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Extinction learning of rewards in the rat: is there a role for CB1 receptors?

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Abstract

Rationale—Endocannabinoids have been widely studied in the context of addiction and reward due to their role in reinstatement. However, little is known about the role of CB1 receptors during extinction learning of an appetitively motivated task.

Objective—The aim of this study was to evaluate the role of endocannabinoids at different stages of extinction learning.

Methods—Endocannabinoid signaling was disrupted by injecting the CB1 receptor antagonist rimonabant (0, 200, 300 µg/kg i.v.) during the acquisition or consolidation phases of learning. The rate of extinction and its half-life were analyzed, as well as food-seeking in a reward-induced reinstatement test. We further investigated the interaction between extinction and endocannabinoids in different groups of rats that received drug treatments but did not undergo extinction training (abstinence). In addition, the effects of rimonabant on cue retrieval were investigated in a cue-induced reinstatement test in which rimonabant (0, 300 µg/kg i.v.) was given immediately prior to the reinstatement session.

Results—Blockade of CB1 receptors during acquisition or consolidation of extinction learning had no effect on the rate extinction or its half-life and these pretreatments had no long term consequences on reward-seeking behavior. Furthermore, rats that underwent extinction training responded at lower levels than those that received the drug in the absence of extinction ($p=0.000$, $\eta^2=0.40$). Rimonabant was effective in inhibiting behavior only if it was immediately given before a cue-induced reinstatement session ($p=0.000$, $\eta^2=0.92$).

Conclusion—The present results clarify and isolate the role of endocannabinoids in reinstatement as key mediators of cue retrieval, rather than orchestrators of extinction learning processes.

Keywords

CB1; Endocannabinoid; Conditioning; Reinstatement; motivation; Food consumption

Introduction

Learning theories of addiction emphasize the role of reward-associated environmental cues in compulsive reward-seeking, craving, and relapse after long periods of abstinence (Childress et al. 1988). There has been a remarkable amount of research using the animal reinstatement model (de Wit and Stewart 1981) to study the neurobiological systems

underlying relapse (Shaham et al. 2003). Among these neural substrates, the endocannabinoid system stands out owing to its involvement in relapse across different drug classes and natural rewards (Fattore et al. 2007). Indeed, pharmacological manipulations at CB1 receptors can potentiate or inhibit reward-seeking behavior. For example, De Vries and collaborators showed that a single injection of the potent CB1 receptor agonist HU-210 reinstated drug-seeking following extinction of cocaine self-administration. More importantly, this effect was completely reversed by injections of rimonabant (De Vries et al. 2001).

Rimonabant is not only effective in reversing drug-seeking behavior elicited by cannabinoid agonists but it can also effectively attenuate reinstatement elicited by other drugs of abuse and their respective conditioned cues. Therefore, the endocannabinoid system is pivotal to global reinstatement processing of reward-seeking behavior because similar effects have been obtained with several drugs of abuse including heroin (De Vries et al. 2003; Fattore et al. 2003), methamphetamine (Anggadiredja et al. 2004), nicotine (Cohen et al. 2002, 2005; De Vries et al. 2005), ethanol (Cippitelli et al. 2005; Economidou et al. 2006), as well as with natural rewards (De Vries et al. 2005; Ward et al. 2007). The inhibition of cue-induced reinstatement by blockade of CB1 receptors suggests that these receptors modulate the incentive salience and retrieval of reward-associated cues.

An overlooked factor in the study of endocannabinoids and relapse is their role in extinction learning. During extinction, decreases in responding observed after the reward is withheld do not reflect forgetting due to weakening of neural connections, but a re-learning in which new contingencies between the stimuli and the absence of reward come to exert control over behavior (Rescorla 1996; Bouton 2004). Consequently, through the elucidation of neural mechanisms that enhance extinction learning, it may be possible to reduce the likelihood of relapse by increasing response inhibition at the presentation of drug-associated cues. Various lines of evidence suggest that endocannabinoid signaling could be an important component of such neural mechanisms.

