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Context-Specific Reversal of Cocaine Sensitization by the CB_1 Cannabinoid Receptor Antagonist Rimonabant

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The CB_1 cannabinoid receptor is implicated in the rewarding properties of many drugs of abuse, including cocaine. While CB_1 receptor involvement in the acute rewarding properties of cocaine is controversial, CB_1 antagonists such as SR141716 (rimonabant) have clearly been found to prevent cue- and cocaine-elicited reinstatement of cocaine self-administration in rodents. Here we demonstrate the novel involvement of CB_1 receptors in the maintenance of behavioral sensitization to cocaine in C57BL/6 mice. Consistent with previous reports, the induction of locomotor sensitization following repeated daily cocaine was not prevented by systemic pretreatment of either rimonabant, Δ^9-tetrahydrocannabinol (THC), or a 1:1 mixture of THC and cannabidiol (CBD). In contrast, established cocaine sensitization was markedly disrupted following subchronic treatment with rimonabant alone. This effect was notably context-dependent, in that rimonabant did not diminish established cocaine sensitization if delivered in the home cage, but only if the rimonabant-injected mice were exposed to activity chambers previously paired with cocaine. These findings are consistent with CB_1 receptor involvement in conditioned cocaine-seeking behaviors, and further suggest that endocannabinoid (eCB)-mediated synaptic plasticity may act specifically within drug-paired environments to maintain cocaine-directed behavioral responses.
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INTRODUCTION

Repetitive use of psychostimulant drugs promotes processes of neural plasticity that are both long lasting and thought to underlie the development of compulsive drug taking and addiction (Everitt and Robbins, 2005; Vanderschuren and Everitt, 2005; Gerdeman *et al*, 2003; Wolf *et al*, 2004). In rodents, subchronic treatment with cocaine or amphetamine leads to a behavioral sensitization to the acute psychomotor activating effects of the drug, such that locomotor responses to a drug challenge remain elevated even following extended periods of abstinence (Vanderschuren and Kalivas, 2000; Wolf *et al*, 2004). Importantly, this model poses that the readily observable locomotor hyperactivation following repeated psychostimulant exposure is paralleled by a sensitization of the incentive salience or motivational properties of the drug (Robinson and Berridge, 1993). Cocaine-induced adaptations within cortical-basal ganglia-midbrain circuits increase both the motor response to subsequent cocaine exposures, and the propensity for drug-seeking behaviors such as those measured by cocaine self-administration (Everitt and Robbins, 2005). In humans, cocaine addiction is a persistent and chronically relapsing condition, making long-term abstinence difficult to achieve. Identifying neural mechanisms related to craving and relapse may therefore be critical to developing effective therapeutic approaches.

The neuroanatomical and physiological substrates of behavioral sensitization have been the subject of extensive research. Cocaine sensitization involves multiple brain areas related to drug reward and addiction, especially the ventral tegmental area (VTA) and the nucleus accumbens (NAc/ventral striatum; Vanderschuren and Kalivas, 2000). These interconnected nuclei are distinctly important during progressive stages of sensitization to chronic cocaine. Behavioral sensitization can be functionally dissociated into discrete phases of induction and expression, or maintenance. There is substantial evidence that neuronal activity and plasticity within the VTA is critical for the *induction* of sensitization during repeated cocaine exposures, whereas the NAc mediates the lasting *maintenance* of sensitization once it has been established (Vanderschuren and Kalivas, 2000; Kalivas *et al*, 2005).

In particular, among the enduring neuronal changes responsible for cocaine sensitization, persistent alterations in glutamate signaling within the VTA and NAc play a primary role (Kalivas *et al*, 2005; Gerdeman *et al*, 2003; Wolf *et al*, 2004; Everitt and Robbins, 2005). In NAc, chronic cocaine has been associated with changes in glutamate

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transport (Kalivas *et al*, 2005), postsynaptic glutamate receptor function (Boudreau and Wolf, 2005; Wolf *et al*, 2004), and alterations in the density of dendritic spines receiving excitatory synaptic contacts (Ferrario *et al*, 2005). Behavioral sensitization to cocaine or amphetamine has been correlated to the expression and occlusion of long-term depression (LTD) of excitatory synaptic transmission in the NAc (Thomas *et al*, 2001; Brebner *et al*, 2005). It was also recently reported that excitatory neurotransmission was reduced (Schramm-Sapyta *et al*, 2006) and LTD was abolished (Martin *et al*, 2006) in the NAc after animals were trained to self-administer cocaine, but not following noncontingent cocaine administration.

In the present study, we focused on the endocannabinoid (eCB) system and its potential role in modulating behavioral sensitization to cocaine. Activation of presynaptic CB_1 cannabinoid receptors inhibits corticostriatal glutamate release in both the ventral (Robbe *et al*, 2001) and dorsal striatum (Gerdeman and Lovinger, 2001), where the activity-evoked release of eCBs from striatal neurons serves as a retrograde signal mediating presynaptic forms of LTD (Robbe *et al*, 2002b; Gerdeman *et al*, 2002; Kreitzer and Malenka, 2005). Recent studies have shown that eCB-mediated LTD in NAc is altered by *in vivo* exposure to cocaine (Fourgeaud *et al*, 2004). The eCB system may therefore underlie some behavioral adaptations to cocaine mediated by this brain area, such as the maintenance phase of behavioral sensitization (Vanderschuren and Kalivas, 2000) or relapse (Kalivas *et al*, 2005).

