_{19}=5.28$, $p<0.0001$), indicating that preference for the nicotine-paired texture was still present 1 week after conditioning. As indicated by the ANOVA, a main treatment effect fell short of significance ($F_{(4,86)}=2.367$, $p=0.059$). This effect was only due to differences between the "Saline control" group and the "Veh/Veh" and "R3/ Veh" groups ($p<0.05$). The time spent on the paired texture

was significantly longer than that spent on the unpaired texture in the four nicotine-conditioned groups, i.e., rats given chronic vehicle plus a single 3 mg/kg dose of rimonabant 30 min before the test ("Veh/R3" group: 711±51 s; t_{19}=2.17, $p<0.05$), rats given chronic rimonabant plus vehicle pretest ("R3/Veh" group: 779±50 s; t_{19}=3.57, $p<0.002$), and those given seven injections of rimonabant ("R3/R3" group: 701±39 s; t_{19}=2.62, $p<0.02$). Thus, six daily injections of rimonabant without acute pretest dose ("R3/Veh" group) did not antagonize the expression of nicotine-induced CPP. Moreover, the rats given rimonabant before the test ("Veh/R3" and "R3/R3" groups) exhibited similar intermediate preference scores (significant paired vs unpaired time differences) that were not statistically different from either the nicotine "Veh/Veh" or the "Saline control" groups.

Experiment 2

All of the 48 rats included in this experiment were given nicotine (0.06 mg/kg, s.c.) during the conditioning phase. During the probe test, they spent 747±24 s on the floor texture previously associated with nicotine (vs unpaired texture: t_{47}=6.15, $p<0.0001$), indicating a marked conditioned preference for the nicotine-paired texture.

Figure 1b depicts the results of the test session conducted 14 days after the probe test. Although the ANOVA failed to yield a significant main treatment effect ($F_{(3,44)}$=1.11, NS), the time spent on the texture previously paired with nicotine was significantly longer than on the unpaired texture in rats of the "Veh/Veh" group (746±46 s; t_{11}=3.20, $p<0.01$), the "Veh/R3" group (715±51 s; t_{11}=2.28, $p<0.05$), and the "R3/Veh" group (781±46 s; t_{11}=3.95, $p<0.005$), indicating that CPP was still present in these animals. In contrast, the rats of the "R3/R3" group only spent 658±58 s on the nicotine-paired texture, a value which did not significantly differ from that spent on the unpaired texture (t_{11}=1.04). Thus, despite the absence of significant between-group differences, 14 days after conditioning, the expression of nicotine–CPP was reduced by daily injections of rimonabant (3 mg/kg) provided that an additional dose was administered 30 min before the test session.

Experiment 3

The 16 "Saline control" rats spent 586±49 s on the vehicle-paired floor texture during the probe test and 561±49 s during the test session conducted 3 weeks later. These values, not significantly different from those for the
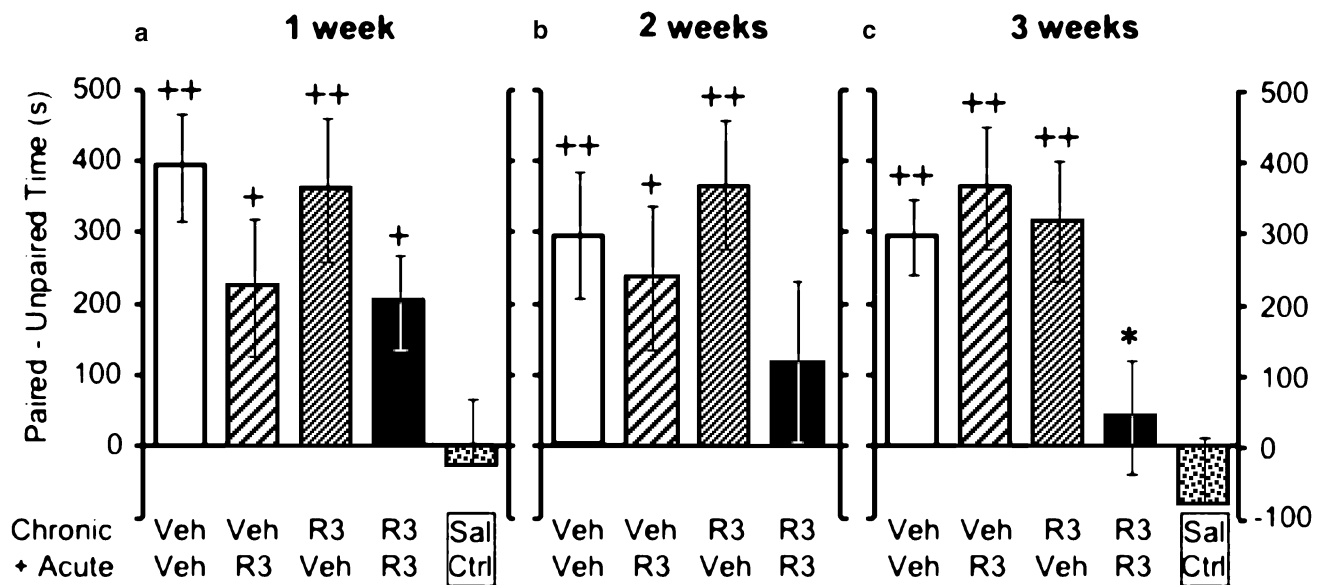