Endocannabinoids and CB1 receptors are ubiquitous in several brain areas related to memory, and they modulate some aspects of learning. For example, when CB1^{-/-} mice are trained in the Morris water maze, they are able to learn the location of the hidden platform at a similar speed compared to their wild-type litter mates, yet when the platform is relocated, CB1^{-/-} mice persevere longer than wild-type mice, suggesting impaired learning of information about the new position (Varvel and Lichtman 2002). Similar results have been observed following blockade of CB1 receptor (Pamplona et al. 2006).

Studies on spatial memory and recognition memory suggest that endocannabinoids play a significant role on memory consolidation and decay (Varvel and Lichtman 2002). When CB1 receptors are blocked, the rate of memory decay slows down; this reduction in the rate of decay may explain enhanced performance on tasks that require retrieval of previous acquired information; however, this characteristic is disadvantageous when the experimental task requires flexibility and adaptability (Varvel and Lichtman 2002; Shiflett et al. 2004). The reduction in memory decay brought about by blockade of CB1 receptors provides a common explanatory framework to these results and the rich literature on the extinction of aversive memories (Marsicano et al. 2002; Varvel and Lichtman 2002; Suzuki et al. 2004; Chhatwal et al. 2005; Varvel et al. 2005a; Chhatwal and Ressler 2007; Niyuhire et al. 2007).

In a seminal paper by Marsicano et al. (2002), it was demonstrated that the endocannabinoid system plays an important role in the extinction of aversive memories. Specifically, it was shown that CB1 deficient mice present normal acquisition and consolidation of fear memories but impairment in short-term and long-term extinction of conditional cues

associated with the aversive stimulus. Similar results were observed when wild-type mice were treated with rimonabant before the first extinction trial. Others have observed similar results in different aversive tasks (Varvel and Lichtman 2002; Niyuhire et al. 2007).

Several attempts have been made to evaluate the contribution of endocannabinoids in the acquisition of extinction of appetitively motivated task, but the results have been elusive (Holter et al. 2005; Niyuhire et al. 2007; Ward et al. 2007). Endocannabinoids are theorized to participate in extinction learning of appetitively motivated tasks through the modulation of the mesolimbic dopaminergic system. Several studies have shown that during learning, phasic dopamine (DA) firing follows the rules of the prediction-error hypothesis (Schultz et al. 1997). Once the reward is paired with a predicting cue, phasic DA firing activity is transferred from the reward to its first predictor (Schultz and Romo 1990). When the reward is omitted, as is the case during extinction, a transient suppression of DA neural activity is observed at the time of the expected reward (Tobler et al. 2003). In support of a role for endocannabinoids in these neural processes, in vitro studies show that DA neurons release endocannabinoids when they undergo phasic activation (Melis et al. 2004; Lupica and Riegel 2005), an activity pattern shown to accompany the presentation of cues that predict access to drug (Phillips et al. 2003; Stuber et al. 2005) natural (Roitman et al. 2004) and brain stimulation reward (Cheer et al. 2007).

Here, we evaluated the role of endocannabinoids in extinction learning and reinstatement of a highly palatable reward. To achieve this goal, we pharmacologically manipulated the endocannabinoid system by administering rimonabant during acquisition and consolidation of extinction learning of an appetitively motivated task, as well as on cue-induced reinstatement. In addition, we assessed the interaction between extinction and the endocannabinoid system by evaluating the effect of rimonabant given in the absence of extinction training (during forced abstinence), a condition that more closely reflects human detoxification.

Materials and methods

Animals

Sixty-four (64) male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) with indwelling jugular vein catheters were maintained at $85\pm 5\%$ of their free-feeding weight throughout all the experiment. Rats were individually housed in a temperature- and humidity-controlled room with a 12-h light–dark cycle (lights on at 07:00 h). All procedures were carried out in accordance with established practices as described in the NIH Guide for Care and Use of Laboratory Animals. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of University of Maryland School of Medicine.

Drugs

SR141716A (rimonabant) was provided by the National Institute on Drug Abuse Drug Supply Program (Research Triangle Park, Raleigh, NC, USA) dissolved in a solution of (1:1:18) ethanol, Emulphor (Rhodia, Cranbury, NJ, USA), and saline and injected intravenously (i.v.) at 0, 200, or 300 $\mu\text{g/kg}$; doses selected on their inability to alter locomotor activity (Fattore et al. 2003; Cippitelli et al. 2005). Also, the higher dose used here, through this administration route, blocks the effects of a CB1 agonist, reduces dopamine transients elicited by drugs of abuse (Cheer et al. 2004, 2006, 2007), and it is effective in preventing reinstatement to heroin seeking (Fattore et al. 2003).