Several lines of evidence have implicated the eCB system in behavioral responses to drugs of abuse, especially in relation to conditioned drug seeking and relapse (De Vries and Schoffelmeer, 2005; Maldonado *et al*, 2006). Regarding psychostimulants, however, studies of CB_1 receptor involvement in self-administration or conditioned place preference (CPP) paradigms have been inconsistent (for review, see Maldonado *et al*, 2006; Arnold, 2005). Several studies have found that pharmacological blockade or genetic deletion of the CB_1 receptor fails to influence cocaine-induced efflux of NAc dopamine (Soria *et al*, 2005; Caille and Parsons, 2006; Houchi *et al*, 2005), suggesting a lack of effect on acute cocaine reward (but see Vlachou *et al*, 2003; Cheer *et al*, 2007). Locomotor sensitization to cocaine is also reportedly independent of CB_1 receptor activation (Martin *et al*, 2000; Lesscher *et al*, 2005), yet these studies have primarily focused only on the induction of behavioral sensitization, which may be more directly related to actions on dopamine release from VTA neurons. In contrast, pharmacological blockade of CB_1 receptors prevents cue-induced reinstatement of cocaine (De Vries *et al*, 2001; Xi *et al*, 2006), nicotine (De Vries *et al*, 2005; Cohen *et al*, 2005) or methamphetamine (Anggadiredja *et al*, 2004) self-administration behavior, indicating a role for eCBs in mediating recall of learned stimulant-related information (De Vries and Schoffelmeer, 2005).

We therefore revisited the relationship between CB_1 receptor signaling and behavioral sensitization to cocaine—investigating both the induction and maintenance phases—using a mouse strain and cocaine treatment schedule that was previously correlated to alterations in LTD induction within the NAc (Thomas *et al*, 2001). We utilized systemic injections of the selective CB_1 receptor antagonist rimonabant, at a concentration that is maximally effective at suppressing relapse to cocaine seeking (De Vries and Schoffelmeer, 2005).

A subset of experiments also investigated effects of THC and CBD, two primary phytocannabinoid constituents of cannabis (Pertwee, 2005). This was to test the related question of whether or not cannabinoid agonists can influence cocaine reward in animals. It is popularly debated whether or not chronic cannabis use in humans can promote a neurobiological vulnerability to other drugs of abuse (Kandel *et al*, 2006; Tarter *et al*, 2006). In laboratory animal models, experiments have tested the notion that CB_1 receptor agonists such as THC can influence self-administration (Panlilio *et al*, 2007), conditioned place preference (CPP; Parker *et al*, 2004) or cross-sensitization to psychostimulants (Arnold *et al*, 1998; Ellgren *et al*, 2004). These studies have largely found no evidence that previous exposure to cannabinoids can enhance the rewarding efficacy of cocaine (Arnold, 2005; Panlilio *et al*, 2007). However, THC has been found to disrupt synaptic LTD in the NAc (Hoffman *et al*, 2003; Mato *et al*, 2004), which may be important for the development of psychostimulant sensitization (Thomas *et al*, 2001; Gerdeman *et al*, 2003; Brebner *et al*, 2005). We, therefore, investigated the effects of THC—alone, or in combination with CBD — on the induction of cocaine sensitization under our experimental conditions.

We report that subchronic treatment with rimonabant blocked maintenance, but not the induction, of behavioral sensitization to cocaine. This effect was only observed, however, when delivery of the CB_1 receptor antagonist occurred within the context previously paired to induction of cocaine sensitization (the activity cage). By demonstrating that eCB signaling plays a distinctly context-specific role in maintaining cocaine sensitization, these results help to clarify previously discrepant findings, and lend further support to the particular involvement of CB_1 receptors in conditioned behavioral responses to psychostimulants.

MATERIALS AND METHODS

Subjects

Male C57BL/6 mice were obtained from Harlan (Indianapolis, IN, USA), weighing 20–26 g on arrival. Mice were housed two per cage in a temperature-controlled vivarium on a 12:12 h light/dark cycle, with lights on at 0800 hours. Throughout the experiments, subjects were allowed *ad libitum* access to food and water. Beginning 2–3 days after arrival, subjects were handled daily within a micro isolation hood located in the vivarium facility until the first experimental day (5–7 days after arrival). On each experimental day, mice were transported from the vivarium, in their home cages, to a temperature controlled, sound attenuated room, with standard fluorescent illumination and a house ventilation fan providing background white noise. All experiments were conducted between 1100 and 1600 hours. All procedures were in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee of the University of Arizona.

Drugs

Cocaine HCl (Sigma, St Louis, MO, USA) was dissolved in 0.9% sterile saline. THC (Sigma) was obtained as an ethanol

solution, dried under a stream of argon gas, and re-dissolved in a vehicle solution of DMSO (20% final volume), Tween 80 (10% final volume) and sterile saline (70% final volume). Vehicle solutions were added in the order listed, with vigorous vortexing between steps. We have previously characterized the use of this vehicle solution for the *in vivo* delivery of cannabinoid compounds (Wu and French, 2000). CBD (Sigma) and rimonabant (SR141716 from the NIDA Drug Supply Program, Baltimore, MD, USA) were obtained in powder form, and dissolved in a DMSO (20%), Tween 80 (10%), and physiological saline (70%) vehicle. All drug solutions were prepared fresh immediately prior to use and were administered via intraperitoneal (i.p.) injection.

Apparatus and Behavioral Measures

Horizontal locomotor activity was detected using a Digiscan Animal Activity Monitoring System (Columbus Instruments, Columbus, OH, USA), equipped with photosensor arrays (16×16 photocells) located 3 cm above a wire grid floor insert. The insert was stably situated, 3 cm above fresh bedding material (Sani-Chips, Harlan) spread over the floor of the activity cage prior to each experiment. Boxes and inserts were cleaned thoroughly between each experimental session. Activity was recorded using Digipro acquisition software and raw data files were exported to Microsoft Excel for analysis. Measures reported include horizontal activity (distance traveled, cm), time spent in center field (open field crossings, as a relative measure of anxiety), and behavioral stereotypies. Stereotypy counts were scored by the Digipro software each time an individual photobeam was repetitively interrupted. Total time spent engaging in such stereotyped beam breaks were also recorded. Area under the curve (AUC) was calculated for horizontal activity and stereotypy times with Flashcalc software (Dr Michael Ossipov, University of Arizona) using a trapezoid rule: $\mathrm{AUC} = \Sigma_2^1\ [(y_{i+1} + y_i)/(x_{i+1} - x_i)]$, where i represents each successive point until the next-to-last ($i+1$ being the last). This transform (to units of cm $\times$ min or sec $\times$ min) allows a reliable analysis of multiple samples (averaged time points from multiple subjects) over time. AUC was determined for the first 15 min of each session following cocaine injection. In similar mouse strains, cocaine sensitization is more pronounced (Michel *et al*, 2003) and persistent (Tirelli *et al*, 2005) during this early timeframe. An experimenter blinded to drug treatments conducted all data analysis.

