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# THE CB1 ANTAGONIST RIMONABANT (SR141716) BLOCKS CUE-INDUCED REINSTATEMENT OF COCAINE SEEKING AND OTHER CONTEXT AND EXTINCTION PHENOMENA PREDICTIVE OF RELAPSE

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## Abstract

Cannabinoid CB1 antagonists decrease self-administration of palatable food and several abused drugs in animals and modulate extinction of conditioned fear responses. Less is known, however, about whether and how CB1 antagonists might modulate the extinction of appetitive behavior. Therefore, this study examined the effects of the CB1 receptor antagonist rimonabant (SR141716) during extinction of responding maintained either by cocaine or by palatable foods (corn oil or Ensure), as well as responding elicited by stimulus cues that had been paired with the presentation of cocaine (i.e., cue-induced reinstatement) or a prime (presentation of cocaine or food). The effect of rimonabant on high rate responding in water-deprived mice trained to self-administer water was also examined. In mice self-administering cocaine, rimonabant attenuated cue-induced reinstatement of cocaine self-administration, the initial burst of responding during cocaine extinction and responding during spontaneous recovery. In mice self-administering corn oil, rimonabant decreased responding during extinction and also attenuated responding that had been reinstated by a priming presentation of corn oil. Moreover, mice treated with rimonabant required fewer daily sessions to reach criterion for extinction of cocaine-maintained responding than vehicle treated mice. Also, rimonabant had no effect on rate of operant responding in mice trained to respond for water under an FR5 schedule of reinforcement. Taken together, these data suggest that in addition to attenuating the primary reinforcing effects of both palatable foods and drugs of abuse, CB1 receptor antagonism can attenuate context and cue reactivity during extinction learning and potentially enhance extinction learning in this way.

### Keywords

CB1 receptors; rimonabant; SR141716; cocaine; self-administration; extinction; reinstatement

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## 1. Introduction

Obesity has reached epidemic proportions globally, yet few anti-obesity drugs are currently available, and those that are have limited clinical efficacy (Bray and Greenway, 2007). Likewise, yearly estimates classify 2.1 million Americans as dependent on or abusing cocaine (Results from the 2007 National Survey on Drug Use and Health), yet cocaine abuse has been particularly challenging to treat, with no pharmacological treatments currently available to treat cocaine addiction specifically. The behavioral approach of cue exposure, in which cue reactivity is extinguished over several therapy sessions, has been shown to decrease both binge eating (Jansen et al 1992) and cocaine craving (O'Brien et al 1988; O'Brien et al 1990; Childress et al 1993). Extinction therapy has met with limited success for long term addiction treatment, however. In addition, research on extinction has uncovered several context and cue reactivity phenomena which arise during extinction learning that can act as potential mechanisms of relapse, including extinction burst (Lerman and Iwata 1995), spontaneous recovery and reinstatement elicited by a context, cue or prime (see Bouton 2002 for review). Therefore, an improved treatment strategy for drug abuse as well as uncontrolled eating might combine extinction therapy with pharmacotherapy to enhance learning and/or attenuate relapse during therapy.

A wealth of evidence demonstrates that the cannabinoid CB1 receptor system is involved in both food and drug reward generally and cue reactivity specifically. The CB1 receptor antagonist/inverse agonist rimonabant (SR141716) decreases self-administration of palatable foods (Arnone et al., 1997; Higgs et al., 2003; Thornton-Jones et al., 2005; Ward and Dykstra 2005, Ward et al 2008), heroin (De Vries et al 2003; Solinas et al 2003), ethanol (Arnone et al 1997) and nicotine (Cohen et al 2002). CB1 receptor blockade also attenuates seeking for palatable food in mice (Ward et al 2007) and rats (De Vries et al 2005) as measured by rodent models of cue-induced reinstatement, a paradigm widely used to model craving in humans (see Katz and Higgins 2003 for review). Likewise, CB1 receptor antagonism attenuates cue-induced reinstatement of sucrose seeking in rats. CB1 receptor antagonism also attenuates reinstatement of heroin (De Vries et al 2003), nicotine (De Vries et al 2005), methamphetamine (Anggadiredja et al 2004), and ethanol (Cippitelli et al 2005) self-administration. Also, although CB1 receptor blockade is generally ineffective in reducing the self-administration of cocaine in rodents and primates under fixed ratio schedules, CB1 receptor blockade can reduce cue-induced reinstatement of cocaine-seeking behavior in rats (De Vries et al 2001; Filip et al 2006) and motivation for cocaine as measured by progressive ratio responding (Xi et al 2008, Soria et al 2005).

Interestingly, a growing literature also implicates the endogenous cannabinoid (eCB) system as a neural substrate involved in extinction of aversively-motivated learning. For example, extinction of auditory fear conditioning is impaired in CB1 knockout (KO) mice (Marsicano et al 2002). Both CB1 KO and rimonabant impair extinction of escape behavior in aversively-motivated spatial tasks (Varvel et al 2005; Harloe et al 2008), and rimonabant impairs extinction of conditioned freezing and extinction of passive avoidance of foot shock (Niyuhire et al 2007). Conversely, it appears that activation of the eCB system may facilitate extinction of fearful memories (Pamplona et al 2006) and has been suggested as a potential pharmacotherapy for extinction of phobias and post-traumatic stress disorder (see Lutz 2007 for review).

Although few studies have investigated the role of the eCB system in extinction of positively-reinforced behaviors, early evidence suggests that CB1 receptor antagonism does not impair extinction of appetitively conditioned behaviors, and may even enhance this type of extinction learning. Initially, Hölter et al (2005) reported that CB1 KO mice did not show impairment in extinction of a classically conditioned appetitive task, and Harloe et al (2008) reported that

rimonabant did not show impairment of extinction in an appetitively-motivated spatial learning task. Furthermore, Niyuhire et al (2007) reported that pretreatment with rimonabant during extinction of an appetitively motivated food self-administration task attenuated initial extinction burst responding, a phenomenon that has been suggested as one of several important triggers for relapse behavior that arise during extinction.

The goals of the present set of studies were three-fold. First, we examined the role of CB1 receptors in cue-induced reinstatement of cocaine seeking using a mouse model of relapse to determine whether the reported attenuation of this behavior by rimonabant in rats would be replicated in mice. Second, the effect of a CB1 receptor antagonist on extinction was examined on a range of measures, including initial extinction burst behavior, spontaneous recovery of the reinforced behavior upon re-exposure to context and cues following extinction, and prime-induced reinstatement following brief re-exposure to a reinforcer and its interoceptive cues. Based on preliminary findings from our laboratory and the work of Niyuhire et al (2007), we hypothesized that the CB1 receptor antagonist rimonabant would block the cue-induced reinstatement of cocaine seeking as well as attenuate behaviors arising during the extinction of corn oil, vanilla-flavored Ensure, and cocaine self-administration in mice. Finally, to address whether any decreases in responding during extinction sessions could be attributed to attenuation of context/cue reactivity versus a general decrease in the animals' rate of responding during an operant task, an additional group of animals were trained to self-administer water and the effect of several doses of rimonabant on this behavior was examined.