Fig. 1 Effects of chronic administration of rimonabant on long-term expression of nicotine-induced conditioned place preference. In three independent experiments, rats were given alternately nicotine (0.06 mg/kg, s.c.) and saline during the conditioning phase and were subjected to a probe test 24 h later. Then, they received 6 (a), 13 (b), or 20 (c) daily injections of rimonabant [3 mg/kg/day, i.p. (R3)], or vehicle (Veh), plus an additional administration of the same dose of rimonabant (R3), or vehicle (Veh), 30 min before a 20-min test session that was conducted 1, 2, or 3 weeks after the probe test, respectively. Saline control rats (Sal Ctrl) received saline (s.c.) before each conditioning session and chronic vehicle (i.p.) between the probe test and the test session. Histograms represent the mean (±SEM) difference between the time (in seconds) spent on the floor texture previously paired with nicotine and the time spent on the unpaired texture (preference time) during the test session. The number of rats per group is indicated in Table 1. +$p<0.05$; ++$p<0.01$; time spent on the nicotine-paired vs unpaired texture (paired Student's t test). *$p<0.05$; "R3/R3" group vs "Veh/Veh", "Veh/R3" and "R3/Veh" groups (Fisher's PLSD test after ANOVA)

unpaired texture (t_{15}=0.29 and 0.83, respectively), confirmed that there was no spontaneous preference for either floor texture.

The 102 rats administered with nicotine (0.06 mg/kg, s.c.) during the conditioning phase spent 745±18 s on the nicotine-paired texture during the probe test (vs unpaired time: t_{101}=7.88; p<0.0001), indicating that they did develop preference for the nicotine-associated texture.

Figure 1c illustrates the results obtained during the test session conducted 21 days after the probe test. The time spent on the nicotine-paired texture was significantly above that for unpaired texture in rats of the "Veh/Veh" group (746±26 s; t_{25}=5.65, p<0.0001), the "Veh/R3" group (780±43 s; t_{25}=4.16, p<0.0003), and the "R3/Veh" group (757±42 s; t_{23}=3.75, p<0.001). In contrast, the rats of the "R3/R3" group only spent 623±46 s on the texture previously paired with nicotine, a time not significantly different from that spent on the unpaired texture (t_{25}=0.41). The ANOVA indicated a main treatment effect on the time differences ($F_{(4,113)}$=4.24; p=0.0031). Post hoc comparisons revealed that performance of the nicotine-conditioned "Veh/Veh", "Veh/R3", and "R3/Veh" groups were significantly different from the "Saline control" group (p_s<0.01) and that the "R3/R3" group differed significantly from the other three nicotine-conditioned groups (vs Veh/Veh: p<0.05; vs "Veh/R3": p<0.01; vs "R3/Veh": p<0.05) but not from the "Saline control" group. Overall, these results indicate that the incentive value of the nicotine-paired texture was still present 3 weeks after the conditioning phase and was prevented by neither a single pretest 3 mg/kg dose of rimonabant ("Veh/R3" group), as previously shown (Forget et al. 2005), nor by 20 daily injections of the CB1 receptor antagonist without additional pretest dosing ("R3/Veh" group). In contrast, the 3-week chronic administration of rimonabant followed by an acute dose before the test session ("R3/R3" group) abolished the long-term expression of nicotine-induced CPP.

As illustrated in Fig. 2, simple linear regressions calculated on percent of the preference time plotted against the delay between the probe test and the test session (1, 7, 14, or 21 days) indicated that the time spent on the nicotine-paired texture was significantly related to the retention interval in rats given a single pretest injection of rimonabant (24 h—R3 group in the study by Forget et al. (2005), and "Veh/R3" group in the present study) ($F_{(1,67)}$=9.04; r=+0.35; p<0.004). There were no such significant time–effect relationships in the other rimonabant-injected groups ("R3/Veh": $F_{(1,54)}$=0.11, r=+0.05; "R3/R3": $F_{(1,56)}$=0.99, r=−0.13; NS). Thus, the ability of a single acute dose of rimonabant (3 mg/kg) to counteract the expression of nicotine-induced CPP progressively vanished within 3 weeks.