Operant procedure

Apparatus: Experiments were conducted in rat operant conditioning chambers (12.5" L×13.5" W×13.5" H; Med Associates, Georgia, VT, USA) located within ventilated sound attenuation chambers. The operant boxes were equipped with a house light, two cue lights above two retractable levers, a modular pellet dispenser and receptacle, a Sonalert module that delivers a 2900 Hz tone, and a white-noise amplifier.

Rats were trained under a fixed-ratio 1 schedule with an inter-trial interval of 10 s. Both retractable levers were present during the experiment, but only one was associated with an illuminated cue light and reward delivery (active lever). Responses on the other lever (inactive lever) were recorded but did not have any scheduled consequences. A trial began with the cue light on top of the active lever and the house light on and the extension of the active and inactive levers. Once the rat pressed down the active lever both levers retracted and a 45 mg sucrose chocolate-flavored pellet (Bio-Serv, Frenchtown, NJ, USA) was delivered, the cue and house lights were turned off, and a 2,900-Hz tone started. At the end of the 10-s inter-trial interval, the tone was muted and a new trial began. White noise and fans of the attenuation chambers were on throughout the experimental sessions. Once rats reliably acquired lever press behavior, the maintenance phase began. Maintenance sessions were carried out daily; each session lasted 1 h and were run until behavior stabilized, no more than 15% variation from the mean of three consecutive sessions. Data from the final 10 sessions were used for analysis; most subjects acquired stable behavior after 13 (± 2) sessions.

Following maintenance, rats were divided into 11 groups of $n=6$ per group (unless otherwise stated) (see Table 1). Three groups of rats constituted the *acquisition of extinction groups*; subjects in these groups were injected daily with 0, 200, or 300 $\mu\text{g/kg}$ of rimonabant for 7 days immediately before starting the extinction sessions. Three groups of rats constituted the *consolidation of extinction groups*; subjects in these groups received similar drug dosing of daily rimonabant for 7 days immediately after the end of the experimental session. During extinction training of these groups, all stimuli associated with lever presentation and reward delivery were presented as during maintenance; but the food pellet was withheld. Extinction training lasted for 7 days and daily sessions ended after an hour had elapsed or following 10 min of inactivity. Three groups of rats constituted the *forced abstinence groups*; the subjects belonging to these groups received similar drug dosing to the acquisition and consolidation groups but did not undergo extinction training sessions. Following rimonabant treatment, rats in the acquisition, consolidation, and abstinence groups were kept food-restricted in the animal colony for 21 days, after which reinstatement sessions began. For these groups of rats, five pellets were delivered randomly during the first 10 min of the reinstatement session and the cues associated with the lever extension and reward delivery were withheld. Finally, to confirm prior reports, two *cue-reinstatement groups* ($n=5$) were run. During extinction training of these groups, all cues associated with the lever presentation and the reward were removed, and the food pellet was not delivered upon a lever press. The experimental subjects in these groups received 0 or 300 $\mu\text{g/kg}$ of rimonabant *immediately* before the start of cue-induced reinstatement, which was run 1 day after the last extinction session. All cues associated with the presentation of the lever and the reward were present during reinstatement sessions which lasted for 1 h.

Data analysis

Data for maintenance phase was analyzed using a between–within-subject analysis of variance (mixed ANOVA). The responses of each subject were normalized to the maximum number of presses during the maintenance phase. Data for the extinction session of the acquisition and consolidation groups were fitted using a single phase exponential decay

curve to obtain extinction rate and half-life values. Normalized responses during extinction were analyzed using a mixed ANOVA; the data obtained during the reinstatement for the acquisition and consolidation groups, as well as the rate and half-life of extinction, were analyzed using a one-way ANOVA. Comparison of the reinstatement data of the different groups against the data obtained from the abstinence groups was performed with a two-way ANOVA. Tukey's honestly significant difference (HSD) post hoc test was used to evaluate any difference between the treatments. Data from cue-induced reinstatement was analyzed using conventional Student's *t* tests. Statistical significance was accepted if $p < 0.05$. Preliminary data analysis and graphs were done in GraphPad Prism (GraphPad Software, San Diego, CA), final data analysis was carried out using Statistica (Statsoft, Inc., Tulsa, OK, USA) and graphs using Origin (OriginLab, Northampton, MA, USA).