## 2. Methods

### 2.1. Animals

C57/Bl6 male mice (ACE Animals, Royersford PA, The Jackson Laboratory, Raleigh NC) were used in the present experiments and in all cases sample sizes include an N of 5–8 per group. Following their arrival at the animal facilities at UNC Chapel Hill or Temple University, mice were housed in groups of four, with *ad libitum* access to food and water throughout the duration of the operant studies, except where indicated in the Operant Procedures section. Lights were programmed on a 12 hr light/dark cycle with lights off at 7 a.m., so that all experimental testing occurred during the dark cycle of the animals' diurnal cycle. Mice weighed 20–25 g at the beginning of the experiments. Animal protocols were approved by Institutional Animal Care and Use Committees at both Universities, and the methods were in accord with the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council 2003).

### 2.2. Drugs

Cocaine hydrochloride (National Institute on Drug Abuse, Rockville, MD) was dissolved in saline at a concentration of 1.8 mg/mL for intravenous administration. Pump time was adjusted for each mouse so that the proper infusion was delivered for administration of 0.3 mg/kg/infusion, with an average pump time of approximately 1 second. For priming, cocaine hydrochloride was dissolved in saline at a concentration of 2.0 mg/mL for IP administration of 20 mg/kg.

SR141716A (Research Triangle Institute, Research Triangle Park NC) was dissolved in cremophor vehicle (1:1:18 ratio of ETOH, cremophor, and saline) at concentrations of 0.1 mg/mL and 0.3 mg/mL for IP administration of 1.0 mg/kg (for cocaine studies) or 3.0 mg/kg (for food studies). Pretreatment time for rimonabant was 15 min in all experiments.

### 2.3. Operant Chambers

Food and cocaine self-administration experiments were conducted in mouse operant conditioning chambers (21.6 cm × 17.8 cm × 12.7 cm, Model ENV-307W, Med Associates, Georgia, VT, USA) located within ventilated sound attenuation chambers. Chambers used at UNC Chapel Hill and Temple University were identical to one another. The operant conditioning chambers were equipped with two nose-poke holes (1.2 cm diameter) equipped with internal amber stimulus lights (ENV-313W), a house light (ENV-315M), ventilator fan, and tone generator (ENV-323AW). The operant chambers used in food experiments were also equipped with a motor-driven dipper (ENV-302W) for liquid food presentation. The receptacle opening for access to the food was located between the two nose-poke holes, and an amber stimulus light was located above the receptacle opening (ENV-221M). For operant chambers used in cocaine experiments, drug delivery was controlled by an electronic circuit that operates a computer-controlled syringe that is connected to a single-channel fluid swivel mounted on a counter-balanced arm above the operant chamber (MED-307A-CT-B2).

### 2.4. Experimental Design

#### 2.4.1. Experiment 1: Cue-induced reinstatement of cocaine seeking

**Acquisition and extinction of nose-poke behavior:** In **Experiment 1**, mice were initially trained to respond for 50% vanilla-flavored Ensure® diluted in tap water under a fixed ratio 1 (FR1) schedule during daily 30-min acquisition sessions (see Ward and Dykstra 2005 for details) in the operant chambers equipped for food self-administration. Acquisition training generally lasted for 10–15 days and concluded once stable responding was reached for each mouse.

**Cocaine self-administration:** Following acquisition of food self-administration, mice were anesthetized (ketamine 145 mg/kg, xylazine 10 mg/kg; IP) and implanted with chronic indwelling jugular cannulae as described by Caine et al 1999. The cannula lines were maintained by daily antibiotic and heparin treatment. After 5 days of recovery from surgery, mice were trained to self-administer cocaine under an FR1 schedule of reinforcement. Responses in the active nose-poke hole were reinforced with a 0.3 mg/kg cocaine infusion; the active nose-poke hole was lit with an internal amber stimulus light. Initiation of drug delivery was signaled by the simultaneous illumination of the cue light above the nose-poke hole for 3 sec and onset of a 1 sec tone, and the active nose-poke stimulus light was extinguished. Also, a 20 sec timeout period was imposed following each nose-poke response in the active hole, during which time the house light was extinguished as well. Each session was terminated after 2 hr had elapsed. A maximum number of 40 infusions/session was set until patterns of self-administration looked stable for each mouse, at which point no maximum was set. Acquisition of cocaine self-administration for each mouse was defined as three consecutive days of at least 40 infusions/2 hr session. Stable responding is defined as three consecutive sessions with less than 20% variation in the total number of infusions earned.

**Extinction:** Following 10 days of stable cocaine self-administration under the FR1 schedule, responding was extinguished. All cues associated with the delivery of cocaine were removed and responses had no scheduled consequences (i.e. the stimulus light was not activated, there was no presentation of the tone, and the syringe pump was not activated). The house light and fan remained on throughout the session. Extinction sessions were conducted daily until mice reached a criterion of ≤33% of the number of active nose-poke hole responses made on the last self-administration day. This criterion was chosen to engender a response rate on the final extinction session comparable to that described by Fuchs *et al* (2003) (also see Ward et al 2007). Retaining this level of responding at the end of extinction helps guarantee that mice will

respond in the active nose poke hole at the start of the reinstatement session, which is required for the reintroduction of the previously associated cues.

**Cue-induced reinstatement test:** Mice were treated with either vehicle or 1.0 mg/kg rimonabant 15-min prior to the reinstatement test to assess the effect of CB1 receptor antagonism on cue-induced reinstatement of cocaine seeking (dose of rimonabant selected based on a smaller pilot study revealing a maximal effect on cue-induced reinstatement behavior at this dose). During the 2-hr test session, responses on the active nose-poke hole resulted in the illumination of the stimulus light, the 1 sec tone, and activation of the syringe pump with delivery of saline.

### 2.4.2. Experiment 2: Extinction of corn oil, Ensure, or cocaine self-administration—

In **Experiment 2**, three groups of mice were trained to respond under an FR1 schedule for either 32% corn oil (emulsified in tap water with xanthan gum) or 50% Ensure® during daily 30 min sessions as described in Ward and Dykstra (2005) or 0.3 mg/kg/inf cocaine for daily 2-hr sessions as described above. Following 10 days of stable food or cocaine self-administration under the FR1 schedule, responding was extinguished in all groups by the removal of the reinforcer and all cues associated with its delivery as described above. Prior to each daily 30-min food extinction session or daily 2-hr cocaine self-administration session, all mice were pretreated with either vehicle or rimonabant IP The 1.0 mg/kg dose of rimonabant was used for the cocaine group as it was the minimal effective dose identified by pilot studies and based on results from Experiment 1; the 3.0 mg/kg dose of rimonabant was used for corn oil and Ensure groups as it has produced the most reliable effects on operant food self-administration in our laboratory). Daily extinction sessions were conducted for each mouse until it reached a criterion of ≤33% of the number of active nose-poke hole responses made on the last self-administration day.

