British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01459.x
www.brjpharmacol.org

Themed Issue: Cannabinoids in Biology and Medicine, Part I

RESEARCH PAPER

Differential effect of opioid and cannabinoid receptor blockade on heroin-seeking reinstatement and cannabinoid substitution in heroin-abstinent rats

L Fattore[1,2], MS Spano[3], V Melis[3]*, P Fadda[2,3] and W Fratta[2,3]

[1]*Institute of Neuroscience-Cagliari, CNR National Research Council of Italy, Cagliari, Italy,* [2]*Centre of Excellence 'Neurobiology of Dependence', Cagliari, Italy, and* [3]*Department of Neuroscience, University of Cagliari, Cittadella Universitaria di Monserrato, Monserrato, Italy*

Correspondence
Liana Fattore, CNR National Research Council of Italy, Institute of Neuroscience-Cagliari c/o Department of Neuroscience, University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (Cagliari), Italy. E-mail: lfattore@in.cnr.it

*Present address: School of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK.

Keywords
Cannabinoid CB_1 receptor; opioid; self-administration; reinstatement; drug-seeking; naloxone; rimonabant; drug substitution; craving incubation

Received
30 November 2010
Revised
6 April 2011
Accepted
19 April 2011

BACKGROUND AND PURPOSE

Opioids and cannabinoids interact in drug addiction and relapse. We investigated the effect of the opioid receptor antagonist naloxone and/or the cannabinoid CB_1 receptor antagonist rimonabant on cannabinoid-induced reinstatement of heroin seeking and on cannabinoid substitution in heroin-abstinent rats.

EXPERIMENTAL APPROACH

Rats were trained to self-administer heroin (30 $\mu g \cdot kg^{-1}$ per infusion) under a fixed-ratio 1 reinforcement schedule. After extinction of self-administration (SA) behaviour, we confirmed the effect of naloxone (0.1–1 $mg \cdot kg^{-1}$) and rimonabant (0.3–3 $mg \cdot kg^{-1}$) on the reinstatement of heroin seeking induced by priming with the CB_1 receptor agonist WIN55,212-2 (WIN, 0.15–0.3 $mg \cdot kg^{-1}$). Then, in a parallel set of heroin-trained rats, we evaluated whether WIN (12.5 $\mu g \cdot kg^{-1}$ per infusion) SA substituted for heroin SA after different periods of extinction. In groups of rats in which substitution occurred, we studied the effect of both antagonists on cannabinoid intake.

KEY RESULTS

Cannabinoid-induced reinstatement of heroin seeking was significantly attenuated by naloxone (1 $mg \cdot kg^{-1}$) and rimonabant (3 $mg \cdot kg^{-1}$) and fully blocked by co-administration of sub-threshold doses of the two antagonists. Moreover, contrary to immediate (1 day) or delayed (90 days) drug substitution, rats readily self-administered WIN when access was given after 7, 14 or 21 days of extinction from heroin, and showed a response rate that was positively correlated with the extinction period. In these animals, cannabinoid intake was increased by naloxone (1 $mg \cdot kg^{-1}$) and decreased by rimonabant (3 $mg \cdot kg^{-1}$).

CONCLUSIONS AND IMPLICATIONS

Our findings extend previous research on the crosstalk between cannabinoid and opioid receptors in relapse mechanisms, which suggests a differential role in heroin-seeking reinstatement and cannabinoid substitution in heroin-abstinent rats.

LINKED ARTICLES

This article is part of a themed issue on Cannabinoids in Biology and Medicine. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

ERK, extracellular signal-regulated kinase; SA, self-administration; WIN55,212-2 (WIN), (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone

Introduction

Opioid abuse in humans is characterized by alternating periods of drug consumption and abstinence. With time, the likelihood of falling into a new use of the drug becomes extremely high and constitutes a substantial problem in the management of heroin addicts (O'Brien, 2005). Previous studies have suggested that some heroin addicts manage to detoxify and recover from their addiction without any medical assistance (Greaven and Greaven, 1983), although the majority attempt self-detoxification with the help of diazepam (43%), alcohol (25%) or cannabis (22%) (Noble *et al.*, 2002). Besides heroin, cannabis is the most prevalent type of illicit drug used among heroin addicts, and its use seems not to affect methadone treatment outcome, nor does it facilitate heroin resumption in polydrug users (Seivewright, 2003; Weizman *et al.*, 2004; Nava *et al.*, 2007). However, whether these studies support a harm reduction approach as opposed to a strict abstinence-oriented approach is still unclear.

It is now widely acknowledged that many pharmacological effects of opioids are affected by cannabinoid agents and that opioid receptors interact with the cannabinoid CB_1 receptors (nomenclature follows Alexander *et al.*, 2009) at molecular/cellular (Shapira *et al.*, 2003; Viganò *et al.*, 2005; Fattore *et al.*, 2007d; Butler *et al.*, 2008), neurochemical (Tanda *et al.*, 1997; Manzanares *et al.*, 1999; Schoffelmeer *et al.*, 2006) and behavioural (De Vries *et al.*, 2003; Fattore *et al.*, 2004; 2005a; Trezza and Vanderschuren, 2008) levels. Opioid and cannabinoid receptors act largely via the same group of G-proteins and are not only expressed in similar brain areas, but are also co-expressed in individual neurons in the rat caudate putamen (Rodriguez *et al.*, 2001), nucleus accumbens (Pickel *et al.*, 2004) and dorsal horn (Salio *et al.*, 2001). Intriguingly, chronic cannabinoid exposure blocks synaptic plasticity in the nucleus accumbens and reduces the sensitivity of GABAergic and glutamatergic synapses to both cannabinoids and opioids (Hoffman *et al.*, 2003). As the neural circuitry that underlies the reinstatement of heroin-seeking behaviour is more diffusely distributed than for other drugs of abuse, such as cocaine (Rogers *et al.*, 2008), it is conceivable that it might be under the control of an endogenous cannabinoid tone.

Functional interactions with the endogenous cannabinoid system are considered of primary importance in the modulation of the opioid rewarding effects (Mas-Nieto *et al.*, 2001; Navarro *et al.*, 2001; Solinas *et al.*, 2003; 2005; Caillé and Parsons, 2006). That is, the integrity of central cannabinoid CB_1 receptors is essential for adaptive responses produced by chronic morphine (Martin *et al.*, 2000), as well as for acute opioid self-administration (SA) (Ledent *et al.*, 1999). Moreover, deletion of the CB_1 receptor gene affects the availability of μ-opioid receptors and/or dopamine innervation in the mouse nucleus accumbens shell (Lane *et al.*, 2010). Nevertheless, preclinical studies that have investigated opioid–cannabinoid interactions in drug craving and relapse are still limited in number (De Vries and Schoffelmeer, 2005; Fattore *et al.*, 2007a,b; Robledo *et al.*, 2008). In our earlier work, we have shown that the opioid antagonist naloxone is able to reduce the reinstatement of cannabinoid seeking in rats (Spano *et al.*, 2004), and that the cannabinoid antagonist/inverse agonist rimonabant is able to attenuate drug-induced reinstatement of heroin-seeking behaviour (Fattore *et al.*, 2005b). In addition, a durable reinstating effect of cannabinoid priming is found over a few days after the acute reinstatement test session (Fattore *et al.*, 2003). Whether such an effect might be mediated by the CB_1 and/or the opioid receptors remains to be investigated. Moreover, it has been shown that craving for heroin grows with time, which results in goal-directed heroin-seeking behaviour in rats following 14 days, but not just 1 day, of abstinence (Kuntz *et al.*, 2008). This increased expression of heroin-seeking (i.e. incubation) is accompanied by important time-dependent changes in the expression of genes that are important for neuroplasticity (Kuntz-Melcavage *et al.*, 2009). In keeping with this, incubation of morphine-conditioned place preference after 14 days of withdrawal is accompanied by increased phosphorylation of extracellular signal-regulated kinase (ERK) (a measure of ERK activity) and cAMP response element binding protein (a downstream target of ERK) (Li *et al.*, 2008).

In light of these findings, we first assessed the effect of naloxone and rimonabant on reinstatement of extinguished heroin-seeking triggered by cannabinoid priming (reinstatement study). Then, in a parallel set of heroin-trained animals, we assessed the possibility that incubation of heroin-seeking might alter the hedonic value of cannabinoids, and hence facilitate the intake of the CB_1 receptor agonist WIN55,212-2 (WIN), which, contrary to the natural component of cannabis, Δ^9-tetrahydrocannabinol, has been reported to reliably sustain SA behaviour in both drug-naïve mice (Martellotta *et al.*, 1998) and trained rats (Spano *et al.*, 2004; Fadda *et al.*, 2006), in a dose-related manner (Fattore *et al.*, 2001) and under different schedules of reinforcement and response-like operanda (Deiana *et al.*, 2007; Solinas *et al.*, 2007). When cannabinoid reliably substituted for heroin, we tested the effect of naloxone and rimonabant on cannabinoid intake (substitution study). Altogether, our data shed new light on the crosstalk between cannabinoid and opioid receptors in craving and relapse mechanisms, and suggest that they may play different roles in heroin-seeking reinstatement and cannabinoid substitution in heroin-abstinent rats.