Results

Comparison of maintenance responses across groups

Acquisition of extinction—During the maintenance phase, the groups of rats that underwent acquisition of extinction, taken as a whole, pressed 311.8 ± 2.27 times on the active lever, compared to 1.26 ± 0.23 times on the inactive lever. When responses were divided according to the treatment regimen, the rats that received vehicle presented an average of 296.05 ± 5.53 lever presses on the active lever, whereas they presented an average of 1.05 ± 0.30 lever presses on the inactive lever. The rats that received 200 $\mu\text{g/kg}$ of rimonabant during the acquisition of extinction presented an average of 317.38 ± 2.18 lever presses on the active lever, whereas they presented an average of 1 ± 0.39 lever presses on the inactive lever. Finally, the group that received 300 $\mu\text{g/kg}$ of rimonabant during the acquisition of extinction presented an average of 321.98 ± 2.30 lever presses on the active lever, whereas they presented an average of 1.73 ± 0.50 lever presses on the inactive lever. A mixed ANOVA on active lever presses showed that there was no statistical difference between the different groups, nor there was a significant interaction between the drug groups and sessions. There was, however, a significant difference across sessions ($F_{(9, 135)} = 2.71$, $p = 0.006$, $\eta_p^2 = 0.15$). Tukey's post hoc test showed that sessions 1 and 2 were statistically different from session 7. Globally, the first two sessions were the ones with comparatively less lever presses.

Consolidation of extinction—Rats that underwent consolidation of extinction manipulations emitted an average of 302.7 ± 2.15 lever presses on the active lever. On the inactive lever the average was 2.91 ± 0.40 lever presses. When responses were divided according to the different regimens, the rats that received vehicle responded on average 294.83 ± 4.39 times on the active lever and 3.15 ± 0.75 on the inactive lever. The group of rats that received 200 $\mu\text{g/kg}$ rimonabant responded on average of 310.76 ± 3.05 times on the active lever and 3.63 ± 0.72 times on the inactive lever. The rats that received 300 $\mu\text{g/kg}$ rimonabant responded on average 302.5 ± 3.37 times on the active lever and 1.96 ± 0.59 times on the inactive lever. A mixed ANOVA on active lever presses showed that there was no statistical difference between groups or the interaction between the drug groups and sessions. There is also no significant difference between sessions.

Forced abstinence—Rats that did not undergo extinction training, but received different doses of rimonabant in their home cages, emitted an average of 296.39 ± 2.36 responses on the active lever and an average of 2.62 ± 0.35 responses on the inactive lever. When the total number of responses was divided according to the dosing regimen, rats in the vehicle group responded on average 286.45 ± 4.27 on the active lever and 3.9 ± 0.75 on the inactive lever. The group of rats that received 200 $\mu\text{g/kg}$ rimonabant responded on average 292.76 ± 4.30 times on the active lever and 2.18 ± 0.57 times on the inactive lever. The rats that received

300 µg/kg of rimonabant responded on average 310.5 ± 2.9 times on the active lever and 1.78 ± 0.49 times on the inactive lever. A mixed ANOVA on active lever presses showed that there was no statistical difference between the different groups, or the interaction between the drug groups and sessions, but there was a significant difference between sessions ($F_{(9, 135)} = 2.36, p = 0.016, \eta_p^2 = 0.13$). Tukey's post hoc test shows that there were statistically less lever presses during the third session compared to the fourth and 10th sessions.

Cue-induced reinstatement—Rats that underwent extinction training but received different doses of rimonabant immediately prior to the cue-induced reinstatement session emitted an average of 311.98 ± 1.97 responses on the active lever and an average of 1.36 ± 0.50 responses on the inactive lever. When responses were divided according to the dosing regimen, rats in the vehicle group responded 311.98 ± 1.97 times on the active lever and 1.36 ± 0.50 times on the inactive lever. The group of rats that received 300 µg/kg of rimonabant responded on average of 313.4 ± 1.29 times on the active lever and 1.06 ± 0.25 times on the inactive lever. A mixed ANOVA on active lever presses showed that there was no statistical difference between the groups. There was also no significant interaction between the drug groups and sessions, and there were no significant differences between sessions.