### 2.4.3. Experiment 3: Extinction burst responding, spontaneous recovery, and prime-induced reinstatement—

In **Experiment 3**, two groups of mice were trained to respond for either 32% corn oil or 0.3 mg/kg/inf cocaine under an FR1 schedule of reinforcement. Following 10 days of stable corn oil or cocaine self-administration, the effect of pretreatment with vehicle or rimonabant (1.0 mg/kg for cocaine group, 3.0 mg/kg for corn oil group) on extinction behaviors was determined in an experimental design modified from Rhodes and Killcross (2004). Specifically, the experiment was designed to assess the effect of daily CB1 antagonist treatment on bursting, spontaneous recovery, and prime-induced reinstatement behaviors. Also, the last two corn oil or cocaine self-administration sessions were divided into discrete trials in an identical manner as is described below for extinction sessions.

**Initial extinction burst responding** was investigated during the first extinction session (E1) by extinguishing self-administration behavior in discrete trials. Five-min trials were implemented for extinction of corn-oil self-administration and 15-min trials were implemented for extinction of cocaine self-administration in order to engender similar baseline levels of responding per trial across reinforcers. A 30-sec time out was implemented between trials wherein the house and stimulus lights were turned off. During each extinction trial, nose-pokes into the previously active hole resulted in the delivery of the reinforcer-paired auditory and visual cues but were not reinforced with corn oil or cocaine. The extinction session was terminated for each mouse immediately following a trial in which it reached an extinction criterion of equal to or less than 2 nose-pokes in the previously active hole. Each mouse was removed from the operant chamber immediately following the trial in which the extinction criterion was met. This extinction criterion was selected for Experiment 3 in order to investigate spontaneous recovery of a well-extinguished response during the subsequent session. Mice were pretreated with vehicle or rimonabant 15 min prior to the beginning of E1.

**Spontaneous recovery** of operant behavior was investigated during the second extinction session (E2) by returning the mice to the chambers and assessing behavior during discrete trials throughout the extinction session. During each extinction trial, nose-pokes into the previously active hole resulted in the delivery of reinforcer-paired auditory and visual cues but were not reinforced with corn oil or cocaine. The extinction criterion for each mouse was identical to that for E1. Mice were pretreated with vehicle or rimonabant 15 min prior to the beginning of the second extinction session.

The cocaine self-administration groups received four subsequent extinction sessions (E3–E6) conducted identical to the two previously described, in order to fully extinguish responding prior to the final cocaine prime reinstatement session. These extinction sessions were also comprised of discrete 15-min trials, and mice were pretreated with vehicle or rimonabant 15 min prior to the beginning of each extinction session.

**Prime-induced reinstatement** of operant behavior was investigated during the final extinction session. First, baseline response levels were assessed in a single trial (5-min for corn oil; 15-min for cocaine) to demonstrate that responding was extinguished in both the corn oil and cocaine groups. Mice were then given non-contingent presentation of corn-oil or an IP injection of cocaine, and operant responding was reassessed in a succeeding discrete trial. Non-contingent corn oil was presented as two manual deliveries of corn oil via the dipper arm into the chamber paired with both the auditory and visual cues. Consumption of the corn oil was confirmed in each animal by leaving the dipper arm in the food receptacle of the chamber until a head entry in the receptacle registered on the computer. Non-contingent cocaine was administered as a single IP injection of 20 mg/kg cocaine. During both the baseline and reinstatement trials, nose-pokes into the previously active hole resulted in the delivery of reinforcer-paired auditory and visual cues but were not reinforced with corn oil or cocaine. Mice were pretreated with vehicle or rimonabant 15 min prior to the beginning of the final extinction session.

#### 2.4.4. Experiment 4: Effect of rimonabant on response rate—

In **Experiment 4**, eight mice were water restricted and trained to respond for 100% tap water. Each daily session consisted of five 5-min trials wherein a maximum of 5 presentations of water were available under an FR5 schedule, and each trial was separated by a 15-min time out period. Once responding stabilized so that response rates were equivalent and robust across all 5 trials, the effect of vehicle or rimonabant (0.3–30 mg/kg IP) on rate of responding was assessed using a multiple-trial cumulative-dosing design. For cumulative-dosing, mice were first injected with 0.3 mg/kg rimonabant, and following a 15 minute pretreatment time, were tested in the first 5 min trial. Thereafter, mice were removed from the experimental chambers and injected with a dose of rimonabant that when added to the previously administered dose, resulting in the next 0.5 log unit dose, and placed back into the experimental chamber for the 15 min pretreatment period. Injections continued for a total of 5 trials.

The Ensure and corn oil extinction studies from Experiment 2 were conducted at the University of North Carolina. The cocaine extinction study from Experiment 2 and all other Experiments were conducted at Temple University.

### 2.5. Statistical Analyses

Food or cocaine self-administration behavior is represented as the mean number of responses made in the active nose-poke hole for each group of mice. ‘Days to extinguish’ is represented as the mean number of days necessary for each group to reach extinction criterion. In **Experiment 1**, the effect of vehicle versus rimonabant on cue-induced reinstatement of cocaine seeking was analyzed by two-tailed Student’s *t*-test. In **Experiment 2**, the effects of vehicle versus rimonabant on extinction of corn oil, Ensure, or cocaine self-administration were

analyzed using repeated measures two-way analysis of variance (ANOVA) with treatment (vehicle vs rimonabant) and day (extinction session) as factors. If a significant effect of treatment was detected, Bonferroni *post-hoc* analyses were run to determine which days(s) reached statistical significance. Also, effect of vehicle versus rimonabant on the number of days to reach extinction criteria for each reinforcer was analyzed using two-tailed Student's *t*-test. In **Experiment 3**, the effects of vehicle versus rimonabant on extinction burst responding and spontaneous recovery were analyzed using repeated measures two-way ANOVA with treatment (vehicle vs rimonabant) and trial as factors. A modified Bonferroni post test corrected for planned comparisons (GraphPad QuickCalcs) was also used to specifically test the stated hypothesis that rimonabant would attenuate 1) the burst in responding seen during **the first trial of the first extinction session** and 2) the spontaneous recovery of responding seen in **the first trial of the second extinction session**. Effect of vehicle versus rimonabant on prime-induced reinstatement of corn oil or cocaine seeking was analyzed using two-tailed Student's *t*-test. Also, effect of vehicle versus rimonabant on the number of trials to reach extinction criteria for each day was analyzed using two-tailed Student's *t*-test. In **Experiment 4**, response rates for water self-administration under an FR5 schedule of reinforcement were recorded and the number of nose poke responses per second for each mouse was calculated. Mean response rates were then calculated for baseline responding (day prior to vehicle test) and following both vehicle and rimonabant (0.3–30 mg/kg) treatment. For rimonabant data, percent baseline response rate was calculated for each of 5 cumulative dosing trials by dividing each trial's response rate by the response rate of the corresponding baseline trial and then multiplying by 100. For vehicle data, percent baseline response rate was calculated for each of 5 vehicle trials by dividing that trial's response rate by the response rate of the corresponding baseline trial and then multiplying by 100. The effect of vehicle versus rimonabant on rate of responding for water reinforcement under an FR5 schedule was then statistically analyzed using a repeated measures one-way ANOVA.