Methods

Animals

All animal care and experimental procedures complied with the E.C. regulations for animal use in research (86/609/EEC) and were approved by the local Animal Care Committee. We used male Lister Hooded rats (Harlan Nossan, Udine, Italy) that weighed 260–280 g at the beginning of the experiments. Animals were housed four per cage and maintained at a temperature of 21 ± 1°C (60% humidity) under a reversed 12 h light/dark cycle (lights on 19:00 h) with free access to food and water. After implantation of an intravenous catheter (see below), rats were individually housed in hanging stainless steel home cages and maintained at about 85% of free feeding with 20 g per day Purina laboratory chow shortly after the end of each daily SA session, with water being available *ad libitum*. Experiments took place at the same time each day during the dark phase of the cycle (between 09:00 and 12:00 h), 6 days per week.

Surgery for implantation of venous catheters

Following 1 week of acclimation and handling, animals were prepared with chronic indwelling venous catheters (Cam-Caths, Ely, UK) under deep anaesthesia with equithesin (5 mL·kg^{-1}, i.p.) [Na-pentobarbital (0.97 g), Mg-sulphate (2.1 g), chloral hydrate (4.25 g), propylene glycol (42.6 mL) and ethanol (11.5 mL)]. One end of the catheter was inserted into the right atrium via the right jugular vein, whereas the distal end was passed s.c. and exited in the mid-scapular region. Animals recovered for 6 days with food and water freely available and received daily s.c. administration of 0.1 mL Baytrill (Bayer, Milan, Italy). Anaesthetics and antibiotics were purchased as sterile solutions from local distributors.

Apparatus

Heroin SA and cannabinoid substitution were carried out in 12 operant chambers (29.5 × 32.5 × 23.5 cm; Med Associates, St Albans, VT, USA) equipped with infrared locomotor sensors and two retractable levers that were 4 cm wide, positioned 12 cm apart and 8 cm from the grid, and extended 1.5 cm into the box. A central stimulus light was located between the two levers, and a single house light was located on the opposite wall. The catheter was mounted on a counterbalanced single-channel swivel apparatus that allowed unrestricted movement within the operant chamber. The swivel was connected to a software-operated infusion pump (Med Associates) that delivered drug solution at a rate of 0.02 mL·s^{-1}. An IBM-compatible computer with Med-PC interface (Med Associates), which was located in the same experimental room, was used for programming, data collection and storage.

Experimental procedure

Rats were trained to self-administer heroin (30 μg·kg^{-1} per infusion) intravenously in 2 h daily sessions under a continuous (fixed-ratio 1) schedule of reinforcement, as previously described (Fattore *et al.*, 2003; 2005b; Spano *et al.*, 2007). At the beginning of the session, the house light was illuminated to signal the start. Depression of one lever, defined as 'active', resulted in: (i) extinction of the house light and illumination of the stimulus light, which remained on for 5 s; (ii) retraction of both levers; and (iii) activation of the infusion pump for 5 s, which delivered a total of 0.1 mL drug solution. There was a 15 s time-out after each drug infusion, after which, the two levers were re-extended into the chamber, the stimulus light went out, and the house light was illuminated. Depressions of the other lever, defined as 'inactive', had no programmed consequences but were always recorded to provide an index of basal level activity. The assignment of the active (drug-paired) and the inactive (no drug-paired) levers was counterbalanced and remained constant for each subject throughout all phases of the study.

To ensure patency, after each training session, 0.1 mL heparinized sterile saline (30 U·mL^{-1}) was flushed through the catheter, which was sealed with a stainless steel cap when not in use. When a catheter was obstructed or damaged, a new one was implanted into the left jugular vein, and testing resumed 6 days after the animal recovered from surgery. At the end of the study, catheter patency was confirmed by intravenous infusion of the short-acting barbiturate methohexital sodium (Brevital®, 10 mg·mL^{-1}, 0.2 mL per rat); a positive test was indicated by loss of righting reflex within 5 s after injection.

Reinstatement study

Heroin SA was considered to be acquired if an animal displayed accurate discrimination between the active and the inactive lever, with ≥15 active lever-presses per session not differing by more than 20% for five consecutive days, and ≤5 inactive lever-presses per session. The extinction condition was introduced over the subsequent 21 days, by replacing drug solution with sterile saline and leaving all the other experimental parameters unchanged. Drug priming test for heroin-seeking reinstatement took place from extinction day 22 onwards. A between-session model of extinction/reinstatement was used as previously described (Fattore *et al.*, 2003; 2005b; Spano *et al.*, 2007). On extinction days 16 and 19, each rat received saline injections either s.c. (1 mL·kg^{-1}) or i.p. (5 mL·kg^{-1}) to habituate them to subsequent drug priming administrations. Starting from extinction day 22, each animal received one injection of saline (s.c.) or cannabinoid vehicle (i.p.), and two out of the following drug priming injections: heroin (0.1 mg·kg^{-1}, i.p.), WIN (0.15 and 0.3 mg·kg^{-1}, i.p.), rimonabant (0.3 and 3 mg·kg^{-1}, i.p.) or naloxone (0.1 and 1 mg·kg^{-1}, s.c.), alone or in combination. Treatments were assigned using a Latin square design, and at least three extinction training sessions were intercalated between each priming test for assessment of carry-over effects. The order of presentation of different test drugs was varied between animals, and each treatment group included six animals. In a separate set of experiments, three groups of rats (n = 6 each) were given 3 weeks extinction, at the end of which they received an acute priming with WIN 0.3 mg·kg^{-1} (i.p.). Extinction training was continued for an extra 5 days, during which animals received daily injections of naloxone (0.1 and 1 mg·kg^{-1}, s.c.) and/or rimonabant (0.3 and 3 mg·kg^{-1}, i.p.), before starting the session, in order to assess the effect of the two antagonists on the residual increased response induced by cannabinoid priming (Fattore *et al.*, 2003).

Substitution study

Five groups of rats (n = 6 each) were given heroin (30 μg·kg^{-1} per infusion) SA training until they showed stable drug intake; then, the extinction condition was introduced. After different periods of extinction training, namely 1, 7, 14, 21 or 90 days, each group was shifted to WIN (12.5 μg·kg^{-1} per infusion) SA. Animals were allowed to lever-press for WIN for seven consecutive days under the same experimental conditions (i.e. fixed-ratio 1 reinforcement schedule, 2 h session). Criteria for acquisition of WIN SA were as previously reported: (i) animals displayed four consecutive days of firm response within ±20% of variation from the mean number of reinforcers obtained; (ii) a minimum of 16 drug infusions gained per session; and (iii) ≤6 responses made on the inactive lever (Fattore *et al.*, 2010). Parallel control groups of rats (n = 5 each) were switched to vehicle (Tween 80 + saline) SA after the same time intervals. In groups of rats in which substitution occurred, that is, in animals that showed stable WIN intake, the effect of daily pre-treatment with rimona-

bant (0.3 and 3 mg·kg^{-1}, i.p.) and naloxone (0.1 and 1 mg·kg^{-1}, s.c.) on animal response was tested.

Locomotor activity

Throughout all phases of the study (heroin SA, extinction, reinstatement test, cannabinoid substitution), the locomotor activity of rats within the operant boxes was constantly monitored by means of four series of photocells that were located at 3.5 cm above the cage floor. The number of photocell beam breaks was recorded and used as a measure of general horizontal locomotor activity of the rats.

Statistical analysis

All data are presented as mean ± SEM. The number of responses on both the active and inactive levers, as well as the motor activity counts, was evaluated. Data were analysed by means of one-way or two-way ANOVA, followed by Newman–Keuls or Bonferroni test respectively. Comparisons between different experimental groups were evaluated by the unpaired Student's *t*-test. Significance level was set at $P \leq 0.05$.

Materials

For SA training, heroin (Sigma, Milan, Italy) was diluted in heparinized (1%) sterile saline solution, and WIN (Tocris, Bristol, UK) was first dissolved in one drop of Tween 80 and then diluted in heparinized (1%) sterile saline solution. Intravenous infusions of heroin (30 µg·kg^{-1} per infusion) and WIN (12.5 µg·kg^{-1} per infusion) were delivered at a rate of 20 µL·s^{-1} over 5 s. Drug solutions were made weekly, refrigerated and filtered through 22 µm syringe filters prior to use to ensure sterility. This dosing procedure has been previously shown to sustain stable SA behaviour under our experimental conditions (Fattore *et al.*, 2007d; Solinas *et al.*, 2007).