Each experiment began with 2 days in which subjects were habituated to the testing room and activity cages. Mice were placed in the activity cages for 30 min each, and returned to home cages, remaining in the testing room for 30–60 min before and after behavioral sessions to minimize behavioral stresses related to transport. On the following day ('Day 1' in data figures; initial habituation days have been omitted for simplicity of presentation), mice were injected intraperitoneally with 0.1 ml sterile saline prior to behavioral sessions, to minimize the stress, and novelty of an intraperitoneal injection on subsequent days. Results did not vary on the basis of whether rimonabant-treated mice were housed together or with a vehicle-treated littermate. Throughout all experiments, subjects were returned to the same activity chamber matched to that particular animal.

Experiment 1: Effect of Rimonabant Pretreatment on Induction of Cocaine Sensitization

To carefully observe effects of rimonabant on the 5-day development of sensitized responses to cocaine, two different protocols were utilized within experiment 1. In both protocols, mice received cocaine (15 mg/kg, i.p.) once daily for 5 days, and were tested for the expression of behavioral sensitization with an identical challenge dose of cocaine (15 mg/kg, i.p.) on day 13. In the first protocol, mice always received a pretreatment of rimonabant (3.0 mg/kg, i.p.) or its vehicle, delivered 30 min prior to cocaine, with the exception of a control group of mice, which received only saline (0.1 ml, i.p.) on days 2–6, and were cocaine-naive until the challenge day 13 (Figure 1a). In protocol 2, subjects' initial exposure to cocaine (day 2) was without any pretreatment. Thus, mice within protocol 1 never received cocaine without a rimonabant (or vehicle) pretreatment until the first cocaine challenge on day 13. For mice in protocol 2, the cocaine challenge on day 13 was compared to a baseline initial cocaine exposure (day 2) that was uncomplicated by coincident blockade of CB_1 receptor signaling. Also, the negative control subjects in protocol 2 were injected with cocaine on days 2 and 13, but only with saline on days 3–6 (Figure 1b).

Experiment 2: Effect of Phytocannabinoid Pretreatment on Induction of Cocaine Sensitization

Mice in this experiment were divided into two groups, receiving a pretreatment of either THC (10 mg/kg, i.p., or its vehicle) or a 1:1 mixture of both THC and CBD (each at 10 mg/kg, i.p., or vehicle), 30 min prior to receiving cocaine (15 mg/kg, i.p.). The design was otherwise similar to protocol 2 of the rimonabant pretreatment experiment above. In order that cocaine sensitization could be compared to a naive cocaine-alone baseline, mice received only cocaine (15 mg/kg, i.p.) on days 2 and 13, while identical injections of cocaine on days 3–6 were preceded by 30 min with THC or THC + CBD pretreatment (or vehicle).

Experiment 3: Effect of Rimonabant on Maintenance of Cocaine Sensitization

Behavioral sensitization was induced using once-daily injections of cocaine (15 mg/kg, i.p.) for 5 consecutive days, as detailed above (see Figure 3a). Expression of cocaine sensitization was observed on day 13. Two mice were excluded from further testing, due to a failure to exhibit significant sensitization. Both were high-responders to cocaine at baseline day 2 (15-min locomotor activity was comparable to sensitized responding in other subjects within our experiments). Subsequently, subjects were left undisturbed in the vivarium for 5 days (days 14–18). On days 19–23, mice were transported to the testing room. Following an acclimation period (30–60 min), subjects were injected with either rimonabant (3 mg/kg, i.p.) or its vehicle. In one subset of experiments, mice were immediately placed in activity chambers for 1 h of locomotor testing. This was initially done to monitor the possibility of hyperlocomotor or sensitizing responses to rimonabant, which was never observed. In a second subset of experiments, subjects were