Table 1 reports the distance traveled by rats during the test session. Depending on the experiment, the "Veh/Veh"
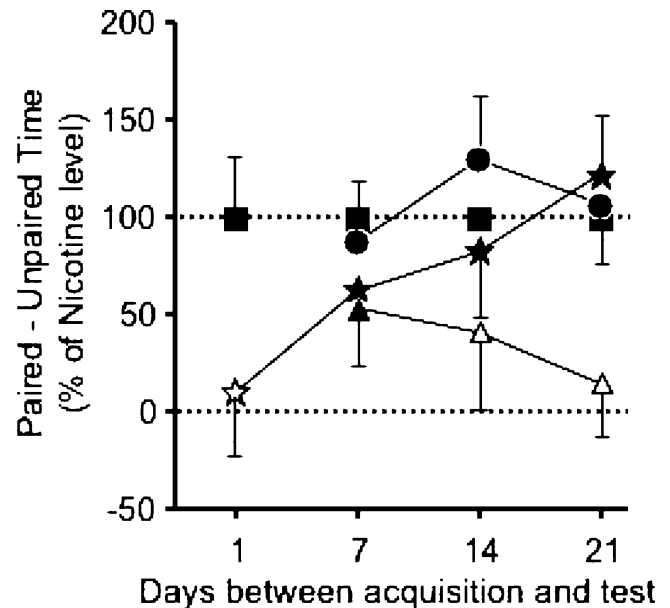

Fig. 2 Evolution of the expression of nicotine-induced conditioned place preference according to both the time interval between the acquisition phase and the test session and the number of injections of rimonabant. Results are those of nicotine-conditioned rats presented in Fig. 1. They are expressed as percent (±SEM) of the preference time of the respective nicotine-conditioned "Veh/Veh" control rats. Results of day 1 (a single 3 mg/kg dose of rimonabant, 30 min before the test session conducted 24 h after the last conditioning session) are from Forget et al. (2005). The *horizontal dashed lines* indicate the no preference (0%) and the CPP induced by nicotine alone (100%). For the sake of clarity, some *error bars* are omitted. *Squares* "Veh/Veh" (chronic + acute vehicle), *stars* "Veh/R3" (chronic vehicle + acute rimonabant, 3 mg/kg), *circles* "R3/Veh" (chronic R3 + acute vehicle), *triangles* "R3/R3" (chronic + acute R3) groups. *Black symbols* Groups exhibiting a conditioned preference as indicated by the time spent on the nicotine-paired texture significantly longer than that on the unpaired texture (p<0.05)

rats traveled 35–45 m in 20 min, a distance that did not differ from that of the "Saline control" animals. In nicotine-conditioned rats, an overall treatment effect was observed in "Experiment 1" ($F_{(3,76)}$=4.73, p<0.005) and "Experiment 3" ($F_{(3,98)}$=10.53, p<0.0001). Subsequent pairwise comparisons indicated that this was due to a significant reduction of the distance traveled by rats of the "Veh/R3" and "R3/R3" groups compared to the "Veh/Veh" group in "Experiments 1 and 3", and also by rats of the "Veh/R3" compared to the "R3/Veh" group in "Experiment 3". Thus, in these two experiments, a significant reduction of motor activity that ranged from −30 to −40% of control levels was observed in rats given rimonabant 30 min before the test session whatever the previous chronic treatment. In "Experiment 2", though there was a trend for a reduction in motor activity in the corresponding groups (−20 and −25%), this effect did not reach the level of statistical significance ($F_{(3,44)}$=1.49, NS).

Table 1 Distance traveled during the test session conducted either 7 days ("Experiment 1"), 14 days ("Experiment 2"), or 21 days ("Experiment 3") after the probe test for nicotine-induced conditioned place preference

Treatment groups (chronic/acute)	Distance (cm) traveled during the 20-min test session (mean±SEM)		
	Experiment 1 (7 days)	Experiment 2 (14 days)	Experiment 3 (21 days)
(1) Veh/Veh	(n=20) 3664±300	(n=12) 4104±369	(n=26) 4538±287
(2) Veh/R3	(n=20) 2326±382*	(n=12) 3297±419	(n=26) 2917±243*·**
(3) R3/Veh	(n=20) 2979±243	(n=12) 3886±350	(n=24) 4381±274
(4) R3/R3	(n=20) 2409±166*	(n=12) 3140±379	(n=26) 3114±231*
(5) Saline control	(n=11) 3921±371	–	(n=16) 4038±395

Rats of groups (1) to (4) were given alternately nicotine (0.06 mg/kg, s.c.) and saline during the conditioning phase, and subsequently, they received 6, 13, or 20 daily injections of rimonabant (3 mg/kg/day, i.p.; R3), or vehicle (Veh), plus an additional administration of the same dose of rimonabant (R3), or vehicle (Veh), 30 min before the second test session. The rats of the "Saline control" group (5) were nicotine-naïve and received daily i.p. injections of vehicle (see "Materials and methods" section). The number of rats per group is indicated in parentheses.
*$p<0.01$ vs respective "Veh/Veh" group
**$p<0.05$ vs respective "R3/Veh" group (Fisher's PLSD test after ANOVA)