Comparison of extinction responses for groups that received rimonabant during extinction training

Acquisition of extinction groups—Groups that underwent acquisition of extinction treatment (Fig. 1a) showed maximal responding on the first day of extinction and then behavior gradually decreased so that by the fourth extinction day all rats pressed below 10% of their maintenance level ($F_{(6, 90)} = 69.82, p = 0.000, \eta_p^2 = 0.82$). Tukey's post hoc test showed that the first extinction session was significantly different from all the other extinction days and that the second and third extinction sessions were significantly different from the sixth and seventh sessions. The drug treatment had no effect on responding during extinction, but there was a statistically significant effect in the interaction between drug dose and extinction day ($F_{(12, 90)} = 2.09, p = 0.02, \eta_p^2 = 0.22$). Post hoc tests for the interaction between drug dose and extinction session showed that the only significant difference was between the first extinction day in the 300 µg/kg group. Responses from this group were significantly lower than those of the vehicle group.

Consolidation of extinction groups—Similar to the acquisition of extinction, groups that underwent consolidation of extinction treatment (Fig. 1b) showed maximal responding on the first extinction session and then showed a steady decline so that by the fourth extinction session responding among all groups was below 10% of their maintenance responses ($F_{(6, 90)} = 109.35, p = 0.000, \eta_p^2 = 0.88$). Tukey's post hoc test showed that the first extinction session was significantly different from all the other extinction days and that the second extinction session was significantly different from the fifth extinction session onward. There was no main effect of drug dose or of the interaction between drug dose and extinction session.

Analysis of quantitatively derived parameters of extinction—Extinction rate and half-life for each subject were obtained by fitting a single exponential decay function to the extinction data (Fig. 2a, b). R^2 of the fit for each of the subjects' data varied between 0.80 and 0.99. ANOVA showed no significant effect of treatment on extinction rate nor half-life.

Analysis of responses given during the reinstatement session—A reinstatement session was run to evaluate if the pharmacological treatments had a long-term effect on food-induced reinstatement. Groups that underwent extinction training (acquisition and

consolidation of extinction) showed similar responses during reinstatement, between 16% and 23% of the responding observed during maintenance, whereas groups that did not undergo extinction training (forced abstinence) showed more lever press activity, between 36% and 43% (see Fig. 3). Two-way ANOVA showed a significant difference between treatments ($F_{(2, 45)}=15.28, p=0.000, \eta^2=0.40$), but there was no main effect of drug dose. The interaction between drug dose and the experimental group was also not significant. Post hoc analysis confirms that the forced abstinence groups performed at a higher level than the groups that underwent extinction training. Moreover, performance in the forced abstinence groups was similar to that observed during the first extinction day (graph not shown) regardless of the extinction training group. Two-way ANOVA showed no significant differences between drug dose, group, or the interaction between the drug dose and the experimental group.

Figure 4a shows responses on the active lever during maintenance and extinction phases for the two groups of rats that received either vehicle or rimonabant (300 $\mu\text{g/kg}$ i.v.) immediately before a cue-induced reinstatement session; both groups of rats performed at a very similar level during these two phases. When given immediately prior to the reinstatement session, rimonabant produced a substantial inhibition of lever press behavior upon cue-induced reinstatement ($t_{(8)}=10.09, p=0.000, \eta^2=0.92$) when compared against the vehicle group (see Fig. 4b).

Discussion

It has been demonstrated that manipulation of CB1 receptors dramatically affects reward-seeking behavior during reinstatement (De Vries et al. 2001, 2003, 2005; Cohen et al. 2005; Ward et al. 2007, 2009). The goal of the present series of experiments was to study the role of endocannabinoids in extinction learning of an operant appetitively motivated task by systemically administering rimonabant at different doses and at different time points during extinction. We hypothesized that CB1 receptor blockade influences the rate of extinction, and the effect of this blockade on memory decay may in turn affect response inhibition upon reinstatement. Systemic treatment with rimonabant failed to produce immediate or long-term changes in the acquisition or consolidation of extinction learning. The extinction rate and the activation of behavior produced by food-induced reinstatement were not statistically different from that of controls. Moreover, rimonabant treatment without extinction training did not affect reward-seeking behavior during reinstatement; indeed, responding in rats that did not undergo extinction was similar to that of the first extinction session observed in all the other groups tested.