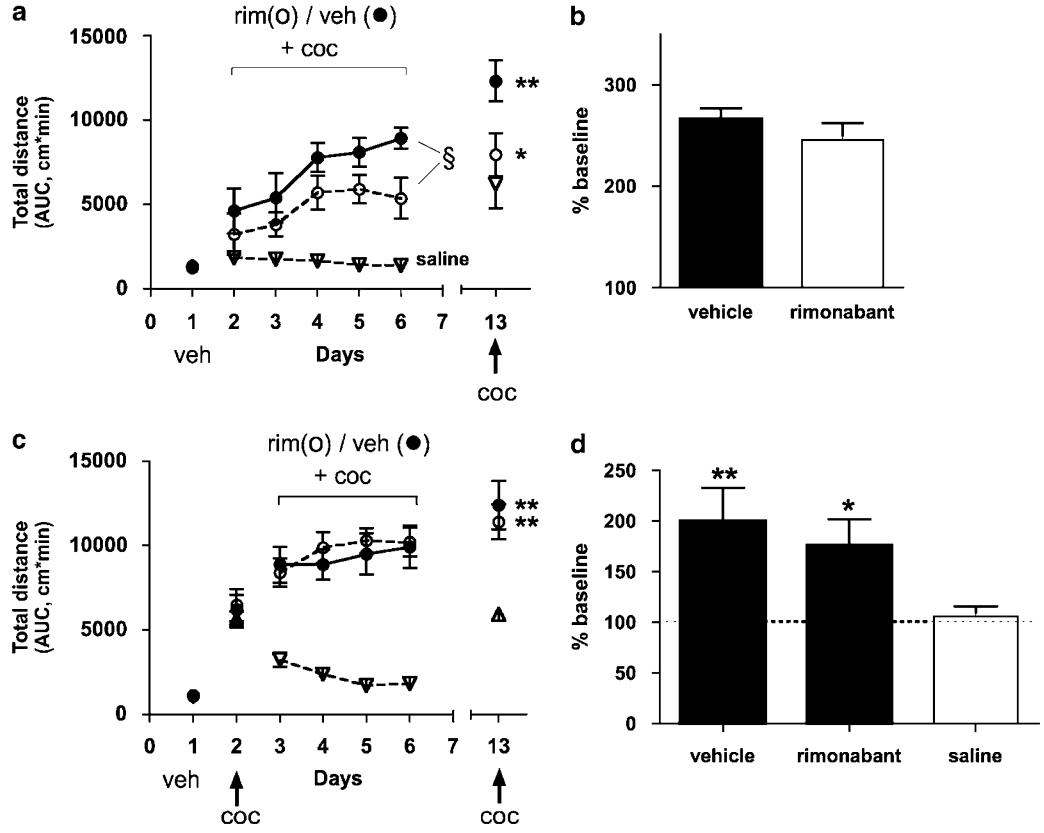

Figure 1 Rimonabant pretreatment has variable effects on cocaine-induced locomotion, but does not prevent behavioral sensitization. (a) When rimonabant (rim) was administered 30 min prior to cocaine (coc) on each of five daily injections (open circles), activating effects of cocaine were reduced compared to vehicle-treated controls (closed circles). $^{\S}p<0.01$ main effect of treatment, using 2-way ANOVA. All mice received coc alone on day 13. Behavioral sensitization to the coc challenge was reduced in rimonabant-pretreated mice ($p<0.05$ compared to vehicle group, unpaired *t*-test), but responses were still significantly enhanced relative to initial coc exposure (comparing activities on day 2 *vs* 13 using paired *t*-tests within each group: $^{*}p<0.05$, $^{**}p<0.01$, $n=10$ mice per group). Day 13 locomotion in rimonabant-pretreated animals was not significantly higher than acute coc effects in a separate group of saline-treated mice (inverted triangles, $n=8$). (b) Day 13 responses to coc in protocol 1, graphed as percent baseline (Day 2) activity. (c) Rimonabant pretreatment did not alter coc sensitization when administered only on days 3–6. Day 13 responses were significantly elevated in comparison to baseline (Day 2, using paired *t*-tests within each group: $^{**}p<0.01$, $n=10$ mice per group). A single coc injection on day 2 followed by subchronic saline (inverted triangles, $n=8$) was not sufficient to induce behavioral sensitization. (d) Day 13 responses to coc in protocol 2, graphed as percent baseline activity (Day 2). $^{*}p<0.05$, $^{**}p<0.01$ compared to saline group, unpaired *t*-test.

returned to their home cages directly following injection of rimonabant or its vehicle. In both cases, four replicate experiments were independently conducted, each with $n=4$ mice per group (rimonabant or vehicle, $n=16$ total per group). In the second subset, home cages remained in the testing room for $\geqslant 1$ h postinjection, and mice were frequently monitored (every 3–5 min, through a viewing window in the door to the activity room) for any aggressive, or otherwise unusual, behaviors between littermates. Occasional bouts of fighting were infrequent and not abnormal for the C57BL/6 mouse strain, and could not be attributed to either drug or vehicle treatment (in all, aggressive behaviors (chasing, biting) were observed 4 and 3 times in vehicle- and rimonabant-treated subjects, respectively). Mice were then returned to the vivarium where they remained undisturbed for another 5 days—until experimental day 28, when a challenge dose of cocaine (15 mg/kg, i.p.) was given to assess the maintenance of sensitized locomotor responding. During the challenge tests, subjects were observed frequently ($\leqslant$ every 2 min) for signs of behavioral stereotypies, following the general descriptions of (Michel and Tirelli, 2002): *eg*, orofacial stereotypies, sniffing, rearing, or grooming.

Statistical Analyses

Statistical tests reported are analyses of AUC values. When comparing the effects of various pretreatments on the time course of inducing cocaine sensitization, two-way ANOVA was performed using GraphPad Prism 4.0 for Macintosh (Graphpad Software; San Diego, CA, USA), with pretreatment and day as independent variables and day as a repeated measure. To analyze the levels of sensitized activity on cocaine challenge days, comparisons were made either between groups, or relative to the first day of cocaine exposure within a given group. Comparisons between treatment groups utilized unpaired *t*-tests. Within-groups comparisons employed paired *t*-tests so that each mouse was analyzed in comparison to its own first cocaine exposure. In experiment 1, locomotor activity on cocaine challenge days was also transformed to % baseline [= (day 13

AUC/day 2 AUC) × 100%]. In all cases, $p<0.05$ was the criterion for statistical significance.

RESULTS

Effect of Rimonabant on Induction of Cocaine Sensitization

Mice that were injected with rimonabant prior to every daily cocaine exposure exhibited an overall lower cocaine-induced hyperlocomotion than vehicle treated animals (Figure 1a). Two-way ANOVA revealed a significant main effect of Treatment over days 2–6 ($F_{(2,135)}=49.28$; $p<0.01$, $n=10$ per group). Rimonabant pretreatment was also associated with a significantly lower activity level following a challenge injection of cocaine on day 13 (7942 ± 1274 cm × min *vs* $12\,308\pm1207$ cm × min for the vehicle group; AUC measures ± SEM). However, when subjects' activity following the cocaine challenge (day 13) was compared to their own initial responding to cocaine (day 2) using paired *t*-tests within groups, a significant behavioral sensitization was seen in both groups ($p<0.05$ in rimonabant-treated group; $p<0.01$ in vehicle-treated group). When responses to cocaine on day 13 were analyzed as percent of baseline cocaine-induced activity (day 2), no difference was observed between groups (Figure 1b; $p>0.05$, unpaired *t*-test). Nonetheless, responses to cocaine on day 13 in the rimonabant-treated group were not significantly higher than cocaine-naive control mice that received only saline on days 2–6 (Figure 1a; AUC = 6208 ± 1435 cm × min; $p>0.05$, unpaired *t*-test). Thus sensitized responding in the rimonabant pretreatment group was not distinguishable from acute cocaine effects in the negative control (chronic saline) group, in apparent disagreement with previous findings (Lesscher *et al*, 2005), but consistent with another recent report (Corbillé *et al*, 2007).