For priming tests, naloxone (Sigma) was dissolved in sterile saline solution and administered subcutaneously (s.c.) 20 min before starting the session (volume of injection: 1 mL·kg^{-1}). WIN (Tocris) and rimonabant (Sanofi, Paris, France) were freshly dissolved in one drop of Tween 80 and diluted in sterile saline solution. The CB_1 receptor agonist and antagonist were administered i.p. 10 and 30 min, respectively, before starting the session (volume of injection: 5 mL·kg^{-1}). Doses, timing and routes of administration of drug priming were chosen based on previous studies performed in our laboratory (Spano *et al.*, 2004; 2007; Fattore *et al.*, 2005b). As a control study, one group of animals were injected with saline, and an additional one with the vehicle of the cannabinoids (Tween 80 + saline).

Results

Reinstatement study

Experiment 1. Synergistic effect of rimonabant and naloxone on cannabinoid-induced reinstatement of heroin-seeking behaviour. Figure 1 illustrates the mean number of active responses over the last 3 days of training (heroin SA), the last 3 days of extinction, and following acute priming with saline, cannabinoid vehicle, WIN, rimonabant and naloxone, given alone or in combination.

In line with our previous observations (Fattore *et al.*, 2003), acute priming with WIN (0.15 and 0.3 mg·kg^{-1}, i.p.) dose-dependently reinstated extinguished heroin-seeking

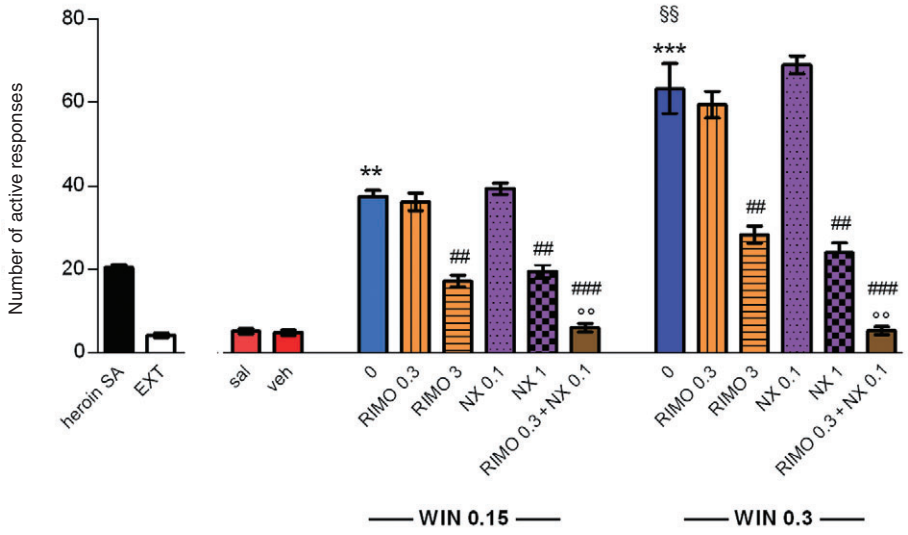

Figure 1

Effect of rimonabant (RIMO; 0.3 and 3 mg·kg^{-1}, i.p.) and/or naloxone (NX; 0.1 and 1 mg·kg^{-1}, s.c.) on the reinstatement of heroin-seeking behaviour triggered by an acute priming with WIN (0.15 and 0.3 mg·kg^{-1}, i.p.). Each bar represents the mean ± SEM of active lever-presses over the last three consecutive days of heroin SA, over the last three consecutive sessions of extinction (EXT), and during the reinstatement test sessions, that is, following priming with saline (sal) or the cannabinoid vehicle (veh), and with WIN alone or in combination with RIMO and/or NX ($n = 6$). $^{**}P < 0.01$, $^{***}P < 0.001$ significantly different from heroin SA,; $\#\#P < 0.01$, $\#\#\#P < 0.001$ significantly different from corresponding WIN only group (blue bars), $^{\circ\circ}P < 0.01$ significantly different from corresponding single antagonists, $\S\S P < 0.01$ significantly different from WIN 0.15 mg·kg^{-1} priming.

Table 1

Reinstatement study

A		heroin SA	EXT		sal	veh	
	Mean	302	245		276	269	
	SEM	7.22	6.55		9.14	10.14	
B	**WIN 0.15**	**0**	**RIMO 0.3**	**RIMO 3**	**NX 0.1**	**NX 1**	**RIMO + NX**
	Mean	291	258	306	270	299	304
	SEM	11.61	7.36	9.31	9.14	8.14	11.55
C	**WIN 0.3**	**0**	**RIMO 0.3**	**RIMO 3**	**NX 0.1**	**NX 1**	**RIMO + NX**
	Mean	308	269	267	270	292	299
	SEM	10.55	11.97	6.23	9.32	7.04	6.83

SA, self-administration; EXT, extinction; sal, saline; veh, cannabinoid vehicle; WIN, WIN 55,212-2; RIMO, rimonabant; NX, naloxone.

behaviour ($P < 0.01$ and $P < 0.001$, respectively, vs. heroin SA) (blue bars). One-way ANOVA confirmed a significant effect of cannabinoid agonist priming on heroin-seeking reinstatement ($F_{2,15} = 67.72$, $P < 0.0001$). The behavioural effects of cannabinoid priming were probably not due to non-specific arousal, as the response following saline or cannabinoid vehicle remained at extinction level, thus indicating a specific pharmacological action of the drug on animal behaviour. In support of this, responses on the inactive lever were constantly ≤6 following all drug priming.

The effect of the cannabinoid priming was significantly ($P < 0.01$) attenuated by pre-treatment with rimonabant (3 mg·kg^{-1}) or naloxone (1 mg·kg^{-1}), and completely prevented by co-administration of ineffective doses of rimonabant (0.3 mg·kg^{-1}, i.p.) and naloxone (0.1 mg·kg^{-1}, s.c.), which indicated a synergistic action of the two antagonists (brown bars). Overall, ANOVA revealed a significant main effect of Priming ($F_{5,60} = 212.61$, $P < 0.0001$), Antagonist ($F_{1,50} = 121.79$, $P < 0.0001$) and a Priming × Antagonist interaction ($F_{4,50} = 21.25$, $P < 0.0001$). Importantly, these effects were selective and not associated with motor disturbances, because the drug doses used in the present experiment did not significantly affect locomotion (Table 1) nor the pattern of responding (Figure 2) during operant response.

Experiment 2. Lack of effect of rimonabant and naloxone on enduring reinstating effect of cannabinoid primings on heroin-seeking reinstatement. Based on earlier evidence of a residual stimulating effect of cannabinoid priming on heroin-seeking reinstatement (Fattore *et al.*, 2003), three separate groups of rats ($n = 6$ each) were pre-treated daily with rimonabant 0.3 mg·kg^{-1}, naloxone 0.1 mg·kg^{-1} or their combination, for five consecutive days after the priming test (WIN 0.3 mg·kg^{-1}). As shown in Figure 3 (top panel), long-term effect of cannabinoid priming on heroin-seeking reinstatement was not affected by pre-treatment with low doses of the two antagonists, nor by their co-administration or higher doses of naloxone (1 mg·kg^{-1}, s.c.) and/or rimonabant (3 mg·kg^{-1}, i.p.) (bottom panel).

Substitution study

Experiment 3. Cannabinoid SA time-dependently substitutes for heroin SA in abstinent rats. As shown in Figure 4 (top), substitution of WIN for heroin on the day after (24 h) the last heroin SA session produced an extinction-like pattern of response, with an immediate increase of active lever-pressing ($P < 0.01$) that dramatically collapsed to minimal values within a few days. However, drug substitution occurred after 1, 2 or 3 weeks of extinction in all rats tested ($n = 6$ per group), as heroin-trained animals promptly self-administered WIN by the very first day of cannabinoid substitution, and maintained constant behaviour over the seven consecutive days of WIN SA.