## 3. Results

### 3.1. Experiment 1: Cue-induced reinstatement of cocaine seeking

As shown in Figure 1, mice self-administered an average of 54 infusions of cocaine (0.3 mg/kg/inf) per 2 hr session under an FR1 schedule of reinforcement. During the first extinction session wherein saline was substituted for cocaine and cocaine-associated cues were terminated, mice showed an initial burst in responding of 75 nose pokes per 2 hr session. This initial extinction burst was followed by an eventual extinction (≤33%) of responding by extinction day 9 (E9). Reintroduction of cocaine-paired cues reinstated responding in vehicle pretreated mice; however mice pretreated with 1.0 mg/kg rimonabant failed to reinstate cocaine-seeking following reintroduction of the cues. Two tailed Student's t-test revealed a significant decrease in cocaine seeking in the rimonabant-treated group ($p<0.05$). Responses made in the inactive hole remained low throughout self-administration, extinction, and reinstatement of cocaine seeking, and rimonabant treatment produced no significant alterations on responses in the inactive nose poke hole.

### 3.2. Experiment 2: Extinction of corn oil, Ensure, or cocaine self-administration

As shown in Figure 2, mice trained to self-administer corn oil, Ensure, or cocaine exhibited initial bursts in responding on the first day of extinction. This initial extinction burst was followed by an eventual extinction of responding by E4 for corn oil, E8 for Ensure, and E7 for cocaine. In mice trained to self-administer corn oil, rimonabant significantly decreased responding during extinction (Figure 2A). Two-way ANOVA revealed a significant effect of day [$F(3,48) = 23.98$; $p<0.0001$], and a significant interaction [$F(3,48) = 7.63$; $p<0.001$], while the effect of treatment only approached significance [$F(1,48) = 3.59$; $p=0.06$]. Bonferroni post hoc analysis revealed the significant effect occurred on the first day of extinction. In mice

trained to self-administer Ensure, rimonabant significantly decreased responding during extinction (Figure 2B). Two-way ANOVA revealed a significant effect of treatment [$F(1,96) = 6.68$; $p<0.01$], day [$F(7,96) = 14.31$; $p<0.0001$], and a significant interaction [$F(7,96) = 4.09$; $p<0.001$]. Bonferroni post hoc analysis revealed the significant effect occurred on the first day of extinction. In mice trained to self-administer cocaine, rimonabant significantly decreased responding during extinction (Figure 2C). Two-way ANOVA revealed a significant effect of treatment [$F(1,24) = 10.45$; $p<0.01$] and day [$F(2,24) = 3.68$; $p<0.05$], but no significant interaction. Bonferroni post hoc analysis revealed the significant effect occurred on the first day of extinction. Two-tailed Student's t-tests revealed that rimonabant did not significantly decrease the number of days required to meet extinction criterion in mice responding for corn oil or Ensure; however, rimonabant significantly decreased the number of days required to meet extinction criterion in mice responding for cocaine ($p<0.05$) (Figure 2D). Responses made in the inactive hole remained low throughout self-administration and extinction of Ensure, corn oil, and cocaine seeking, and rimonabant treatment produced no significant alterations on responses in the inactive nose poke hole (data not shown).

### 3.3. Experiment 3: Extinction burst responding, spontaneous recovery, and prime-induced reinstatement

Figure 3 shows the time course of extinction of corn oil- or cocaine-maintained responding across trials during extinction sessions. Mice trained to self-administer corn oil show increased levels of responding over baseline for the first five extinction trials (or first 25 min) during the first extinction session (E1) (Figure 3A). Two-way ANOVA revealed a significant effect of rimonabant treatment [$F(1,192) = 40.2$; $p<0.01$] and time [$F(15,192) = 9.12$; $p<0.01$], but no significant interaction. Planned comparisons post test revealed a significant effect of rimonabant on trial 1 of E1 ($p<0.05$). Following extinction of corn oil seeking at the end of E1, mice spontaneously recovered corn oil seeking behavior on the second extinction session (E2), as can be seen in the first 5-min trial. Two-way ANOVA revealed a significant effect of time [$F(8,108) = 6.38$; $p<0.01$], but not of treatment and no significant interaction. Planned comparisons post test revealed a significant effect of rimonabant on trial 1 of E2 ($p<0.05$). Following a priming presentation of non-contingent corn oil, vehicle treated mice reinstated corn oil seeking behavior previously extinguished. Two-tailed Students t-test revealed that pretreatment with 3.0 mg/kg rimonabant significantly attenuated prime-induced reinstatement of corn-oil seeking ($p<0.05$).

Mice trained to self-administer cocaine show increased levels of responding over baseline for the first two extinction trials (or first 30 min) (Figure 3B). Two-way ANOVA revealed a significant effect of time [$F(1,80) = 4.69$; $p<0.01$], but not of rimonabant treatment and no interaction. Planned comparisons post test revealed a significant effect of rimonabant on trial 1 of E1 ($p<0.05$). Following extinction of cocaine seeking at the end of E1, mice spontaneously recovered cocaine seeking behavior on E2. Two-way ANOVA revealed that rimonabant treatment significantly attenuated responding during E2 [$F(1,80) = 12.22$; $p<0.01$], with no significant effect of time or interaction. Planned comparisons post test revealed a significant effect of rimonabant on trial 1 of E2 ($p<0.05$). Following a priming dose of cocaine (20 mg/kg IP), neither vehicle- nor rimonabant-treated mice reinstated the previously extinguished cocaine self-administration.

Two-tailed Student's t-tests revealed that rimonabant significantly decreased the number of trials required to meet extinction criterion during the initial extinction session in mice trained to respond for corn oil and during the spontaneous recovery session in mice trained to respond for cocaine (Figure 3A and 3B insets). Rimonabant also significantly decreased the number of trials required to meet extinction criterion during E3–E6 in mice trained to respond for cocaine (data not shown).

### 3.4. Experiment 4: Effect of rimonabant on response rate for water

Results from Experiment 4 demonstrated that rimonabant did not affect response rate for water in an operant task. Repeated measures ANOVA revealed that pretreatment with rimonabant did not significantly decrease response rate at any dose tested [$F_{(5, 46)} = 1.995$, ns] (data not shown). The average raw response rates (in responses/second ± SEM) for water were as follows: 0.84 ± 0.09, 0.76 ± 0.08, 0.70 ± 0.09, 0.64 ± 0.11, 0.62 ± 0.14, 0.42 ± 0.16 for 0.0–30.0 mg/kg rimonabant respectively.

## 4. Discussion

Extinction of operant learning reflects the decline in a conditioned response (e.g. lever press, nose poke) following the removal of the reinforcer (e.g. delivery of food or drug). Several behavioral phenomena related to context and/or cue reactivity can arise during extinction learning and these may predispose an organism to relapse to reinforcer-seeking. These include both cue- and prime-induced reinstatement, initial extinction burst responding in the immediate absence of the reinforcer, and spontaneous recovery of responding that occurs when the context and/or cues are presented after time has passed following extinction learning. The present results from **Experiment 1** are the first to demonstrate that the CB1 receptor antagonist rimonabant significantly decreases cue-induced reinstatement of cocaine seeking following extinction of this behavior in C57Bl/6 mice, supporting reports that CB1 receptor antagonism can attenuate reinstatement of cocaine seeking induced by re-exposure to cocaine-associated cues in rats (DeVries et al 2001; Filip et al 2006).