Quantitative autoradiography of [^{35}S]GTP-γ-S binding evoked by CB1 receptor stimulation

As illustrated in Fig. 3a, the CB receptor agonist, WIN 55212-2 (1, 10, and 100 μM), induced a concentration-dependent increase of [^{35}S]GTP-γ-S binding in the hippocampal CA3 area, the lateral caudate-putamen (CPu), and the shell of the nucleus accumbens (NAcc), and similar results were obtained in all other brain structures examined (not shown). Regional differences were noted in the maximal increase produced by 100 μM WIN 55212-2, with the hippocampal CA3 area showing the largest increase, followed, in decreasing order, by the CA1 area, dentate gyrus (DG), lateral CPu, cingulate cortex (Cg Cx), medial CPu, NAcc core, and NAcc shell (Fig. 3b). In contrast, the calculated EC$_{50}$ value of WIN 55212-2 did

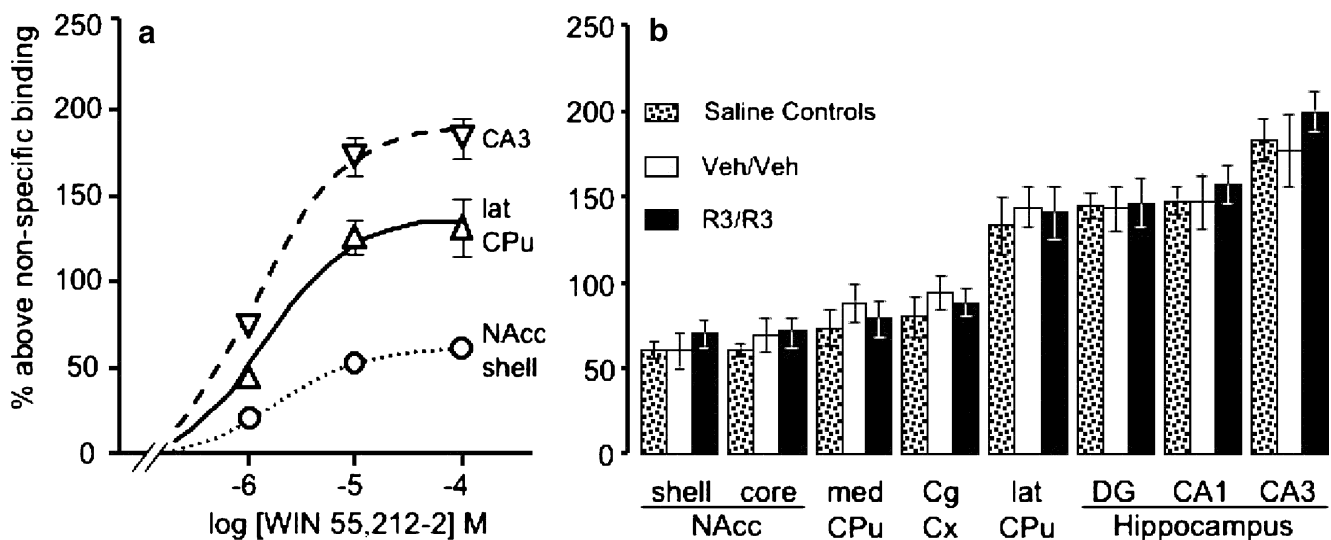## CB1 receptor-stimulated [^{35}S]GTP-γ-S binding

Fig. 3 **a** Concentration-dependent increase by WIN 55212-2 of [^{35}S]GTP-γ-S autoradiographic labeling of three selected areas in brain sections from "Saline control" rats. **b** Maximal increase (B_{max}) in [^{35}S]GTP-γ-S autoradiographic labeling produced by 100 μM of WIN 55212-2 in eight different brain areas of rats from "Experiment 3". "Saline control" group: nicotine-naïve rats given 21 daily injections of vehicle between the first and the second test sessions; "Veh/Veh" group: rats conditioned to nicotine (0.06 mg/kg, s.c.) and given 21 daily i.p. injections of vehicle between the first and the second test sessions; "R3/R3" group: rats conditioned to nicotine and given 21 daily injections of rimonabant (3 mg/kg, i.p.) between the first and the second test sessions. *Each point* or *bar* is the mean (±SEM) WIN 55212-2-induced increase of [^{35}S]GTP-γ-S binding expressed as percentage above nonspecific binding. Some *error bars* are smaller than *symbols* (n=5 rats per group). *NAcc* Nucleus accumbens, *CPu* caudate-putamen, median or lateral, *Cg Cx* cingular cortex, *DG* dentate gyrus. Two-way ANOVAs indicated a statistically significant effect of WIN 55212-2 concentration in each structure considered (all $p<0.0001$), but there were no group effects and no WIN × group interactions