Specifically, after 1 week of extinction, responding level was very similar to that previously shown for heroin (25.5 ± 1.53 vs. 20.6 ± 1.57 active lever-presses). Yet, when rats were given access to the cannabinoid after a 14 day period of extinction, on the first day of WIN substitution their response rate was significantly higher (+60%) than during heroin SA (33 ± 1.67 vs. 20.6 ± 1.57 active responses), and remained fairly stable over the entire duration of testing. Even following a longer (21 day) period of time from the last heroin access, rats exhibited prompt and steady cannabinoid intake as long as WIN was available ($P < 0.001$). Notably, their response rate was significantly higher than that observed after 14 days and, to a greater extent, 7 days of extinction, which revealed a time-related efficacy of WIN to substitute for heroin following extinction. ANOVA confirmed a significant main effect of Group ($F_{12,105} = 284.56$, $P < 0.0001$) and Day ($F_{6,105} = 9.62$, $P < 0.0001$), but not a Group × Day interaction [$F_{12,105} = 0.75$, P = not significant (ns)]. However, WIN lost its ability to substitute for heroin as extinction training was prolonged further, because it was no longer self-administered by heroin-abstinent rats after 3 months of extinction. A separate control group of animals that were given access to the vehicle of the cannabinoid (Tween 80 + saline) instead of WIN did not resume a response, regardless of the time period that had elapsed from the last heroin SA session, and their responses on the active

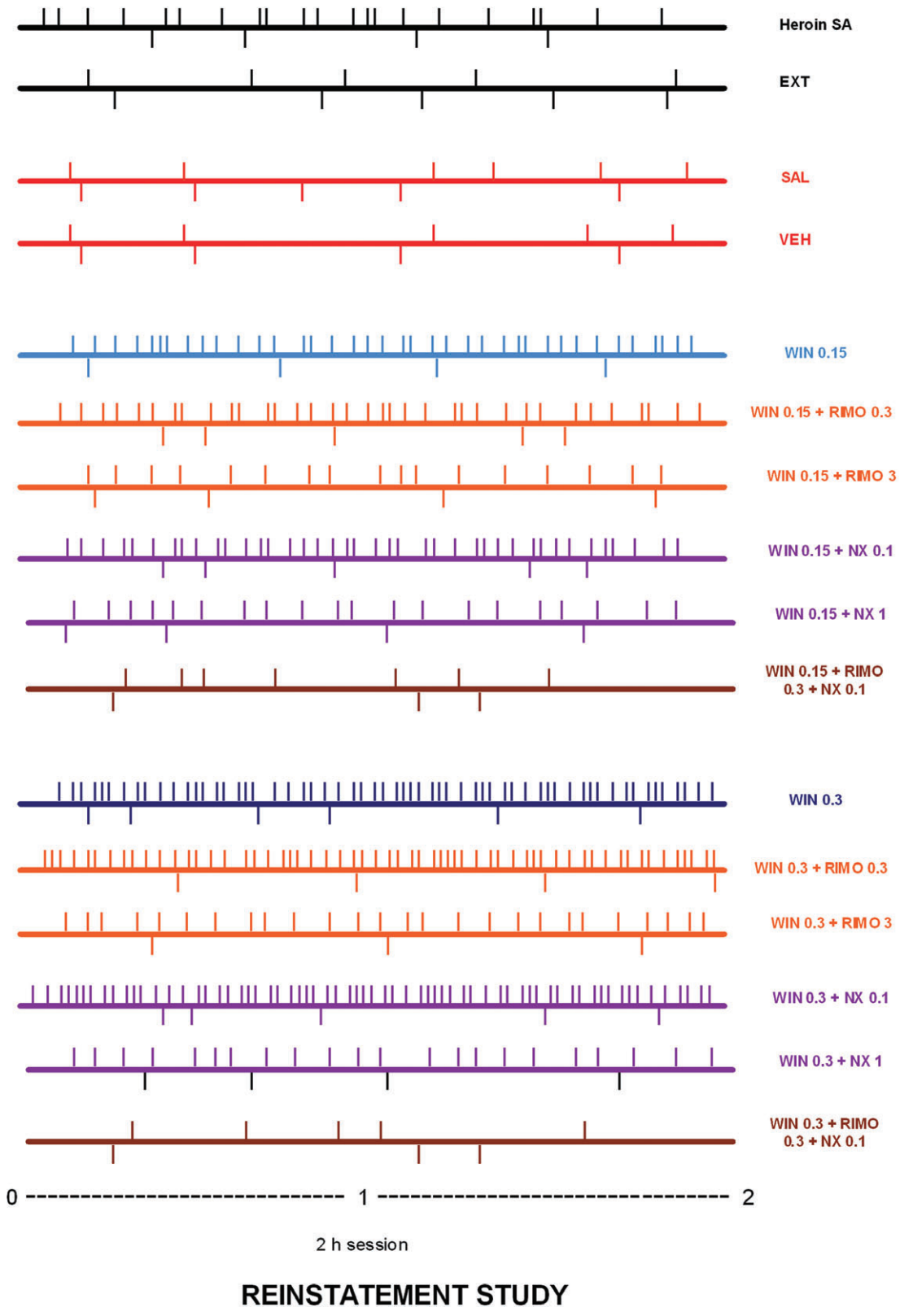

Figure 2

Individual responding patterns during reinstatement of heroin-seeking behaviour. Each record represents a separate 2 h session and each small vertical mark represents an active (upward) or inactive (downward) lever-press over the last day of drug self-administration training (heroin SA), over the last day of extinction (EXT) or after different drug priming (as indicated on the right side of corresponding record). SAL, saline; VEH, cannabinoid vehicle; WIN, WIN 55,212-2; RIMO, rimonabant; NX, naloxone.

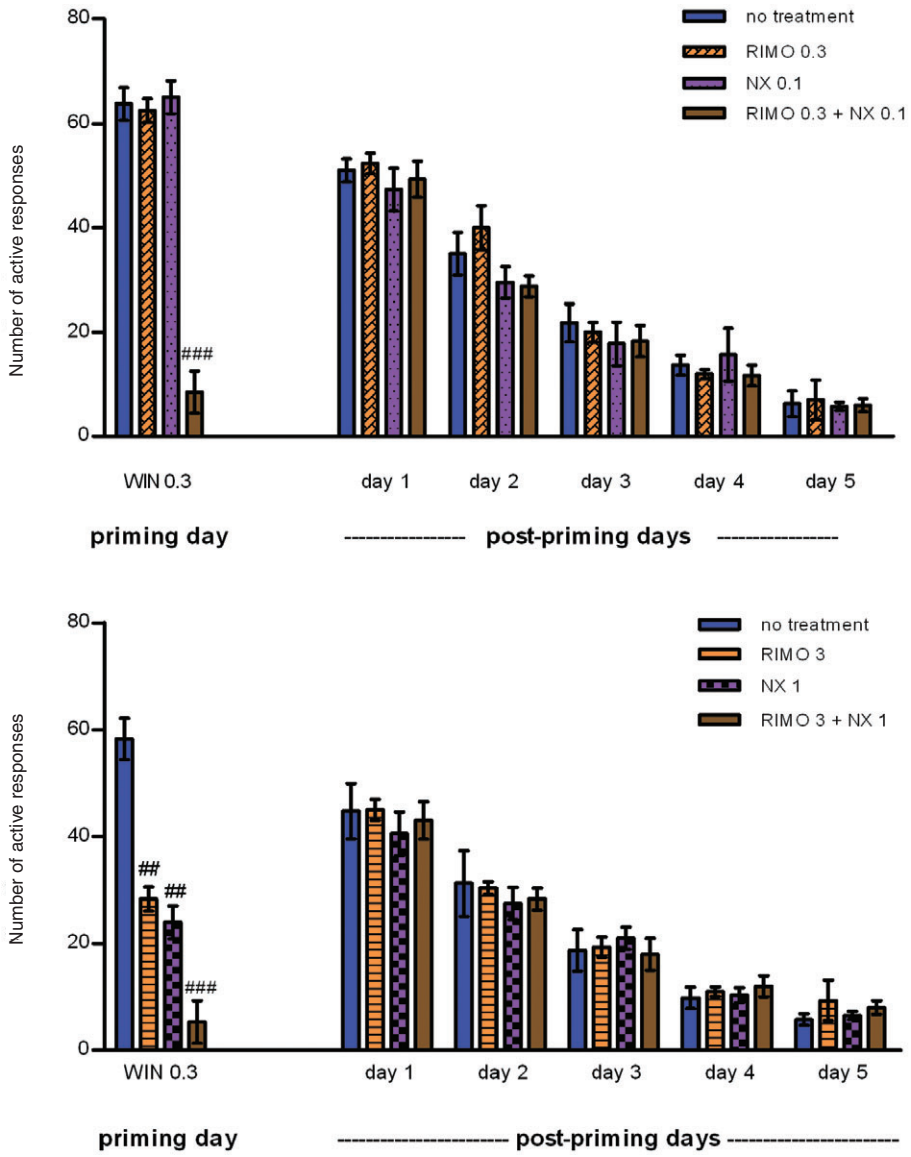

Figure 3

Effect of low (top) and high (bottom) doses of rimonabant (RIMO; 0.3 and 3 mg·kg^{-1}, i.p.) and/or naloxone (NX; 0.1 and 1 mg·kg^{-1}, s.c.) on the long-lasting effect of WIN priming on the reinstatement of heroin-seeking behaviour. Each bar represents the mean ± SEM of the responses on the active lever on the priming test day (left bars), and on the following five post-priming days (groups of bars, right) (n = 6). ##P < 0.01, ###P < 0.001 significantly different from corresponding WIN alone group (blue bar).

lever were constantly ≤8 and not significantly different from those made on the inactive one. The time-dependent enhancement of responsiveness to the cannabinoid agonist in heroin-experienced rats thus appeared to be a long-lasting, yet reversible phenomenon, and not associated with significant alterations in locomotor activity (Table 2) nor in the responding patterns (Figure 5).