This attenuation of cue-induced responding also extended to initial burst responding and spontaneous recovery of cocaine seeking during extinction of cocaine self-administration, as well as initial burst responding, spontaneous recovery, and prime-induced reinstatement during extinction of palatable food self-administration **(Experiments 2 and 3)**. Moreover, mice treated with rimonabant during extinction of cocaine self-administration took significantly fewer daily sessions to reach extinction criterion than vehicle-treated mice, and rimonabant treatment also significantly decreased the number of discrete trials within a session to reach extinction criterion in mice trained to self-administer cocaine as well as corn oil. The attenuation of responding produced by rimonabant during extinction is not likely due to a general decrease in locomotor behavior or a non-specific suppression of high rate-responding, since rimonabant did not decrease responding in water-restricted mice responding for water under an FR5 schedule of reinforcement in **Experiment 4**. Taken together, these data suggest that in addition to attenuating the primary reinforcing effects of both palatable foods and drugs of abuse, CB1 receptor antagonism can attenuate context- and cue-induced reward seeking during extinction learning and potentially enhance extinction learning in this way. As mentioned in the introduction, although rimonabant can be classified as a CB1 antagonist, it produces inverse agonist effects both biochemically and behaviorally (see Bergman et al 2008 for review). The present results support the notion that rimonabant acts as an inverse agonist, in that it produced behavioral effects not only in the absence of a CB1 receptor agonist, but in the absence of a drug or food reinforcer. It remains to be clarified whether the suppression of food and drug-seeking produced by rimonabant and other CB1 antagonists/inverse agonists results from inhibition of constitutively active CB1 receptors versus a dampening of enhanced eCB activity.

As we reported in Ward et al (2007), extinction of both corn oil and Ensure self-administration produced an initial burst of responding, and the present results demonstrate that extinction of cocaine self-administration did as well. Results from **Experiment 2** demonstrate that treatment with rimonabant prior to the first extinction session (E1) significantly attenuated the initial burst in responding seen in all groups. As seen in Ward et al (2007), extinction of corn oil self-administration again elicited a more robust extinction burst than extinction of Ensure, and

although bursting was seen during extinction of cocaine self-administration, this effect is smaller still than those seen for extinction of the palatable foods. These differences in the magnitude of the extinction bursts may be due to initial differences in baseline rates of responding across reinforcers, and it is possible that upon examination of a wider range of cocaine doses more robust extinction burst responding would be seen. CB1 antagonism with rimonabant also significantly decreased the number of daily extinction sessions required to reach the extinction criterion in the cocaine self-administration mice, while rimonabant treatment had no effect on extinction rate in the corn oil- or Ensure- trained mice.

Results from **Experiment 3** further demonstrated that CB1 receptor antagonism blunts the expression of behaviors elicited by context and/or cues under extinction conditions that can provide mechanisms of relapse. Rimonabant attenuated burst behavior in the beginning trials of E1 in both the corn oil and cocaine groups; however this effect was only statistically significant in the corn oil group. These results were also the first to show that rimonabant attenuates subsequent spontaneous recovery of extinguished responding, and the first to show an attenuation of prime-induced reinstatement of palatable food seeking by rimonabant. Lastly, a cocaine prime did not reinstate cocaine-seeking in mice in this study and other pilot studies in our laboratory, replicating the reported failures of several cocaine doses to reinstate extinguished responding in C57Bl/6 mice (Fuchs et al 2003) and 129X1/SvJ mice. More recently, however, Soria et al (2008) reported reinstatement of cocaine seeking in CD1 outbred mice. Lastly, rate of extinction was significantly accelerated by rimonabant in the present study, in that fewer trials were required to reach extinction criterion. Taken together, these results are the first to demonstrate that blockade of CB1 receptors can facilitate extinction learning by 1) suppressing relapse behaviors throughout extinction and 2) decreasing in the number of sessions and/or trials required to reach extinction criterion.

These results add to a complex developing story regarding the seemingly incongruous involvement of CB1 receptors in modulating 1) new learning versus extinction of learned behaviors and 2) the motivational nature of the behaviors to be extinguished. It is well established that CB1 receptor agonists impair working and short term memory in humans and in animal models, an effect that likely results from interference with encoding processes by the hippocampus (Wise et al 2009). Extinction reflects a form of active learning, and in the case the extinction learning the eCB system facilitates extinction of conditioned fear (Pamplona et al 2006). The neuroanatomy and neurochemistry involved in extinction of conditioned fear is well-characterized and in many cases overlaps with that involved in initial acquisition of the task. However, certain differences between these pathways might stem from their excitatory vs inhibitory effects upon these two forms of learning (see Myers and Davis 2007 for review), and the eCB system may be an example of this. By contrast, CB1 antagonism can enhance cognition (Lichtman, 2000; Wolff and Leander, 2003), and the present results suggest that antagonism of the eCB system can also facilitate extinction learning in an appetitively-motivated task (also see Niyuhire et al 2007). Relative to fear extinction, there are few studies on the neural mechanisms of appetitive extinction; however the few studies have shown that appetitive extinction involves the basolateral amygdala (Weiskrantz 1956; McLaughlin and Floresco, 2007) and prefrontal cortex (Butter et al., 1963; Rhodes and Killcross 2004), two regions involved in fear extinction. However, the present data strongly suggest differences in the role of CB1 receptors in the modulation of appetitive versus fear extinction. An alternate interpretation of this apparent opposite role in appetitive versus fear extinction may be that SR141716 is not having an effect on extinction learning *per se*, but is attenuating behavior during extinction learning solely based on its ability to suppress motivated reinforcer-seeking behavior generally. This blunting of motivation may reduce interference between the initial conditioned behavior with the new association being acquired, leading to a facilitation of extinction learning.

In summary, the present results support other findings from rat studies that while the CB1 receptor system may not regulate the primary reinforcing properties of cocaine, CB1 receptor antagonism significantly decreases reinstatement of cocaine seeking, and these findings add to the growing theory that eCBs play a general role in modulating cue reactivity or conditioned reinforcement following extinction of both drug and natural reinforcer seeking behavior. These data are also the first to demonstrate that the CB1 receptor antagonist rimonabant attenuates additional behavioral phenomena arising during extinction of both palatable food and cocaine seeking, including burst responding, spontaneous recovery, and prime-induced reinstatement, and can facilitate extinction of both learned behaviors. These findings are in contrast to the apparent role of the eCB system in extinction of aversive memories. Further research is warranted to explore whether and how modulators of the CB1 system may show promise as novel pharmacotherapies to treat both uncontrollable eating and drug addiction.