In a second set of experiments, we more stringently tested a critical involvement of CB_1 receptors in cocaine sensitization by measuring an initial baseline response to cocaine alone. After experiencing cocaine (15 mg/kg) alone on day 2, mice received either rimonabant or vehicle pretreatment on the remaining 4 days of the sensitization protocol (Figure 1c). In this paradigm, subjects pretreated with rimonabant showed no differences in the induction of cocaine sensitization. Control subjects injected with saline on days 3–6 did not demonstrate any enhanced cocaine-induced activation on day 13 (AUC = 5933 ± 601 cm × min) compared to day 2 (AUC = 5618 ± 1333; $p>0.05$). Analyzed as percent of baseline (day 2), both rimonabant ($176\pm28\%$) and vehicle ($200\pm33\%$) pretreatment groups were significantly sensitized on day 13 relative to saline control ($106\pm10\%$), as shown in Figure 1d. A single injection of cocaine was therefore not capable of inducing lasting sensitization. Rather, repeated exposure during days 3–6 was necessary for cocaine sensitization, which was not influenced by rimonabant pretreatment. In summary, rimonabant pretreatment blunted the behavioral activation to cocaine in mice, including the magnitude of sensitization, but this effect was ablated by a single cocaine injection that was not paired to a pretreatment.

Effect of Phytocannabinoids on Induction of Cocaine Sensitization

After mice received an initial injection of cocaine alone, cocaine injections on the following 4 days were preceded by pretreatment with either the cannabinoid vehicle solution, THC or a combination of THC + CBD (10 mg/kg, i.p. in each case). Neither THC (Figure 2a) nor THC + CBD (Figure 2b) pretreatment had obvious effects on the outcome of cocaine sensitization. Subjects receiving either THC or vehicle pretreatments were sensitized to cocaine over a similar 5-day time course (two-way ANOVA revealed no main effect of pretreatment, $F_{(1,56)}=0.03$; $P=0.8$); hyperlocomotion elicited by a cocaine challenge on day 13 was likewise not different between groups, as determined also by unpaired *t*-test ($p>0.05$, $n=10$ per group). Responses of individual subjects to cocaine on day 13 were significantly greater in comparison to the initial cocaine exposure on day 2 ($p<0.05$; using paired *t*-tests within groups) in both the THC and vehicle treatment groups. Similar results were observed in the THC + CBD experiment. Mice pretreated with the combination of THC + CBD developed a behavioral

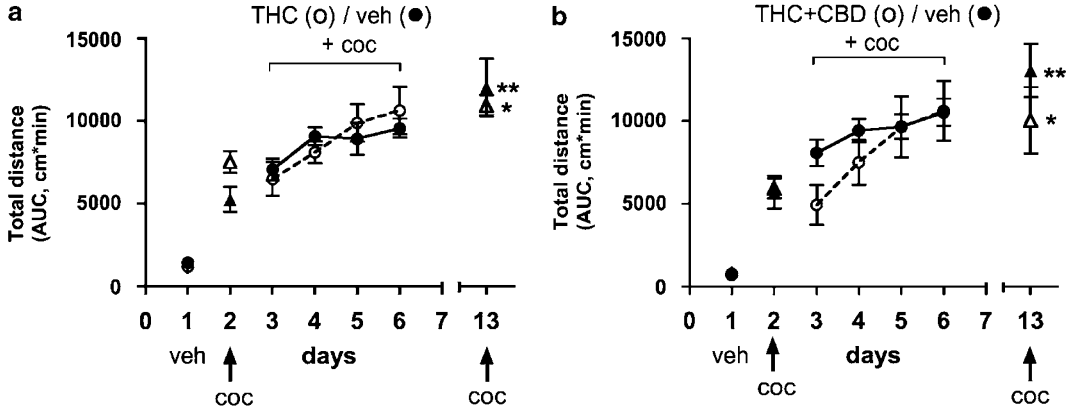

Figure 2 THC or THC + CBD pretreatment does not alter behavioral sensitization to repeated cocaine. (a) THC (open symbols) or vehicle (closed symbols) were injected 30 min prior to cocaine (coc) on days 3–6. Behavioral sensitization to the coc challenge (Day 13) was observed in both groups. (b) THC + CBD (open symbols) pretreatment did not prevent the induction of behavioral sensitization to a challenge dose of cocaine on day 13. Statistics are as in Figure 1c, $^{*}p<0.05$, $^{**}p<0.01$ compared to baseline within group (Day 2), $n=9$–10 mice per group.

sensitization to cocaine (Figure 2b; day 13 compared to day 2, $p<0.05$; paired *t*-test). Responses to cocaine challenge on day 13 were not different from the vehicle group ($p>0.05$; unpaired *t*-test, $n=9$–10 per group). Thus, behavioral sensitization was neither blocked nor enhanced by cannabinoid pretreatment.