The time-dependency of cannabinoid substitution in heroin-trained rats was more obvious when we looked at the mean cumulative intake of the cannabinoid over the week of WIN SA testing (Figure 4, bottom). That is, although rats self-administered only a minimal amount of WIN when they switched to cannabinoid SA 24 h the last heroin session, they self-administered an increasing amount of the cannabinoid as extinction was extended to 7, 14 or 21 days. One-way ANOVA revealed a significant main effect of Group ($F_{4,25}$ = 20.52, P < 0.01 vs. day 1 extinction). Yet, after 90 days of extinction, WIN was no longer self-administered by rats, thus showing that it had lost its ability to substitute for heroin.

Experiment 4. Differential effect of rimonabant and naloxone on cannabinoid SA in heroin-abstinent rats. In groups of rats in which cannabinoid substitution occurred (i.e. after 7, 14 and 21 days of extinction), WIN SA training was prolonged for an extra week to evaluate the effect of subchronic (5 days) pre-treatment with rimonabant or naloxone on the intake of the

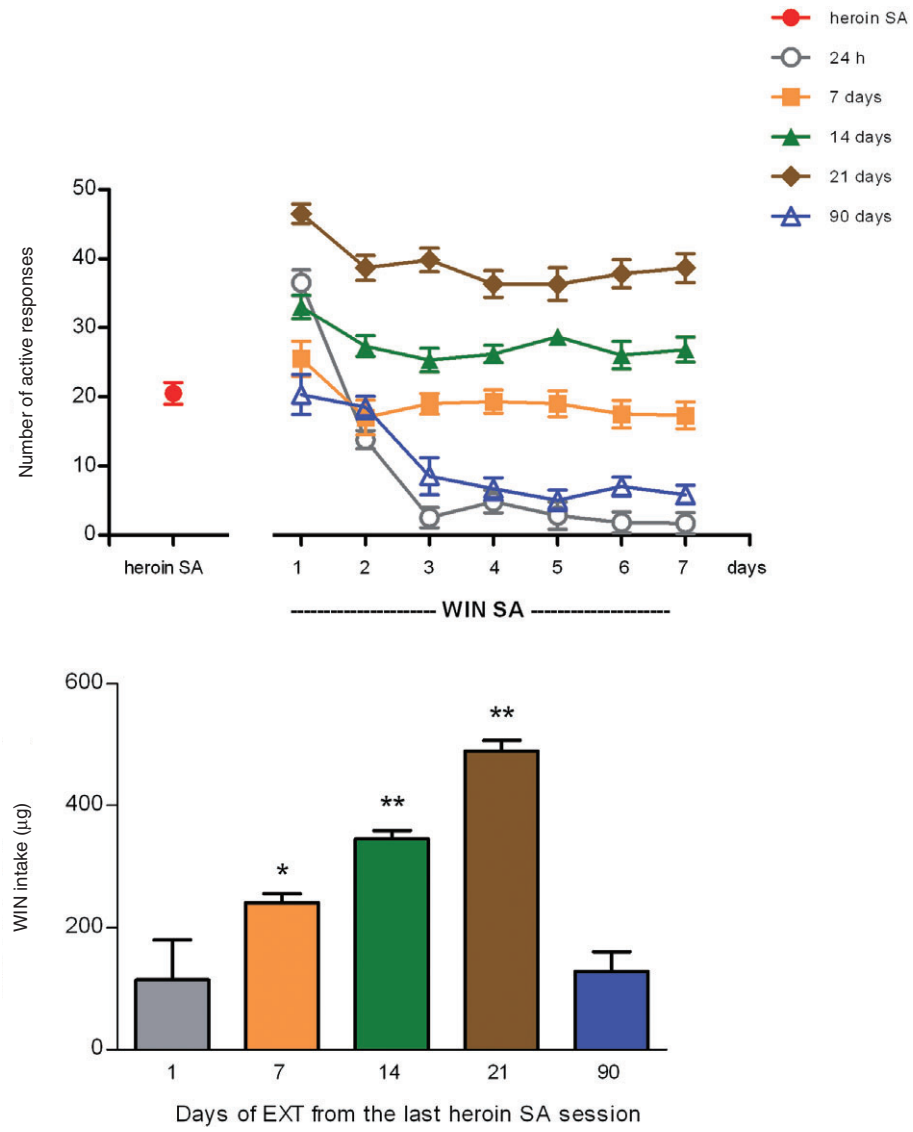

Figure 4

Top: WIN (12.5 µg·kg^{-1} per infusion) SA in heroin-trained rats after different periods of extinction (EXT) training. WIN was substituted for heroin on the day after the last heroin training session (24h), following 1 week (7 days), 2 weeks (14 days), 3 weeks (21 days) or 3 months (90 days) of EXT training. Each point represents the mean ± SEM of active responses during the 7 days of cannabinoid SA (n = 6 each). Heroin SA: mean ± SEM of active responses over the last three consecutive sessions of heroin SA training. Bottom: each bar represents the mean ± SEM of cumulative amounts of WIN self-administered by heroin-trained rats following different periods of EXT training (n = 6). *P < 0.05, **P < 0.01 significantly different from day 1 EXT.

cannabinoid (days 1–5). Two extra days of WIN SA training were conducted to ensure that animals recovered to basal responding level. As shown in Figure 6 (top panel, right), with respect to mean basal response for WIN (WIN SA), daily pre-treatment with naloxone 1.0 mg·kg^{-1} (s.c.) significantly modified cannabinoid SA by enhancing the rate of response in all groups (n = 6 per group) during the 5 days of pre-treatment. ANOVA revealed a significant effect of Group ($F_{6,245}$ = 68.33, P < 0.0001) and Day ($F_{6,245}$ = 3.34, P = 0.0035), but not a Group × Day interaction ($F_{36,245}$ = 0.42, P = ns). Conversely, pre-treatment with rimonabant 3.0 mg·kg^{-1} (i.p.) for five consecutive days slightly but significantly decreased cannabinoid intake in all groups (Figure 6, top panel, left).

Analysis of variance revealed an overall significant effect of Group ($F_{6,245}$ = 47.82, P < 0.001) but not of Day ($F_{6,245}$ = 0.34, P = ns) or a Group × Day interaction ($F_{36,245}$ = 0.18, P = ns). Importantly, after the 5 day period of pre-treatment with naloxone or rimonabant, animals recovered to their basal level of WIN intake as drug pre-treatment was discontinued. In line with their inability to affect cannabinoid-induced reinstatement of heroin-seeking behaviour (Figure 1), lower doses of naloxone (0.1 mg·kg^{-1}, s.c.) or rimonabant (0.3 mg·kg^{-1}, i.p.) had no effect on cannabinoid SA in this drug substitution test, as cannabinoid intake did not differ more than 15% from basal daily intake (Figure 4, bottom panels).

Table 2

Substitution study

WIN SA – first day	Groups	1 day	7 days	14 days	21 days	90 days
	Mean	308	295	308	289	289
	SEM	26.24	9.13	8.65	31.25	5.37
WIN SA – seventh day	**Groups**	**1 day**	**7 days**	**14 days**	**21 days**	**90 days**
	Mean	298	312	299	280	293
	SEM	5.12	8.22	5.34	6.18	7.33

WIN, WIN 55,212-2; SA, self-administration.