not significantly differ from one brain area to another [EC_{50} in micromolars (mean±SEM): CA3=1.35±0.29, CA1=1.57±0.53, DG=1.54±0.31, lateral Cpu=1.54±0.16, Cg Cx=2.47±0.36, medial CPu=1.79±0.22, NAcc core=1.63±0.22, NAcc shell=1.19±0.21, $F_{(7,32)}$=1.56, NS]. In all these regions, the effect of WIN 55212-2 could be ascribed to the activation of CB1 receptors since it was completely prevented by rimonabant (10 μM), which, on its own, did not change [^{35}S]GTP-γ-S binding compared to baseline (data not shown). In each brain area, the ANOVAs indicated an overall significant effect of WIN 55212-2 (all $p<0.0001$) but no treatment effect and no interaction. Thus, previous administration of nicotine alone ("Veh/Veh" group) or nicotine plus rimonabant ("R3/R3" group) had no significant influence on the activation of G-proteins by CB1 receptor stimulation whatever the brain structure considered (Fig. 3b).

Discussions

The present study supports and extends our previous findings (Forget et al. 2005) giving evidence that, in rats subjected to an unbiased procedure, (1) the 0.06 mg/kg dose of nicotine reliably induced robust CPP, (2) the expression of such incentive learning endured for several weeks without additional exposure to the drug and the test apparatus, and (3) a single pretest blockade of CB1 receptors by rimonabant, at the 3 mg/kg dose active to abolish the short-term (24 h) expression of nicotine-induced CPP, was totally ineffective when the test session took place 3 weeks after the conditioning phase. As illustrated in Fig. 2, the results of test sessions conducted at shorter time intervals indicate that the ability of acute rimonabant to inhibit approach behavior elicited by nicotine-associated floor texture was reduced within 7 days and was totally abolished 2 weeks after the conditioning phase ("Veh/R3" group). Thus, whereas the activation of CB1 receptors is likely to be necessary for the short-term expression of nicotine incentive learning, at longer time intervals, the same function seems to be subserved by neurobiological processes, independent of the endocannabinoid system, which developed progressively. It is most interesting that the acute pretest blockade of CB1 receptors retained its capacity to abolish long-term expression of nicotine–CPP in rats that had been given daily injections of rimonabant during the 2- or 3-week postacquisition period ("R3/R3" group). Therefore, the endocannabinoid activation of CB1 receptors seems essential to the development of neuro-adaptive changes responsible for the progressive shift from CB1-dependent to CB1-independent expression of nicotine incentive learning.

The rats given acute rimonabant exhibited a decrease in locomotor activity as previously reported (Chaperon et al. 1998; Singh et al. 2004). This effect was observed in both the "Veh/R3" and "R3/R3" groups in "Experiments 1 and 3", and the possibility that hypoactivity contributed to the observed effects of the blockade of CB1 receptors cannot be discounted. In contrast, in "Experiment 2", the distance traveled by rats was not significantly modified by rimonabant administered either as a single pretest dose ("Veh/R3" group), as already observed (Le Foll and Goldberg 2004; Forget et al. 2005), or as daily injections ("R3/Veh" and "R3/R3" groups). Collectively, these data indicate that rimonabant had rather variable consequences on locomotor activity. It is of interest that it must be emphasized that, whatever the experiment, motor effects were not observed in the "R3/Veh" group, indicating that no long lasting effect occurred even after 20 daily injections. Thus, 3 weeks after conditioning, despite a similar reduction of locomotor activity (Table 1), the rats of the "Veh/R3" group exhibited an unaltered preference for the nicotine-paired texture, whereas rats of the "R3/R3" group no longer expressed preference for that texture. On the other hand, although the present study was not designed to investigate the consequences of chronic rimonabant on food intake, it is of note that body weight gain was significantly attenuated (for instance in "Experiment 3", the 20-day weight gains were: Veh=+55.00±2.48 g; R3=+43.33±2.60 g; $p<0.005$; Saline control=+55.63±4.86 g). This effect is in keeping with the known consequences of CB1 receptor blockade on food consumption and body weight (see, e.g., Ravinet Trillou et al. 2003 for a review). Nevertheless, the expression of nicotine–CPP was clearly different in the "R3/Veh" and "R3/R3" groups in spite of a similar attenuation of body weight gain as compared with the other two groups ("Veh/ Veh" and "Veh/R3"). Collectively, these data indicate that the differential effects of acute and chronic rimonabant on long-term expression of nicotine–CPP are unlikely secondary to nonspecific motor or feeding effects.