At first glance, the difference between the acquisition and consolidation of extinction groups on the first extinction day suggests that rimonabant interfered with early extinction learning. However, subsequent decreases in responding occurred at a rate identical to the other groups. Changes in locomotion are unlikely responsible for this effect because rimonabant at comparable doses to the ones used in the present study failed to reduce locomotion (Fattore et al. 2003; Cippitelli et al. 2005; Niyuhire et al. 2007). Moreover, in the Morris water or Barnes maze tasks, animals treated with equipotent doses of rimonabant show similar latencies and distance travel to target (Varvel et al. 2005b; Harloe et al. 2008).

Alternatively, rimonabant may have decreased responding during the first extinction day because it attenuated the motivation to work for food and/or the salience of cues associated with the reward. This interpretation is supported by several lines of evidence; first, rimonabant at doses equipotent to the ones here used is able to reduce responding for food pellets during higher fixed ratios to the one here employed (Freedland et al. 2000; De Vry et al. 2004). Thus, the reduction observed in the first day of extinction for the acquisition of

extinction group would be consistent with decreased motivation. Second, rimonabant at higher doses than the ones used here effectively reduces breakpoints under a progressive ratio schedule (Ward and Dykstra 2005), which suggests that endocannabinoids play a role in the modulation of motivation to work for food.

Cues associated with rewards acquire incentive salience when (a) they elicit approach behavior towards them, (b) when they maintain a behavior oriented towards their retrieval, and (c) when they elicit seeking behavior towards their associated reward (Yager and Robinson 2010). Thus, the observed decrease in responding during the first extinction day may be due to a decrease in the incentive salience of the set of cues that were present during extinction training, which in turn reduced seeking behavior towards the reward.

The evidence for an effect of rimonabant on the incentive nature of reward-predictive cues is reinforced by the finding that rimonabant obliterated cue-induced reinstatement. This observation replicates prior results that used highly palatable rewards (De Vries et al. 2005; Ward et al. 2007), as well as drugs of abuse (De Vries et al. 2001, 2003, 2005; Cohen et al. 2002, 2005; Fattore et al. 2003; Anggadiredja et al. 2004; Cippitelli et al. 2005; Economidou et al. 2006). The attenuation of cue-induced reinstatement produced by rimonabant when drugs of abuse are used as rewards suggests that there is a very low likelihood that the observed effect in the cue-induced reinstatement experiment was due to rimonabant's anorexigenic properties.

As stated above, it could also be argued that the effect of rimonabant during the cue-induced reinstatement is the product of a reduction in the animal's propensity to perform and active response. If this were the case and assuming that rimonabant effects on performance are constant, we would expect to see a comparatively similar reduction in lever-press behavior as the one observed in the first extinction day between the control subjects and those that received 300 µg/kg of rimonabant in the acquisition of extinction group; the reduction in this group was approximately half of its control. In contrast, the reduction in lever-press behavior observed in the group that received 300 µg/kg of rimonabant in the cue-induced reinstatement group was approximately 10 times lower than that of its respective control. Given these differences in lever-press behavior, and the evidence presented above, the plausibility of a reduction in the animal's propensity to perform an active response as an explanation for the observed results seems very low. The data presented herein suggests that rimonabant alters the retrieval of memories for cues previously associated with rewards, which translates into a goal-directed performance deficit during cue-induced reinstatement and contributes to evidence suggesting that CB1 receptors are crucial for the expression of Pavlovian associations between cues and unconditioned stimuli.