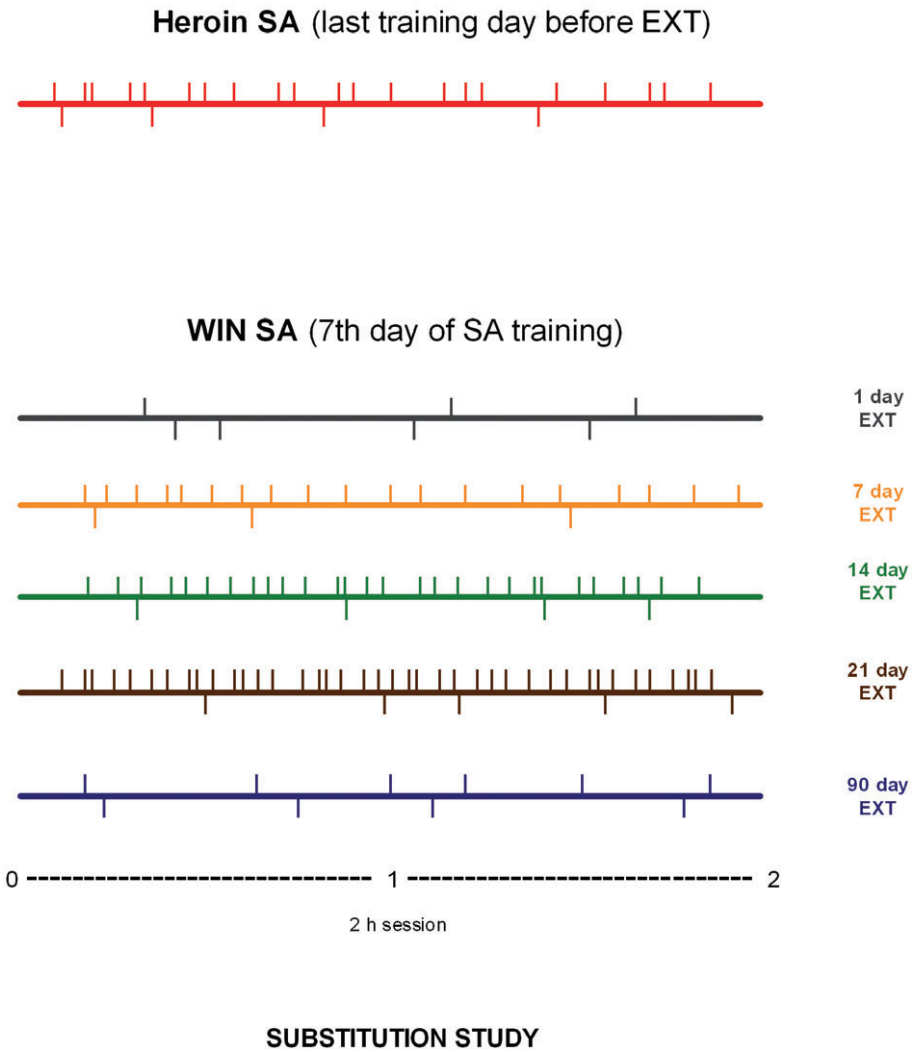

Figure 5

Individual responding patterns during cannabinoid substitution test. Each record represents a separate 2 h session and each small vertical mark represents an active (upward) or inactive (downward) lever-press over the last day of heroin self-administration training (heroin SA) or over the last day (7th) of cannabinoid self-administration training (WIN SA) after different periods of extinction (EXT) from last heroin.

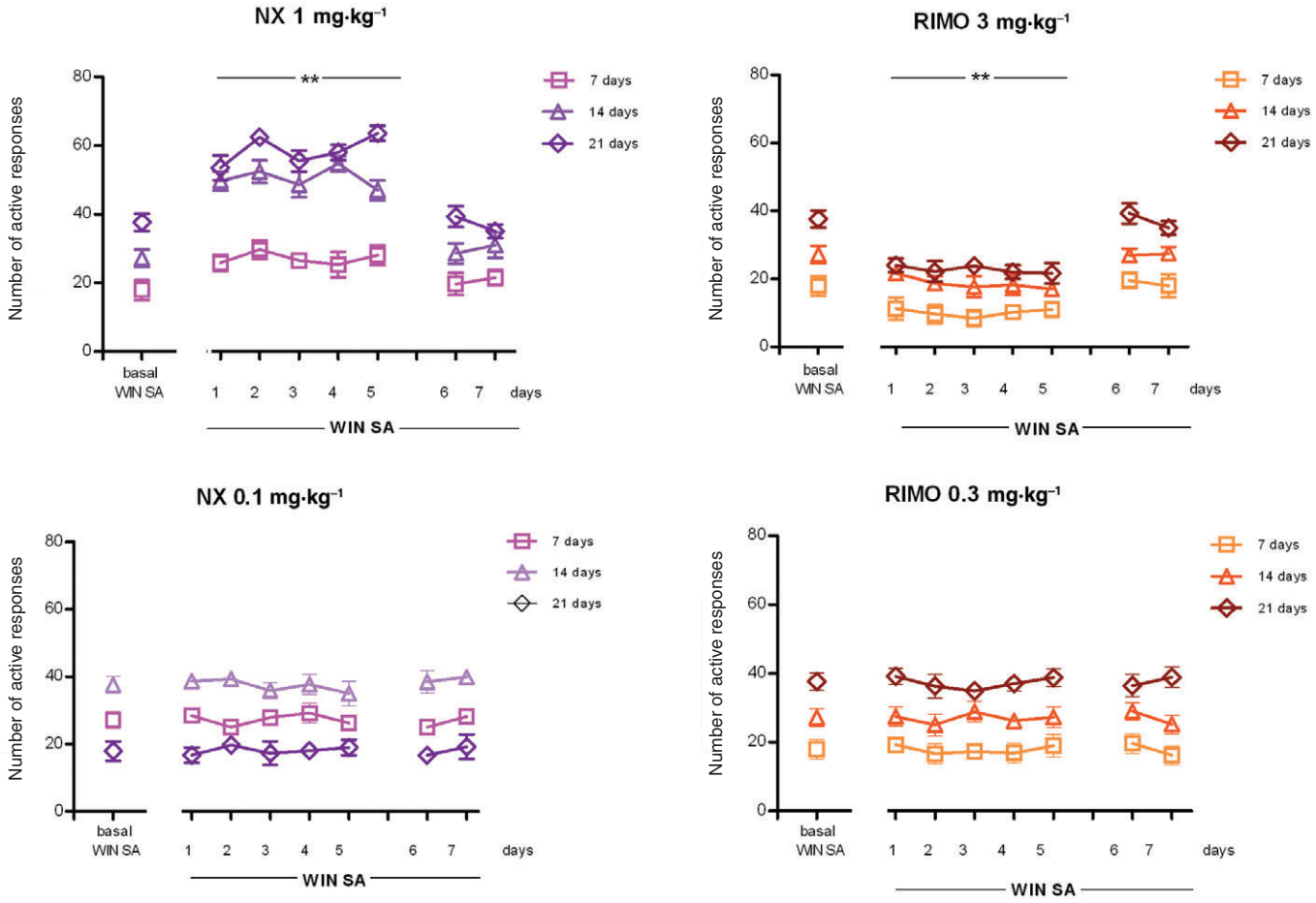

Figure 6

Effect of 5 day pre-treatment with naloxone (NX, 1.0 and 0.1 mg·kg^{-1}, left panels) or rimonabant (RIMO, 3.0 and 0.3 mg·kg^{-1}, right panels) on cannabinoid SA in heroin-trained rats. WIN SA: mean ± SEM of active responses over the last three consecutive sessions of cannabinoid SA training before antagonism study. Naloxone and rimonabant were administered daily at 20 and 30 min before starting the SA session, respectively, over five consecutive days. Cannabinoid SA training was then continued for two extra days to assess recovery of basal response. Each point represents the mean ± SEM of responses on the active lever during the 7 days of testing. **$P < 0.001$ significantly different from WIN SA group ($n = 6$ each).

Discussion

The findings of the present study are fourfold: (i) naloxone and rimonabant attenuated cannabinoid-induced reinstatement of heroin-seeking behaviour when given alone, and fully prevented it when administered together. Notably, (ii) neither antagonist influenced the long-term effect of cannabinoid priming on heroin-seeking reinstatement, which implied that this effect was probably not mediated at the level of the CB_1 or opioid receptors. Finally, (iii) heroin-trained animals self-administered the cannabinoid CB_1 receptor agonist after extinction in a time-dependent manner, a behaviour that (iv) was significantly enhanced by naloxone and attenuated by rimonabant pre-treatment.

Reinstatement study

We first investigated the effect of the two antagonists on the resumption of extinguished heroin-seeking behaviour. Priming with rimonabant and naloxone have been previously reported to prevent completely reinstatement of heroin-seeking (Fattore *et al.*, 2005b) and cannabinoid-seeking behaviour (Spano *et al.*, 2004) elicited by heroin priming. However, when heroin-seeking reinstatement is triggered by cannabinoid priming, pre-treatment with either naloxone or rimonabant resulted in partial inhibition (Fattore *et al.*, 2005b; present study). Here, we showed that the simultaneous blockade of the cannabinoid and opioid receptors with sub-threshold doses of the two antagonists completely inhibited the effect of cannabinoid priming on reinstatement of heroin-seeking, which revealed a synergistic action of naloxone and rimonabant on the cannabinoid-elicited resumption of heroin-seeking behaviour. Although the reducing effect of rimonabant might be ascribed to direct action on the CB_1 receptor, that of naloxone is more likely to be due to its ability to reduce cannabinoid-enhanced dopamine transmission in the mesolimbic circuitry (Chen *et al.*, 1990; Tanda *et al.*, 1997).