Other alternative factors which could be responsible for the effects of rimonabant on conditioned incentive stimuli associated with nicotine (e.g., rimonabant-induced motivational or mnemonic effects, nicotine-like discriminative effects or alteration of nicotine discrimination) were considered in previous publications (Cohen et al. 2002; Le Foll and Goldberg 2004; Forget et al. 2005). It seems unlikely for any of these factors to be a potential confounder under the conditions used in our studies.

To assess whether possible changes in the functional properties of CB1 receptors would account for the shift from CB1-dependent to CB1-independent expression of nicotine incentive learning, we investigated whether the capacity of CB1 receptor stimulation to increase [^{35}S]GTP-γ-S binding exhibited adaptive changes under the long-term

treatment conditions used in our protocols. As expected from its mediation by CB1 receptors, the rimonabant-sensitive WIN 55212-2-induced increase in [^{35}S]GTP-γ-S binding exhibited the same EC_{50} value, whatever the brain area considered, but marked regional differences in B_{max} values that matched the known regional variations in CB1 receptor density (Herkenham et al. 1991). However, CB1 receptor-mediated G-protein activation did not differ between "Veh/Veh" and "Saline control" rats, indicating that, under our conditions, nicotine conditioning that was achieved in "Veh/Veh" rats had no influence on CB1 receptor coupling with G-proteins. Similarly, Balerio et al. (2004) already reported that a 6-day infusion of nicotine (circa 9 mg free base kg^{-1} day^{-1}) affected neither the number and distribution of CB1 receptor binding sites nor the activation of G-proteins by WIN 55212-2 in mice.

Previous studies clearly established that chronic treatments with cannabinoid agonists downregulate and desensitize CB1 receptors in various brain regions in rats (Breivogel et al. 1999). However, opposite changes (supersensitivity and/or upregulation) did not occur after chronic (3 weeks) CB1 receptor blockade by rimonabant under our own conditions. Indeed, B_{max} (and EC_{50}) values of WIN 55212-2-induced [^{35}S]GTP-γ-S binding did not differ in rimonabant-treated rats ("R3/R3" group) compared to the other two groups of animals which did not receive the CB1 antagonist ("Saline control" and "Veh/Veh"). In line with these results, Rubino et al. (2000) showed that, in rats given rimonabant (5 mg/kg, i.p.) once a day for 4 days, cyclic adenosine monophosphate levels and protein kinase A activity did not change in several brain areas, suggesting that repeated blockade of CB1 receptors did not alter their intracellular signaling mechanisms. Therefore, although in the present study rimonabant was administered for longer periods of time, in rats previously given nicotine, the observed long-term behavioral responses were unlikely accounted for by compensatory alterations in CB1 receptor functional activity. They could possibly result from complex adaptive changes in the neuronal reward pathways.

Nicotine shares with other addictive drugs the property of activating the mesolimbic dopaminergic (DA) reward pathway (see, e.g., Balfour 2002 and Pierce and Kumaresan 2006 for reviews). Indeed, at low concentrations comparable to those obtained by smoking, nicotine induces a prolonged increase in firing and burst rates of DA neurons in the ventral tegmental area (VTA) by interacting with different nACh receptors located on these neurons and on GABAergic and glutamatergic afferents. This effect, along with a direct action of nicotine on DAergic terminals, promotes a sustained DA overflow in the NAcc, a brain area that plays a key role in the rewarding effects of drugs (Salamone et al. 2005). It is interesting to note that these responses sensitize upon repeated administrations, suggest-

ing that nicotine induces synaptic plasticity (see, e.g., Pidoplichko et al. 2004, Wonnacott et al. 2005, and Pierce and Kumaresan 2006 for reviews). However, it must be noted that a role for such process in reward-related behavior is still a matter of debate because enhancement of DA release as a mechanism underlying behavioral sensitization to nicotine (Fung and Lau 1989; Benwell and Balfour 1992) has not been consistently demonstrated in sensitized rats (Nisell et al. 1996; Birrell and Balfour 1998). On the other hand, several lines of evidence indicate that the endocannabinoid system is involved in the motivation for a variety of drugs of abuse including nicotine (see "Introduction") through an interaction with the mesolimbic DA pathway (see, e.g., Tanda and Goldberg 2003 for a review). Convergent studies showed that, within the VTA, the local release of DA may induce a transient release of endocannabinoids that act as retrograde messengers on presynaptic CB1 receptors located on afferent terminals to reduce the local release of both GABA (Szabo et al. 2002) and glutamate (Melis et al. 2004). To the best of our knowledge, the respective intensities of these opposing effects have not been assessed in the VTA. However, studies on hippocampal slices showed that the inhibitory transmission is markedly more sensitive than the excitatory transmission to CB receptor agonists (Ohno-Shosaku et al. 2002; Chen et al. 2003). Therefore, it can be hypothesized that CB1-mediated suppression of GABAergic inhibitory control has greater functional consequence on the net excitability of postsynaptic DA neurons than the concomitant relatively mild reduction of glutamatergic excitatory input. Thus, it is striking that both nicotine and endocannabinoids interfere with GABAergic and glutamatergic control over VTA DA neurons, through receptors located on afferent terminals, to enhance (nACh) and suppress (CB1) local neurotransmitter release, respectively.