Reversal of Cocaine Sensitization by Rimonabant

We next tested the hypothesis that CB_1 receptor signaling might play an important role in the maintenance of established behavioral sensitization to cocaine (Figure 3). In this experiment, behavioral sensitization was induced in all subjects by five daily injections of cocaine (sensitized responding was confirmed on day 13, and was significantly greater than initial cocaine exposures on day 2; $p<0.05$ using paired *t*-tests within subjects). Cocaine-sensitized mice were subsequently treated with a 5-day course of either rimonabant or its vehicle on days 19–23. Subjects were monitored in activity boxes for 1 h following each daily injection of the CB_1 receptor antagonist. No direct behavioral effects of rimonabant were observed; mice were neither hyperactive when compared to vehicle-injected controls, nor did rimonabant treatment elicit visually obvious signs of anxiety or distress, such as increased freezing, rearing or gnawing. This is consistent with previous studies that found similar doses of rimonabant to be nonaversive or anxiolytic (Chaperon *et al*, 1998; Haller *et al*, 2002). Accordingly, rimonabant treatment did not alter the occurrence of stereotyped behaviors during these 1 h sessions (number of stereotypies/min $=11.1\pm0.9$ and 10.5 ± 1.1 for rimonabant and vehicle, respectively, $p>0.05$), or open-field crossings (time spent in center arena $=11.9\pm4.9$ and 15.8 ± 6.6 s/min for rimonabant and vehicle treatment, respectively, $p>0.05$), which would have been indicative of stimulant or anxiogenic effects, respectively. Locomotion following injection with rimonabant or its vehicle was also not significantly greater than during habituation to activity boxes ($p>0.05$; unpaired *t*-tests, comparing to saline injection, day 1), indicating a lack of conditioned responding to the experimental context alone. However, when mice were treated with a final challenge dose of cocaine on day 28, those mice that had received subchronic rimonabant exhibited markedly reduced levels of locomotor activity. Day 28 responding to cocaine was significantly less in the rimonabant-treated group (AUC $=8467\pm900$ cm $\times$ min) in comparison to the vehicle-treated group (AUC $=12\,268\pm743$ cm $\times$ min; $p<0.01$; unpaired *t*-test). Indeed, comparing activities of individual subjects on day 28 to activities on day 2, the stimulus effects of cocaine on day 28 were no longer significantly different than on initial cocaine exposure ($P=0.1$; paired *t*-test). Thus, behavioral sensitization to cocaine was largely extinguished in mice that received repeated daily injections of rimonabant during the maintenance phase (Figure 3).

Subsequent experiments revealed that the reversal of cocaine sensitization by rimonabant was dependent upon repeated coupling of the CB_1 antagonist to the behavioral

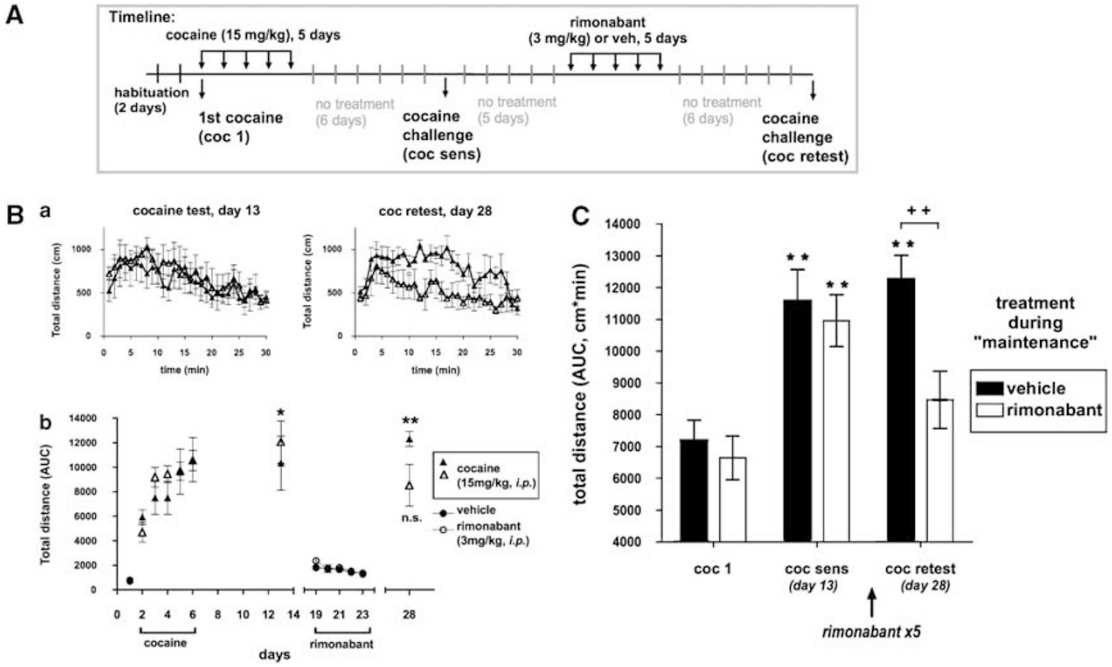

Figure 3 Behavioral sensitization to cocaine is disrupted by repeated rimonabant paired to activity cages. (A) Experimental timeline (see Materials and methods). (Ba) Cocaine-stimulated locomotor activity (total distance traveled, cm) is plotted for challenge days 13 and 28 in a single experiment ($n=4$ mice per group, representative of four replicate experiments run independently). (Bb) Locomotor activity (AUC calculated for first 15 min) is plotted for the entire experimental timeline in the same representative experiment (curves in panel a correspond to data points on days 13 and 28). All mice received single injections of cocaine (triangles) on days 2–6, day 13, and day 28. (*$p<0.05$; **$p<0.01$; NS $=p>0.05$ *vs* activity on day 2 using paired *t*-tests within groups, $n=4$). On days 19–23, mice received either vehicle (closed circles) or rimonabant (open circles), followed by activity monitoring in test cages. Closed and open triangles represent responses to cocaine in mice that received vehicle or rimonabant, respectively, on days 19–23. (C) Summary histogram for all mice tested in all four replicate experiments, $n=16$ mice per group. **$p<0.01$ *vs* activity on day 2 (coc 1), using paired *t*-tests within groups. $^{++}p<0.01$ between groups, using unpaired *t*-test.