## References


Anggadiredja K, Nakamichi M, Hiranita T, Tanaka H, Shoyama Y, Watanabe S, Yamamoto T. Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. Neuropsychopharmacology 2004;29:1470–1478. [PubMed: 15085091]

Arnone M, Maruani J, Chaperon F, Thiébot MH, Poncelet M, Soubrié P, Le Fur G. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology (Berl) 1997;132:104–106. [PubMed: 9272766]

Bergman J, Delatte MS, Paronis CA, Vemuri K, Thakur GA, Makriyannis A. Some effects of CB1 antagonists with inverse agonist and neutral biochemical properties. Physiol Behav 2008;93:666–670. [PubMed: 18076956]

Bray GA, Greenway FL. Pharmacological treatment of the overweight patient. Pharmacol Rev 2007;59:151–184. [PubMed: 17540905]

Bouton ME. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol Psychiatry 2002;52:976–986. [PubMed: 12437938]

Butter CM, Mishkin M, Rosvold HE. Conditioning and extinction of a food-rewarded response after selective ablations of frontal cortex in rhesus monkeys. Exp Neurol 1963;7:65–75. [PubMed: 14017412]

Caine SB, Negus SS, Mello NK. Method for training operant responding and evaluating cocaine self-administration behavior in mutant mice. Psychopharmacology (Berl) 1999;147:22–24. [PubMed: 10591861]

Childress AR, Hole AV, Ehrman RN, Robbins SJ, McLellan AT, O'Brien CP. Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res Monogr 1993;28:613–630.

Cippitelli A, Bilbao A, Hansson AC, del Arco I, Sommer W, Heilig M, Massi M, Bermúdez-Silva FJ, Navarro M, Ciccocioppo R, de Fonseca FR. The European TARGALC Consortium. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. Eur J Neurosci 2005;21:2243–2251. [PubMed: 15869521]

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P. SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol 2002;13:451–463. [PubMed: 12394421]

De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J, Vanderschuren LJ, Schoffelmeer AN. A cannabinoid mechanism in relapse to cocaine seeking. Nat Med 2001;7:1151–1154. [PubMed: 11590440]

De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer AN. Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology 2003;168:164–169. [PubMed: 12669182]

De Vries TJ, Schoffelmeer AN. Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci 2005;26:420–426. [PubMed: 15992935]

Filip M, Go da A, Zaniewska M, McCreary AC, Nowak E, Kolasiewicz W, Przegali ski E. Involvement of cannabinoid CB1 receptors in drug addiction: effects of rimonabant on behavioral responses induced by cocaine. Pharmacol Rep 2006;58:806–819. [PubMed: 17220538]

Fuchs RA, See RE, Middaugh LD. Conditioned stimulus-induced reinstatement of extinguished cocaine seeking in C57BL/6 mice: a mouse model of drug relapse. Brain Res 2003;973:99–106. [PubMed: 12729958]

Harloe JP, Thorpe AJ, Lichtman AH. Differential endocannabinoids regulation of extinction by in appetitive versus aversive Barnes maze tasks. Learn Mem 2008;15:806–809. [PubMed: 18957525]

Higgs S, Williams CM, Kirkham TC. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. Psychopharmacology (Berl) 2003;165:370–377. [PubMed: 12447606]

Hölter SM, Kallnik M, Wurst W, Marsicano G, Lutz B, Wotjak CT. Cannabinoid CB1 receptor is dispensable for memory extinction in an appetitively-motivated learning task. Eur J Pharmacol 2005;510:69–74. [PubMed: 15740726]

Jansen A, Broekmate J, Heymans M. Cue-exposure vs self-control in the treatment of binge eating: a pilot study. Behav Res Ther 1992;30:235–241. [PubMed: 1586360]

Katz JL, Higgins ST. The validity of the reinstatement model of craving and relapse to drug use. Psychopharmacology (Berl) 2003;168:21–30. [PubMed: 12695875]

Lerman DC, Iwata BA. Prevalence of the extinction burst and its attenuation during treatment. J Appl Behav Anal 1995;28:93–94. [PubMed: 16795857]

Lichtman AH. SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. Eur J Pharmacol 2000;15:175–179. [PubMed: 10980277]

Lutz B. The endocannabinoid system and extinction learning. Mol Neurobiol 2007;36:92–101. [PubMed: 17952654]

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530–534. [PubMed: 12152079]

McLaughlin RJ, Floresco SB. The role of different subregions of the basolateral amygdala in cue-induced reinstatement and extinction of food-seeking behavior. Neuroscience 2007;146(4):1484–1494. [PubMed: 17449185]

Myers KM, Davis M. Mechanisms of fear extinction. Mol Psychiatry 2007;12:120–150. [PubMed: 17160066]

National Research Council of the National Academies. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. The National Academies Press; Washington DC: 2003.

Niyuhire F, Varvel SA, Thorpe AJ, Stokes RJ, Wiley JL, Lichtman AH. The disruptive effects of the CB (1) receptor antagonist Rimonabant on extinction learning in mice are task-specific. Psychopharmacology 2007;191:223–231. [PubMed: 17211653]

O'Brien CP, Childress AR, Arndt IO, McLellan AT, Woody GE, Maany I. Pharmacological and behavioral treatments of cocaine dependence: controlled studies. J Clin Psychiatry 1988;49(Suppl): 17–22.

O'Brien CP, Childress AR, McLellan T, Ehrman R. Integrating systemic cue exposure with standard treatment in recovering drug dependent patients. Addict Behav 1990;15:355–365. [PubMed: 2248109]

Pamplona FA, Prediger RD, Pandolfo P, Takahashi RN. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. Psychopharmacology (Berl) 2006;188:641–649. [PubMed: 16947018]

Rhodes SEV, Killcross S. Lesions of rat infralimbic cortex enhance recovery and reinstatement of an appetitive Pavlovian response. Learn Mem 2004;11:611–616. [PubMed: 15466316]

Solinas M, Panlilio LV, Antoniou K, Pappas LA. Goldberg SR. 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 2003;306:93–102. [PubMed: 12660305]

Soria G, Mendizábal V, Touriño C, Robledo P, Ledent C, Parmentier M, Maldonado R, Valverde O. Lack of CB1 cannabinoid receptor impairs cocaine self-administration. Neuropsychopharmacology 2005;30:1670–1680. [PubMed: 15742004]

Soria G, Barbano MF, Maldonado R, Valverde O. A reliable method to study cue-, priming-, and stress-induced reinstatement of cocaine self-administration in mice. Psychopharmacology (Berl) 2008;199:593–603. [PubMed: 18488200]

Substance Abuse and Mental Health Services Administration National Survey on Drug Use and Health 2007. [Accessed on May 9, 2009]. http://www.oas.samhsa.gov/NSDUH/2k7NSDUH/2k7results.cfm

Thornton-Jones ZD, Vickers SP, Clifton PG. The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. Psychopharmacology (Berl) 2005;179:452–460. [PubMed: 15821957]

Varvel SA, Anum EA, Lichtman AH. Disruption of CB(1) receptor signaling impairs extinction of spatial memory in mice. Psychopharmacology 2005;179:863–872. [PubMed: 15619104]

Ward SJ, Dykstra LA. The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). Behav Pharmacol 2005;16:381–388. [PubMed: 16148442]

Ward SJ, Walker EA, Dykstra LA. Effect of cannabinoid CB1 receptor antagonist SR141716A and CB1 receptor knockout on cue-induced reinstatement of Ensure and corn-oil seeking in mice. Neuropsychopharmacology 2007;32:2592–2600. [PubMed: 17392737]