Intriguingly, both naloxone and rimonabant are ineffective against the long-lasting reinstating effect of cannabinoid

priming (Fattore *et al.*, 2003), even when co-administered, which indicates that the persistent response that is observed after cannabinoid priming is unlikely to be due to the residual stimulation of opioid or cannabinoid receptors. The fact that the resumed response for heroin became resistant after priming with cannabinoids suggests that it might have generated long-lasting effects on the nervous system functions that underlie control of goal-oriented responses, or on higher-order cognitive and executive functions (i.e. reversal learning, behavioural flexibility) that are not necessarily under a direct control of the cannabinoid or opioid neurotransmission. Habitual behaviour, which is defined as behaviour that is insensitive to updates in outcome value and action–outcome contingency, might also be involved in the persistence of active lever-pressing.

Substitution study

The cannabinoid substitution study that was performed in rats trained to self-administer heroin demonstrated that the cannabinoid agonist could replace heroin in sustaining SA behaviour, depending on the time that had elapsed from the last heroin intake. In particular, a typical extinction-like response profile, that is, an immediate increase in response followed by cessation of response, was observed when heroin was replaced by the CB_1 receptor agonist on the day immediately after the last heroin session. This finding is in agreement with the notion that WIN is not promptly self-administered by rats, because animals typically require 2–3 weeks of training (acquisition) before showing stable intake of the drug (Fattore *et al.*, 2001), and this effect was independent of sex (Fattore *et al.*, 2007c), rat strain (Fadda *et al.*, 2006) or modus operandi (Deiana *et al.*, 2007). Conversely, substitution of WIN for heroin SA occurred after 7, 14 and 21 days of extinction in a time-dependent manner (i.e. with cannabinoid intake increasing with the length of drug abstinence), and within a range very similar to that typically self-administered by male adult Lister Hooded rats (Fattore *et al.*, 2001; Spano *et al.*, 2004; Fadda *et al.*, 2006; Deiana *et al.*, 2007). The fact that the response was specifically oriented to obtain the drug was corroborated by the observation that vehicle did not substitute for heroin at any of the time points tested, nor rats generalized between the active and inactive levers. However, we cannot exclude the possibility that heroin-abstinent rats might be more responsive to other addictive drugs besides cannabinoids, or that they avidly self-administered WIN to alleviate stress or anxiety-related states, and future studies will be performed to assess the pharmacological specificity of such an interaction.

Craving and relapse are enhanced with increasing periods of abstinence, a phenomenon referred to as incubation, which is defined as an increase in drug-seeking as a function of the time from the last drug exposure. In the case of heroin, such an enhancement in drug-seeking behaviour is positively correlated with the period of abstinence until an arbitrary point, after which a gradual decrease in heroin-seeking behaviour is typically observed (Shalev *et al.*, 2001). More specifically, lever-pressing during extinction was reported to follow a bell-shaped curve with maximal responding occurring after 6, 12 and 25 days of heroin withdrawal, but not after 1 or 66 days of extinction (Shalev *et al.*, 2001). This aligns with our finding that WIN substitutes for heroin after 7, 14 or 21 days, but not after 1 or 90 days, of extinction training, and implies that WIN is substituting for heroin during the period of incubation craving. Similar to heroin, alcohol- and cocaine-seeking behaviour also increases over time, with drug-seeking reaching the highest levels following several weeks of drug removal (Tran-Nguyen *et al.*, 1998; Grimm *et al.*, 2001; Bienkowski *et al.*, 2004).

Our finding that cannabinoid intake increases in proportion to the time of abstinence suggests that the neurochemical events that accompany the development of withdrawal from heroin are crucial factors in determining the impact value of the drug, and consequently, the magnitude of the reinstatement response. The present results thus support the idea that drug-seeking behaviour becomes more intense after long-term abstinence, which renders the cannabinoid agonist a more salient stimulus.

Moreover, the greater salience of the cannabinoid as a positive stimulus for maintaining operant behaviour in this substitution paradigm might result from the super-sensitivity of opioid receptors that occurs in heroin-dependent rats (Bolger *et al.*, 1988), as well as in the reward-related brain areas of rats that self-administer heroin (Fattore *et al.*, 2007d). If such enhanced sensitivity of opioid receptors were to be maintained (if not increased) over time after drug removal, it might account (at least in part) for the amplified impact of the cannabinoid. Alternatively, other populations of neurons or neural circuits that are normally not activated by cannabinoid agonists might become responsive to them after extinction from heroin. In this case, however, recruitment of these neurons or circuits should take place over time, as WIN does not act as a reinforcer when it is presented on the day after the last heroin SA session. Whatever the detailed changes occuring during extinction from heroin, such modifications are likely to be transient and reversible in nature, because drug substitution gradually declines over time when extinction is protracted over 3 months.

Remarkably, in this substitution study, we detected overlapping yet separate roles for the opioid and the CB_1 receptors in regulating drug-taking behaviour, in that daily administration of naloxone and rimonabant significantly enhanced and attenuated, respectively, the intake of the cannabinoid. These opposite effects of the two antagonists were unexpected, because they resemble those found in animals that are self-administering heroin rather than cannabinoid agonists. In fact, systemic administration of opiate receptor antagonists increases heroin SA in rats (Negus *et al.*, 1993; Carrera *et al.*, 1999), and decreases cannabinoid intake (Navarro *et al.*, 2001). Conversely, systemic administration of rimonabant has been show to decrease heroin SA (Navarro *et al.*, 2001) and increase cannabinoid SA (Fattore *et al.*, 2001). Based on these data, the increasing effect of naloxone and the decreasing effect of rimonabant on cannabinoid intake in heroin-abstinent rats found in the present study led us to hypothesize that abstinent rats might perceive cannabinoid and heroin as interchangeable, positive reinforcing stimuli.

In conclusion, our results reveal for the first time the ability of a cannabinoid agonist to substitute for heroin in a SA paradigm after certain drug-free periods, and show that blockade of opioid and cannabinoid receptors has a different outcome on drug-seeking reinstatement and cannabinoid

substitution in heroin-abstinent rats. We cannot exclude, however, that the effectiveness of rimonabant at decreasing WIN-induced reinstating effects (reinstatement study) or WIN SA (substitution study) may result from its activity as CB_1 receptor inverse agonist rather than its pure antagonistic effect. Nevertheless, these findings indicate that the length of extinction is a crucial modulator of drug-seeking behaviour, and that cannabinoid availability following heroin abstinence might represent a stimulus condition that is strong enough to elicit a reliable and persistent response for the drug. If the same phenomenon is found in humans, it might reflect a form of plasticity that contributes to the inability of heroin addicts to remain drug-free.

Acknowledgements

The authors wish to acknowledge the excellent technical and animal assistance of Barbara Tuveri.

Conflicts of interest

None.

References

Alexander SPH, Mathie A, Peters JA (2009). Guide to Receptors and Channels (GRAC), 4th edn. Br J Pharmacol 158 (Suppl. 1): S1–S254.

Bienkowski P, Rogowski A, Korkosz A, Mierzejewski P, Radwanska K, Kaczmarek L *et al*. (2004). Time-dependent changes in alcohol-seeking behaviour during abstinence. Eur Neuropsychopharmacol 14: 355–360.

Bolger GT, Skolnick P, Rice KC, Weissman BA (1988). Differential regulation of mu-opiate receptors in heroin- and morphine-dependent rats. FEBS Lett 234: 22–26.

Butler RK, Rea K, Lang Y, Gavin AM, Finn DP (2008). Endocannabinoid-mediated enhancement of fear-conditioned analgesia in rats: opioid receptor dependency and molecular correlates. Pain 140: 491–500.

Caillé S, Parsons LH (2006). Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. Neuropsychopharmacology 31: 804–813.

Carrera MR, Schulteis G, Koob GF (1999). Heroin self-administration in dependent Wistar rats: increased sensitivity to naloxone. Psychopharmacology 144: 111–120.

Chen JP, Paredes W, Li J, Smith D, Lowinson J, Gardner EL (1990). Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology 102: 156–162.