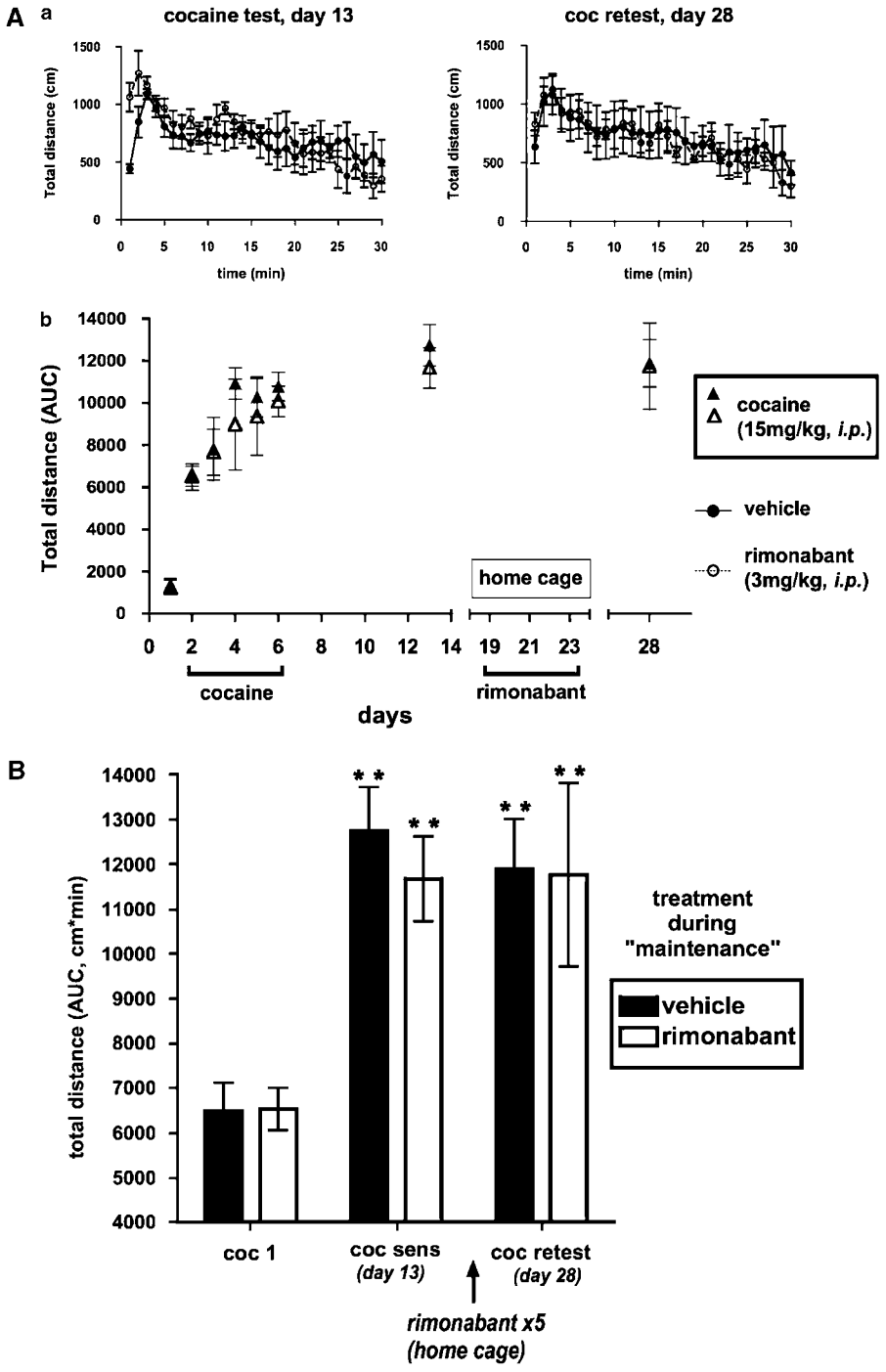

Figure 4 Cocaine sensitization is not disrupted by rimonabant administered repeatedly within the home cage. A(a, b) Activity measures are plotted for a single experiment ($n = 4$ mice per group), representative of four separately repeated experiments. The experiment is exactly as in Figure 3, except that mice received either rimonabant (open symbols) or its vehicle (closed symbols) on days 19–23 and were immediately returned to home cages located within the testing room. (B) Summary histogram for all mice tested, $n = 16$ per group. Statistics are as in Figure 3, $^{**}p < 0.01$ *vs* activity on day 2 (coc1).

context of the activity cages. First, we tested whether a single injection of rimonabant delivered on day 19 would reduce behavioral sensitization to a challenge dose of cocaine administered 6 days later (day 25). Mice receiving a single rimonabant treatment showed no reduction in the expression of cocaine sensitization on day 25 as compared to vehicle-injected controls (AUC = 10 065 ± 1703 cm × min for rimonabant group and 11 644 ± 1926 cm × min for vehicle group; $P = 0.56$, $n = 4$ each, data not shown). This suggested that the maintenance of behavioral sensitization to cocaine is not subject to rapid disruption by blockade of CB_1 receptors, consistent with previous results (Lesscher *et al*, 2005).

Rather, we hypothesized a role for CB_1 receptors in maintaining cocaine sensitization based on a conditioned elevation of eCB signaling during re-exposure to the

cocaine-paired environment. To test this hypothesis, we performed a series of experiments identical to those described above; with the exception that after animals received rimonabant or its vehicle on days 19–23, they were placed immediately back into their home cages for one hour (though still within the behavioral testing room) instead of into the activity cages. When these mice were subsequently tested for cocaine-induced hyperlocomotion on the challenge day 28, *no* differences were observed between groups treated with rimonabant *vs* its vehicle (AUC = 11 763 ± 2051 for rimonabant group *vs* 11 888 ± 1121 for vehicle group; $p > 0.05$; unpaired *t*-test). All subjects remained significantly sensitized to the locomotor effects of cocaine ($p < 0.05$, days 28 *vs* 2, using paired *t*-tests within groups).

This finding is consistent with a gradual reversal of cocaine sensitization by a context-specific blockade of eCB signaling at CB_1 receptors. It is important to consider, however, that behavioral sensitization is often associated with an increase in stereotyped behaviors such as grooming, gnawing, and rearing. In some instances, stereotyped behaviors in sensitized animals can occur at the expense of time spent in ambulatory activity (Michel and Tirelli, 2002), raising the possibility that the reduction observed in horizontal locomotion following context-paired rimonabant was associated with an increase in the time spent in behavioral sterotypies. Although this was not visually confirmed, our frequent observations were not standardized, and stereotypical behaviors such as rearing and sniffing did occur rather frequently following cocaine in every experiment. We therefore analyzed the time spent in stereotyped behaviors as detected by repetitive beam breaks in the activity cages (Figure 5). In our experiments, locomotor sensitization to cocaine in mice was not associated with a significant increase in behavioral stereotypies, and day 28 stereotypy scores were not different between groups ($p > 0.05$). Thus, while our automated methods do not provide the most precise measure of stereotyped behaviors in mice, the markedly reduced expression of locomotor sensitization to cocaine following rimonabant cannot be explained by a greater amount of time spent engaged in such behaviors. We conclude that the repeated and context-specific blockade of CB_1 receptors led to a reversal of established cocaine sensitization, measured by cocaine-induced hyperlocomotion, with no significant effect on stereotyped behaviors.