Ward SJ, Lefever TW, Jackson C, Tallarida RJ, Walker EA. Effects of a Cannabinoid1 receptor antagonist and Serotonin2C receptor agonist alone and in combination on motivation for palatable food: a dose-addition analysis study in mice. J Pharmacol Exp Ther 2008;325:567–576. [PubMed: 18256173]

Weiskrantz L. Behavioral changes associated with ablation of the amygdaloid complex in monkeys. J Comp Physiol Psychol 1956;49:381–391. [PubMed: 13345917]

Wise LE, Thorpe AJ, Lichtman AH. Hippocampal CB(1) Receptors Mediate the Memory Impairing Effects of Delta(9)-Tetrahydrocannabinol. Neuropsychopharmacology. 2009March 25 Epub ahead of print

Wolff MC, Leander JD. SR141716A, a cannabinoid CB1 receptor antagonist, improves memory in a delayed radial maze task. Eur J Pharmacol 2003;477:213–217. [PubMed: 14522359]

Xi ZX, Spiller K, Pak AC, Gilbert J, Dillon C, Li X, Peng XQ, Gardner EL. Cannabinoid CB1 receptor antagonists attenuate cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. Neuropsychopharmacology 2008;33:1735–1745. [PubMed: 17728698]

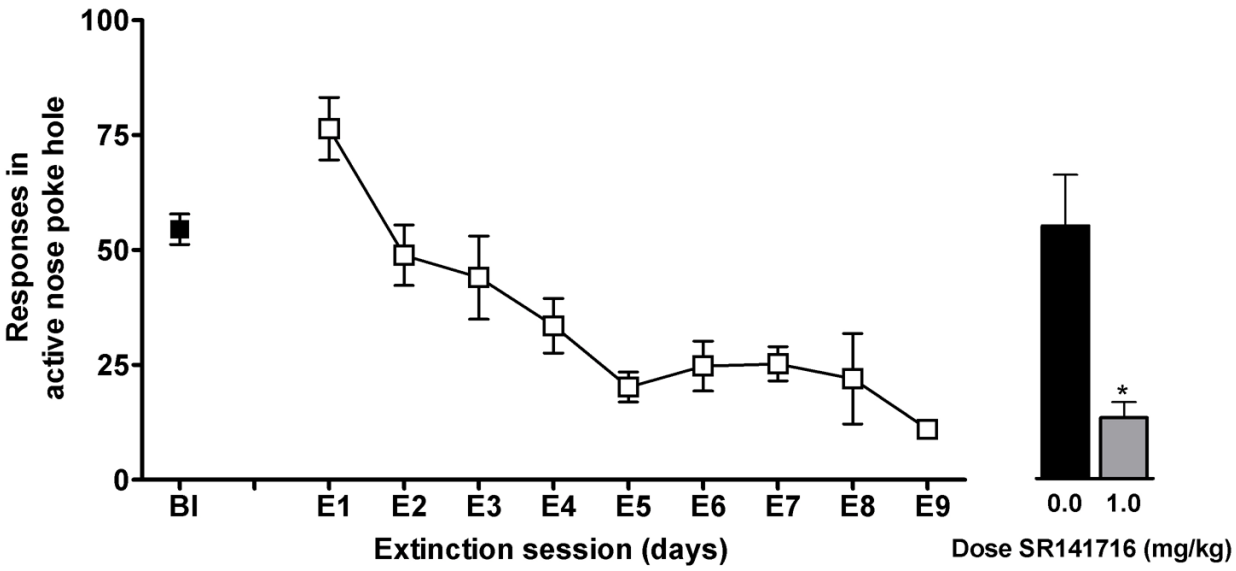


**Figure 1. Rimonabant attenuates cue-induced reinstatement of cocaine seeking in C57Bl/6 mice (Experiment 1)**

The line graph represents mean number of responses in the active nose-poke hole under baseline conditions (■) and during daily extinction sessions (□) in mice trained to self-administer 0.3 mg/kg/inf cocaine (± SEM). The bar graph represents reinstatement of responding in the active nose-poke hole following reintroduction of cocaine-paired cues after administration of vehicle (solid bar, n=8) or 1.0 mg/kg SR147161 (open bar, n=6). Asterisk indicates significant effect of rimonabant on responding during reinstatement as compared to vehicle ($p<0.05$).