Deiana S, Fattore L, Spano MS, Cossu G, Porcu E, Fadda P *et al*. (2007). Strain and schedule-dependent differences in the acquisition, maintenance and extinction of intravenous cannabinoid self-administration in rats. Neuropharmacology 52: 646–654.

De Vries TJ, Schoffelmeer AN (2005). Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci 26: 420–426.

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

Fadda P, Scherma M, Spano MS, Salis P, Melis V, Fattore L *et al*. (2006). Cannabinoid self-administration increases dopamine release in the nucleus accumbens. Neuroreport 17: 1629–1632.

Fattore L, Cossu G, Martellotta MC, Fratta W (2001). Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology 156: 410–416.

Fattore L, Spano MS, Cossu G, Deiana S, Fratta W (2003). Cannabinoid mechanism in reinstatement of heroin-seeking after a long period of abstinence in rats. Eur J Neurosci 17: 1723–1726.

Fattore L, Cossu G, Spano MS, Deiana S, Fadda P, Scherma M *et al*. (2004). Cannabinoids and reward: interactions with the opioid system. Crit Rev Neurobiol 16: 147–158.

Fattore L, Deiana S, Spano MS, Cossu G, Fadda P, Scherma M *et al*. (2005a). Endocannabinoid system and opioid addiction: behavioural aspects. Pharmacol Biochem Behav 81: 343–359.

Fattore L, Spano MS, Cossu G, Deiana S, Fadda P, Fratta W (2005b). Cannabinoid CB(1) antagonist SR 141716A attenuates reinstatement of heroin self-administration in heroin-abstinent rats. Neuropharmacology 48: 1097–1104.

Fattore L, Fadda P, Fratta W (2007a). Endocannabinoid regulation of relapse mechanisms. Pharmacol Res 56: 418–427.

Fattore L, Spano MS, Deiana S, Melis V, Cossu G, Fadda P *et al*. (2007b). An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. Brain Res Rev 53: 1–16.

Fattore L, Spano MS, Altea S, Angius F, Fadda P, Fratta W (2007c). Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. Br J Pharmacol 152: 795–804.

Fattore L, Viganò D, Fadda P, Rubino T, Fratta W, Parolaro D (2007d). Bidirectional regulation of mu-opioid and CB1-cannabinoid receptor in rats self-administering heroin or WIN 55,212-2. Eur J Neurosci 25: 2191–2200.

Fattore L, Spano MS, Altea S, Fadda P, Fratta W (2010). Drug- and cue-induced reinstatement of cannabinoid-seeking behaviour in male and female rats: influence of ovarian hormones. Br J Pharmacol 160: 724–735.

Greaven D, Greaven K (1983). Treated and untreated addicts: factors associated with treatment and cessation of heroin use. J Drug Issues 13: 207–218.

Grimm JW, Hope BT, Wise RA, Shaham Y (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature 412: 141–142.

Hoffman AF, Oz M, Caulder T, Lupica CR (2003). Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. J Neurosci 23: 4815–4820.

Kuntz KL, Twining RC, Baldwin AE, Vrana KE, Grigson PS (2008). Heroin self-administration: I. Incubation of goal-directed behavior in rats. Pharmacol Biochem Behav 90: 344–348.

Kuntz-Melcavage KL, Brucklacher RM, Grigson PS, Freeman WM, Vrana KE (2009). Gene expression changes following extinction testing in a heroin behavioral incubation model. BMC Neurosci 10: 95–105.

Lane DA, Chan J, Lupica CR, Pickel VM (2010). Cannabinoid-1 receptor gene deletion has a compartment-specific affect on the dendritic and axonal availability of μ-opioid receptors and on dopamine axons in the mouse nucleus accumbens. Synapse 64: 886–897.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F *et al*. (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283: 401–404.

Li YQ, Li FQ, Wang XY, Wu P, Zhao M, Xu CM *et al*. (2008). Central amygdala extracellular signal-regulated kinase signaling pathway is critical to incubation of opiate craving. J Neurosci 28: 13248–13257.

Manzanares J, Corchero J, Romero J, Fernández-Ruiz JJ, Ramos JA, Fuentes JA (1999). Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol Sci 20: 287–294.

Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W (1998). Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience 85: 327–330.

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.

Mas-Nieto M, Pommier B, Tzavara ET, Caneparo A, Da Nascimento S, Le Fur G *et al*. (2001). Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction. Br J Pharmacol 132: 1809–1816.

Nava F, Manzato E, Lucchini A (2007). Chronic cannabis use does not affect the normalization of hypothalamic-pituitary-adrenal (HPA) axis induced by methadone in heroin addicts. Prog Neuropsychopharmacol Biol Psychiatry 31: 1089–1094.

Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L *et al*. (2001). Functional interaction between opioid and cannabinoid receptors in drug self-administration. J Neurosci 21: 5344–5350.

Negus SS, Henriksen SJ, Mattox A, Pasternak GW, Portoghese PS, Takemori AE *et al*. (1993). Effect of antagonists selective for mu, delta and kappa opioid receptors on the reinforcing effects of heroin in rats. J Pharmacol Exp Ther 265: 1245–1252.

Noble A, Best D, Man LH, Gossop M, Stang J (2002). Self-detoxification attempts among methadone maintenance patients: what methods and what success? Addict Behav 27: 575–584.

O'Brien CP (2005). Anticraving medications for relapse prevention: a possible new class of psychoactive medications. Am J Psychiatry 162: 1423–1431.

Pickel VM, Chan J, Kash TL, Rodríguez JJ, MacKie K (2004). Compartment-specific localization of cannabinoid 1 (CB1) and mu-opioid receptors in rat nucleus accumbens. Neuroscience 127: 101–112.

Robledo P, Berrendero F, Ozaita A, Maldonado R (2008). Advances in the field of cannabinoid–opioid cross-talk. Addict Biol 13: 213–224.

Rodriguez JJ, Mackie K, Pickel VM (2001). Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. J Neurosci 21: 823–833.

Rogers JL, Ghee S, See RE (2008). The neural circuitry underlying reinstatement of heroin-seeking behavior in an animal model of relapse. Neuroscience 151: 579–588.

Salio C, Fischer J, Franzoni MF, Mackie K, Kaneko T, Conrath M (2001). CB1-cannabinoid and mu-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. Neuroreport 12: 3689–3692.

Schoffelmeer AN, Hogenboom F, Wardeh G, De Vries TJ (2006). Interactions between CB1 cannabinoid and mu opioid receptors mediating inhibition of neurotransmitter release in rat nucleus accumbens core. Neuropharmacology 51: 773–781.

Seivewright N (2003). Methadone treatment outcomes appear mainly unaffected by cannabis use. Addiction 98: 251–252.

Shalev U, Morales M, Hope B, Yap J, Shaham Y (2001). Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. Psychopharmacology 156: 98–107.

Shapira M, Gafni M, Sarne Y (2003). Long-term interactions between opioid and cannabinoid agonists at the cellular level: cross-desensitization and downregulation. Brain Res 960: 190–200.

Solinas M, Panlilio LV, Antoniou K, Pappas LA, Goldberg SR (2003). The cannabinoid CB1 antagonist N-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. J Pharmacol Exp Ther 306: 93–102.

Solinas M, Panlilio LV, Tanda G, Makriyannis A, Matthews SA, Goldberg SR (2005). Cannabinoid agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats. Neuropsychopharmacology 30: 2046–2057.

Solinas M, Scherma M, Fattore L, Stroik J, Wertheim C, Tanda G *et al*. (2007). Nicotinic alpha 7 receptors as a new target for treatment of cannabis abuse. J Neurosci 27: 5615–5620.

Spano MS, Fattore L, Cossu G, Deiana S, Fadda P, Fratta W (2004). CB1 receptor agonist and heroin, but not cocaine, reinstate cannabinoid-seeking behaviour in the rat. Br J Pharmacol 143: 343–350.

Spano MS, Fattore L, Fratta W, Fadda P (2007). The GABAB receptor agonist baclofen prevents heroin-induced reinstatement of heroin-seeking behavior in rats. Neuropharmacology 52: 1555–1562.

Tanda G, Pontieri FE, Di Chiara G (1997). Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276: 2048–2050.

Tran-Nguyen LT, Fuchs RA, Coffey GP, Baker DA, O'Dell LE, Neisewander JL (1998). Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19: 48–59.

Trezza V, Vanderschuren LJ (2008). Cannabinoid and opioid modulation of social play behavior in adolescent rats: differential behavioral mechanisms. Eur Neuropsychopharmacol 18: 519–530.

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

Weizman T, Gelkopf M, Melamed Y, Adelson M, Bleich A (2004). Cannabis use is not a risk factor for treatment outcome in methadone maintenance treatment: a 1-year prospective study in an Israeli clinic. Aust N Z J Psychiatry 38: 42–46.