Endocannabinoids and nicotine might also exert control over VTA DA neuron activity through multisynaptic circuits, in particular from the NAcc. In this brain structure, the activation of nACh receptors located on GABAergic interneurons and neurons projecting into the VTA, and on glutamatergic afferent terminals, enhances neurotransmitter release (Wonnacott et al. 2005). Likewise, endocannabinoids can be released from GABAergic medium spiny neurons in the NAcc and, through retrograde activation of CB1 receptors on glutamatergic afferents, they can induce long-term depression of the excitatory input to the NAcc (Robbe et al. 2002). One consequence of this action is a reduction of the firing rate of GABAergic neurons projecting into the VTA, which, in turn, results in the disinhibition of DA neuron activity (Pistis et al. 2002). In fact, an ex vivo study in rats indicated that chronic nicotine (1 mg kg^{-1} day^{-1} × 7 days) enhanced anandamide levels in limbic forebrain (González et al. 2002). If this effect

generalizes to the dose and the rhythm of injections used in the present study, then it can be speculated that the activity of DA neurons can be modulated by CB1 receptor-mediated trans- and multisynaptic processes. This could represent as many possible mechanisms whereby rimonabant interferes with both the motivational properties of nicotine and the development of long-term synaptic plasticity responsible for the progressive shift from CB1-dependent to CB1-independent expression of CPP supported by nicotine.

To conclude, the present study shows that the capacity of a single pretest injection of rimonabant to abolish approach behavior and the lengthened time contact elicited by the floor texture previously paired with nicotine progressively vanished within 1 to 2 weeks. This indicates that the endocannabinoid system, which plays a pivotal role in the initial neurobiological processes underlying the attribution of incentive salience to cues related to nicotine effects (Forget et al. 2005), is progressively replaced by other system(s) and no longer constitutes a necessary step for the long-term expression of nicotine–CPP. Daily administrations of rimonabant during the postacquisition period allowed an acute pretest injection of this CB1 receptor antagonist to retain its blocking effect on the long-term expression of nicotine-induced CPP without altering the functional G-protein coupling of CB1 receptors in all the brain regions considered. Thus, a sustained (or repeated) activation of CB1 receptors is likely to be necessary for the development of adaptive processes during and/or after the establishment of nicotine–CPP. As a result, the endocannabinoid system no longer controls the dominant influence exerted by nicotine-paired cues on rats' behavior. The neurobiological processes underlying such endocannabinoid-dependent synaptic plasticity remain to be elucidated. However, it can be speculated that the opposite actions exerted by nicotine and endocannabinoids on GABAergic and glutamatergic control over DA neurons in the VTA and limbic projection areas represent possible mechanisms through which rimonabant interferes with nicotine-induced neuronal plasticity.

References

Arnone M, Maruani J, Chaperon F, Thiébot MH, Poncelet M, Soubrié P, Le Fur G (1997) Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology (Berl) 132:104–106

Balerio GN, Aso E, Berrendero F, Murtra P, Maldonado R (2004) Δ^9-Tetrahydrocannabinol decreases somatic and motivational manifestations of nicotine withdrawal in mice. Eur J Neurosci 20:2737–2748

Balfour DJ (2002) Neuroplasticity within the mesoaccumbens dopamine system and its role in tobacco dependence. Curr Drug Targets CNS Neurol Disord 1:413–421

Bell SM, Stewart RB, Thompson SC, Meisch RA (1997) Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology (Berl) 131:1–8

Benwell ME, Balfour DJ (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br J Pharmacol 105:849–856

Birrell CE, Balfour DJ (1998) The influence of nicotine pretreatment on mesoaccumbens dopamine overflow and locomotor responses to D-amphetamine. Psychopharmacology (Berl) 140:142–149

Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Vogt LJ, Sim-Selley LJ (1999) Chronic Δ^9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. J Neurochem 73:2447–2459

Carr KD (2002) Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms. Physiol Behav 76:353–364

Castañé A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O (2002) Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. Neuropharmacology 43:857–867

Chaperon F, Soubrié P, Puech AJ, Thiébot MH (1998) Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. Psychopharmacology (Berl) 135:324–332

Chen K, Ratzliff A, Hilgenberg L, Gulyas A, Freund TF, Smith M, Dinh TP, Piomelli D, Mackie K, Soltesz I (2003) Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. Neuron 39:599–611

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrié P (2002) SR141716, a central cannabinoid (CB$_1$) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol 13:451–463

De Vries TJ, Schoffelmeer ANM (2005) Cannabinoid CB$_1$ receptors control conditioned drug seeking. Trends Pharmacol Sci 26:420–426