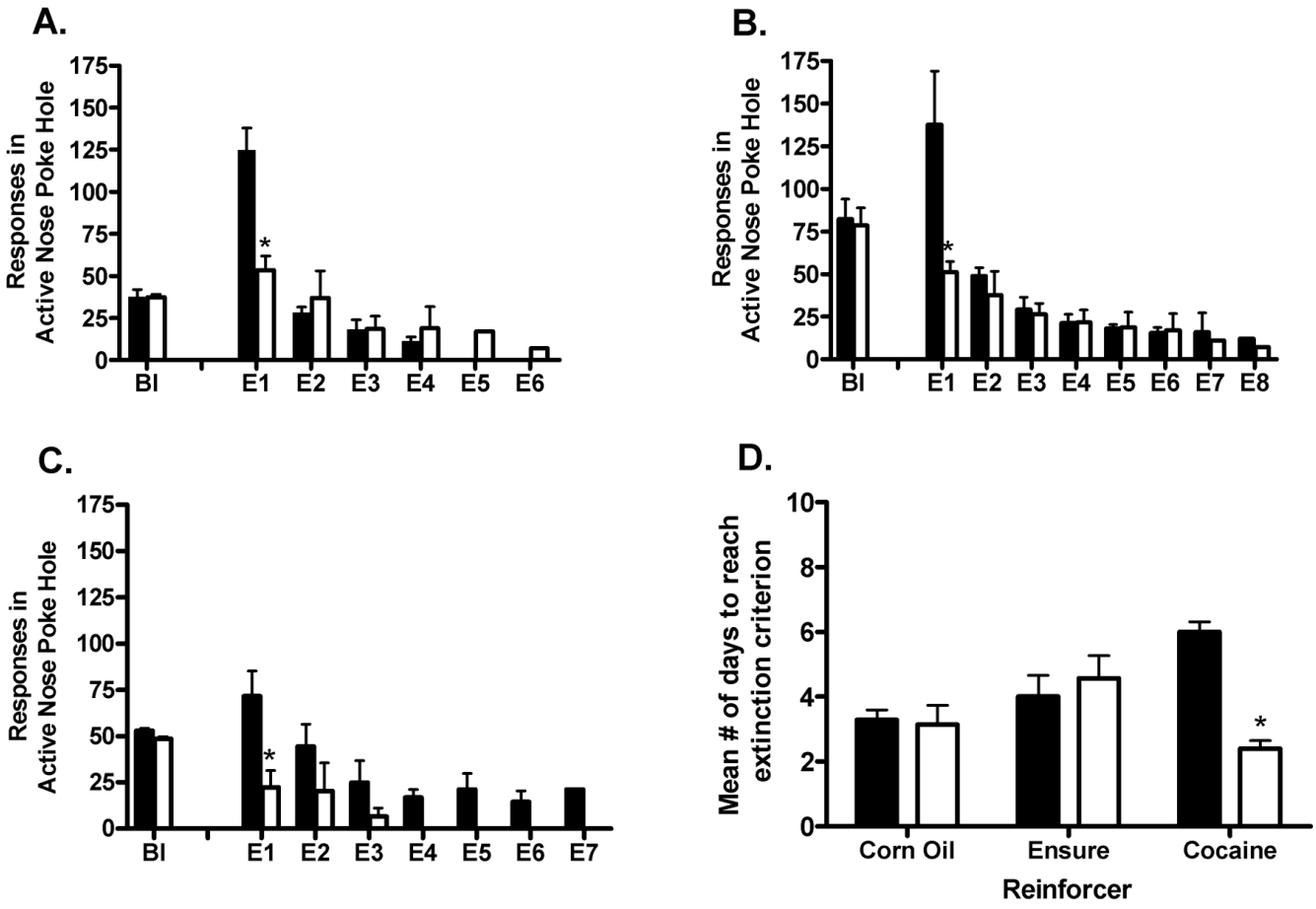


**Figure 2. Rimonabant attenuates extinction burst responding in mice trained to self-administer 32% corn oil, 100% Ensure, and 0.3 mg/kg/inf cocaine (Experiment 2)**

**Figure 2A** illustrates the effect of vehicle (n=7) or rimonabant (n=7) on extinction of 32% corn oil self-administration. **Figure 2B** illustrates the effect of vehicle (n=7) or rimonabant (n=7) on extinction of 100% Ensure self-administration. **Figure 2C** illustrates the effect of vehicle (n=6) or rimonabant (n=6) on extinction of 0.3 mg/kg cocaine self-administration. Solid bars represent mean number of responses in the active nose-poke hole under baseline conditions and during vehicle-pretreated extinction sessions. Open bars represent mean number of responses in the active nose-poke hole under baseline conditions and during rimonabant-pretreated extinction sessions. In **figure 2D**, solid bars represent mean number of extinction sessions required to meet extinction criterion in vehicle-pretreated mice. Open bars represent mean number of extinction sessions required to meet extinction criterion in rimonabant-pretreated mice. Error bars represent standard error of mean. Asterisks indicate significant effect of rimonabant on responding during extinction as compared to vehicle ($p<0.05$).

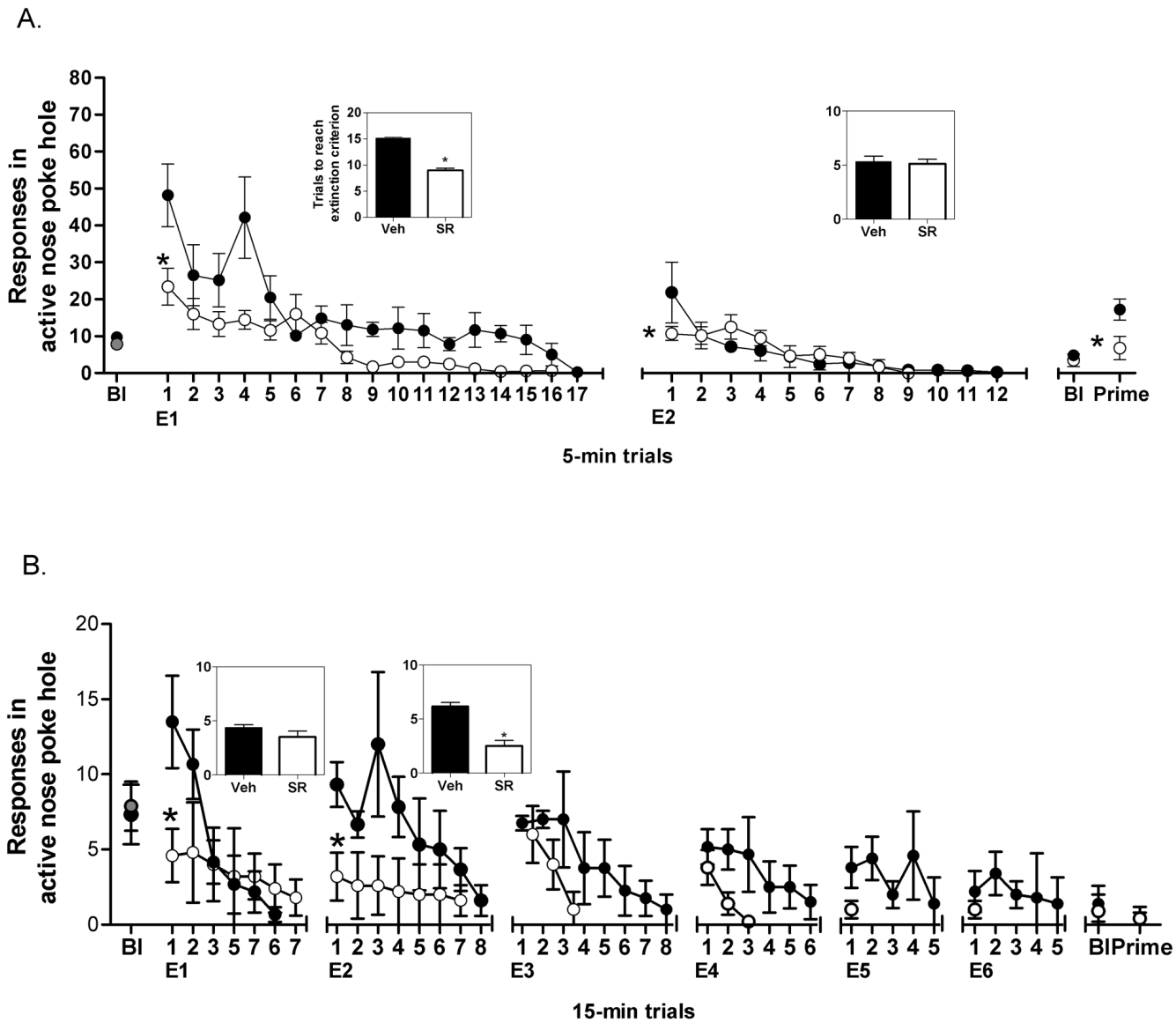


**Figure 3. Rimonabant attenuates context and extinction phenomena associated with relapse in mice trained to self-administer 32% corn oil or 0.3 mg/kg/inf cocaine (Experiment 3)**

The line graphs represent mean number of responses in the active nose-poke hole per discrete trial for 32% corn oil (**figure 3A**) or 0.3 mg/kg/inf cocaine (**figure 3B**) on the last day of baseline self-administration and during daily extinction sessions. The baseline data point represents the mean number of responding during the first discrete trial of the final baseline session. Mice were pretreated with vehicle (●, n=7 for corn oil, n=6 for cocaine) or rimonabant (○, n=7 for corn oil, n=6 for cocaine) 15 min prior to each extinction session. **Figure 3A and 3B insets illustrate the effect of rimonabant on rate of extinction during extinction sessions 1 and 2.** Bars represent the mean number of discrete trials required to meet extinction criterion in vehicle versus rimonabant pretreated mice. Error bars represent standard error of mean. Asterisks indicate significant effect of rimonabant on responding during extinction as compared to vehicle ($p<0.05$).