When blockade of CB1 receptors is studied during the extinction of appetitively motivated task, all the results, including the ones reported here, are suggestive of a lack of effect on extinction rate (Holter et al. 2005; Niyuhire et al. 2007; Ward et al. 2007). To the best of our knowledge, the present study is the first one to investigate the involvement of CB1 receptors at different stages of extinction learning and retrieval. The sharp contrast between the results obtained when appetitively motivated tasks are used and when aversively motivated tasks are used suggests that the endocannabinoid system is dispensable in the extinction of appetitively motivated task, although it plays a critical role in the extinction of aversively motivated memories (Holter et al. 2005). Indeed, when the same behavioral task is used but appetitive or aversive contingencies are used, rimonabant preferentially impairs extinction learning under aversive, but not under appetitive, conditions (Harloe et al. 2008).

However, this assertion seems to be at odds with research on spatial learning and reversal tasks. Extinction learning can be seen as a type of reversal learning in which the relation

between a set of stimuli and the presence of reward changes. During extinction, subjects have to learn that the stimuli that previously signaled the presence of reward now they signal its absence. Thus, it is surprising that given the rich research showing that pharmacological blockade or genetic deletion of the CB1 receptors alters reversal learning on spatial memory tasks, such effects cannot be replicated during operant extinction of an appetitively motivated behavior. One possible interpretation of the lack of effects is that operant extinction and reversal learning require different brain systems in which the former is most dependent on the state of the dorsal striatum, whereas the latter is most dependent on hippocampal activity (Packard et al. 1989; McDonald and White 1993). A problem with this interpretation is that the striatum, like the hippocampus, is very rich in CB1 receptor expression (Pettit et al. 1998; Tsou et al. 1998; Egertova and Elphick 2000; Kofalvi et al. 2005). Given this abundance of CB1 receptors in the striatum, it would be expected that their blockade would produce a measurable effect, yet the results have been elusive. Another factor that could play a role in the lack of effect of blockade of CB1 receptors in the extinction of an operant appetitively motivated task is a procedural one. During extinction of an operant behavior, subjects experience several extinction trials during consecutive sessions, which can be seen as a massive training protocol. Varvel et al. (2005b) have shown that pharmacological blockade and genetic deletion of CB1 receptors impairs extinction in the Morris water maze when extinction trials are spaced with five probe tests over several months. Conversely, extinction is not affected by CB1 receptor manipulations if the extinction procedure consists of many trials in a short time period (20 in 5 days). This result suggests that there are different mechanisms mediating short-term extinction learning and those regulating the consolidation of extinction. It would be of interest to replicate this protocol within an operant paradigm to evaluate if similar results can be obtained.

The lack of a positive result in the extinction of positively motivated task requires that the evidence obtained when other paradigms are used be taken into account in the design of new experiment, extinction sessions in particular. Such experiments should provide evidence supporting differential roles of endocannabinoids in different brain regions and should clarify their role in extinction learning of appetitively motivated tasks.

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Fig. 1.

Responses on the active lever during maintenance and extinction phases. **a** Performance of the acquisition of extinction groups. There is a significant difference, highlighted by an *asterisk*, in the first day of extinction for the group that received the highest dose of rimonabant. **b** Performance of the consolidation of extinction groups

Fig. 2. Fit of single exponential decay to the extinction data. For the acquisition of extinction groups (a) and the consolidation of extinction groups (b)

Fig. 3. Reward-induced reinstatement for the acquisition and consolidation of extinction groups as well as on the groups of rats that did not undergo extinction training. Rimonabant has no long-term effects on reward-seeking behavior; all groups of rats that underwent extinction training presented comparable behavior during the reinstatement test regardless of drug dose. The subjects that did not undergo extinction training showed a significant elevated responding, highlighted by an *asterisk*, when compared to the other groups

Fig. 4.

Responses on the active lever during maintenance and extinction phases (**a**) for two groups of rats that were treated with either vehicle or rimonabant immediately before a cue-induced reinstatement session. The bar graph (**b**) shows that the group treated with rimonabant showed significantly less reward-seeking behavior, highlighted by an *asterisk*, than the group that received vehicle

Table 1

Group assignment by treatment

Group	Rimonabant dose ($\mu\text{g/kg}$)		
	0	200	300
Acquisition of extinction	$n=6$	$n=6$	$n=6$
Consolidation of extinction	$n=6$	$n=6$	$n=6$
Forced abstinence	$n=6$	$n=6$	$n=6$
Cue-induced reinstatement	$n=5$	$n=0$	$n=5$