De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J, Vanderschuren LJ, Schoffelmeer AN (2001) A cannabinoid mechanism in relapse to cocaine seeking. Nat Med 7:1151–1154

De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer AN (2003) Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology (Berl) 168:164–169

Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy DL, Lanfumey L, Hamon M, Martres MP (2000) Altered expression and functions of serotonin 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in knock-out mice lacking the 5-HT transporter. Eur J Neurosci 12:2299–2310

Forget B, Hamon M, Thiébot MH (2005) Cannabinoid CB$_1$ receptors are involved in motivational effects of nicotine in rats. Psychopharmacology (Berl) 181:722–734

Fung YK, Lau YS (1989) Effect of nicotine pretreatment on striatal dopaminergic system in rats. Pharmacol Biochem Behav 32:221–226

González S, Cascio MG, Fernández-Ruiz J, Fezza F, Di Marzo V, Ramos JA (2002) Changes in endocannabinoid contents in the

brain of rats chronically exposed to nicotine, ethanol or cocaine. Brain Res 954:73–81

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563–583

Laitinen JT, Jokinen M (1998) Guanosine $5'$-(γ-[35S]thio)triphosphate autoradiography allows selective detection of histamine H3 receptor-dependent G protein activation in rat brain tissue sections. J Neurochem 71:808–816

Le Foll B, Goldberg SR (2004) Rimonabant, a CB_1 antagonist, blocks nicotine-conditioned place preferences. Neuroreport 15:2139–2143

Le Foll B, Goldberg SR (2005) Cannabinoid CB_1 receptor antagonists as promising new medications for drug dependence. J Pharmacol Exp Ther 312:875–883

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Böhme GA, Imperato A, Fratta W, Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. Science 283:401–404

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

Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 24:53–62

Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L, Chowen JA, Gomez R, del Arco I, Villanua MA, Maldonado R, Koob GF, Rodríguez de Fonseca F (2001) Functional interaction between opioid and cannabinoid receptors in drug self-administration. J Neurosci 21:5344–5350

Nisell M, Nomikos GG, Hertel P, Panagis G, Svensson TH (1996) Condition-independent sensitization of locomotor stimulation and mesocortical dopamine release following chronic nicotine treatment in the rat. Synapse 22:369–381

Ohno-Shosaku T, Tsubokawa H, Mizushima I, Yoneda N, Zimmer A, Kano M (2002) Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. J Neurosci 22:3864–3872

Pertwee RG (1999) Pharmacology of cannabinoid receptor ligands. Curr Med Chem 6:635–664

Pidoplichko VI, Noguchi J, Areola OO, Liang Y, Peterson J, Zhang T, Dani JA (2004) Nicotinic cholinergic synaptic mechanisms in the ventral tegmental area contribute to nicotine addiction. Learn Mem 11:60–69

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30:215–238

Pistis M, Muntoni AL, Pillolla G, Gessa GL (2002) Cannabinoids inhibit excitatory inputs to neurons in the shell of the nucleus accumbens: an in vivo electrophysiological study. Eur J Neurosci 15:1795–1802

Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, Soubrié P (2003) Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am J Physiol Regul Integr Comp Physiol 284:R345–R353

Rinaldi-Carmona M, Barth F, Héaulme M, Alonso R, Shire D, Congy C, Soubrié P, Brelière JC, Le Fur G (1995) Biochemical and pharmacological characterization of SR 141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci 56:1941–1947

Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc Natl Acad Sci USA 99:8384–8388

Rubino T, Vigano D, Zagato E, Sala M, Parolaro D (2000) In vivo characterization of the specific cannabinoid receptor antagonist, SR141716A: behavioral and cellular responses after acute and chronic treatments. Synapse 35:8–14

Salamone JD, Correa M, Mingote SM, Weber SM (2005) Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. Curr Opin Pharmacol 5:34–41

Singh ME, Verty AN, McGregor IS, Mallet PE (2004) A cannabinoid receptor antagonist attenuates conditioned place preference but not behavioural sensitization to morphine. Brain Res 1026:244–253

Szabo B, Siemes S, Wallmichrath I (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15:2057–2061

Tanda G, Goldberg SR (2003) Cannabinoids: reward, dependence, and underlying neurochemical mechanisms—a review of recent preclinical data. Psychopharmacology (Berl) 169:115–134

Tanda G, Munzar P, Goldberg SR (2000) Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci 3:1073–1074

Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA (2004) Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 306:1029–1032

van der Stelt M, Di Marzo V (2003) The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. Eur J Pharmacol 480:133–150

Vinklerová J, Nováková J, Sulcová A (2002) Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM 251. J Psychopharmacol 16:139–143

Wonnacott S, Sidhpura N, Balfour DJ (2005) Nicotine: from molecular mechanisms to behaviour. Curr Opin Pharmacol 5:53–59

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci USA 96:5780–5785