DISCUSSION

Here, we revisited the role of eCB signaling in behavioral sensitization to cocaine, discriminating between the effects of pharmacologically blocking CB_1 receptors during either an induction or maintenance phase of cocaine sensitization. In failing to find a pronounced effect of CB_1 receptor antagonism on the induction of cocaine sensitization, our study is consistent with previous findings (Lesscher *et al*, 2005). However, we found differences between our two protocols, depending upon the timing of the first rimonabant pretreatment. When mice were administered rimonabant 30 min prior to every cocaine treatment, cocaine-induced hyperactivity, and sensitization were blunted as compared to vehicle-injected controls (see Figure 1a), consistent with recent studies (Cheer *et al*, 2007). Yet, within-subjects comparison revealed that when mice were initially exposed to cocaine without pretreatment (as in protocol 2), behavioral sensitization was indistinguishable between rimonabant and vehicle pretreatment groups (see Figure 1b). This was not due to a single-exposure sensitization to cocaine (Ciccocioppo *et al*, 2004), since under our experimental conditions a single injection of cocaine was insufficient to induce a lasting behavioral sensitization. Interestingly, Corbillé *et al* (2007) very recently reported that single-trial sensitization to cocaine was largely absent following pharmacological blockade or genetic deletion of CB_1 receptors in mice. Also, Lesscher *et al* (2005) reported observations (using a substantially different open-field apparatus and sensitization protocol) that initially indicated a reduction of cocaine sensitization when daily cocaine was preceded by rimonabant, but the effect was not maintained in their later experiments when

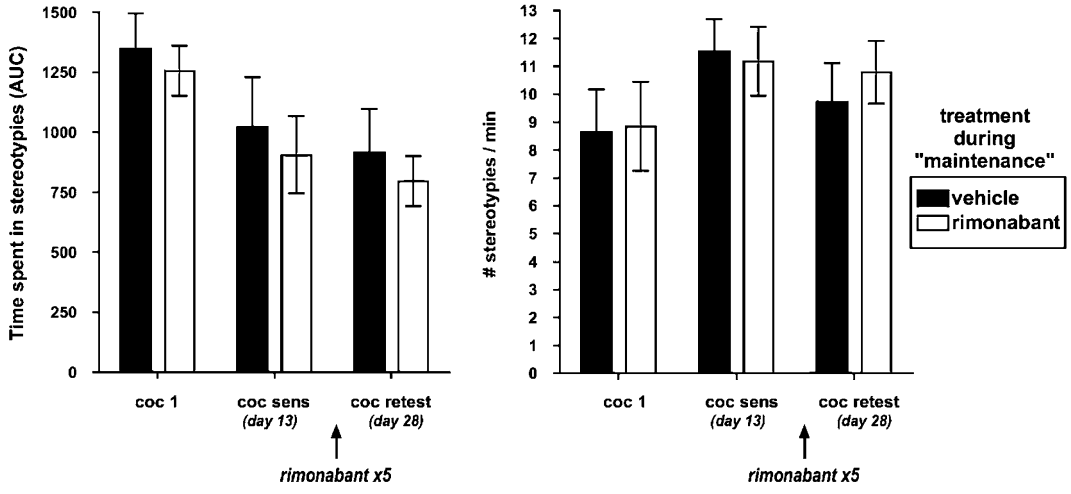

Figure 5 Locomotor sensitization to cocaine was not associated with increased behavioral stereotypies. Summary histograms representing total time spent in repetitive stereotyped behaviors (left panel, see Materials and methods) and average number of stereotypies per min (right panel) for the first 15 min following cocaine injections. Data are from the same experiments as shown in Figure 3.

the concentration of a cocaine challenge dose was varied. Taken together, the CB_1 receptor appears to markedly influence the neural mechanisms of cocaine sensitization without actually mediating the critical cellular adaptations underlying this response. These findings further imply that CB_1 signaling contributes important aspects of the psychomotor/incentive salience of cocaine as some have reported (Chaperon *et al*, 1998; Cheer *et al*, 2007; Soria *et al*, 2005), but in contrast to other findings (Cossu *et al*, 2001; Caille and Parsons, 2006; Houchi *et al*, 2005). We suggest that even subtle differences in subjects' drug history may explain some discrepant behavioral findings.

In contrast to our results, Martin *et al* (2000) reported that both the induction and expression/maintenance of cocaine sensitization was preserved in CB_1 KO mice of the CD1 genetic background. Their conclusions may be due to numerous methodological differences compared with our own: (1) homeostatic mechanisms (Mato *et al*, 2005; Houchi *et al*, 2005) may compensate for the lack of CB_1 receptors during development; (2) reduced baseline locomotion in CB_1 KO mice (Steiner *et al*, 1999; Martin *et al*, 2000) renders direct comparisons with wild-type subjects problematic; (3) ambulatory activity was measured in chambers much smaller than our own ($9 \times 20 \times 11$ cm), resulting in lower levels of activity overall; and (4) whereas chronic cocaine (10 mg/kg, i.p.) was administered more frequently (two times per day for 15 days) in their study, cocaine-induced activity was monitored only once for every six injections (Martin *et al*, 2000). Thus, these authors may have observed a smaller component of sensitization that was relatively uncoupled to environmental associations and genetically dissociable from CB_1-dependent processes (Corbillé *et al*, 2007).

Lack of Phytocannabinoid Effects on Induction of Cocaine Sensitization

Predating the notion that rimonabant might have anti-addictive properties (Carai *et al*, 2005), Kandel and colleagues posited that adolescent cannabis use might increase the likelihood of abusing other drugs, including cocaine (Kandel, 1975; Kandel *et al*, 2006). A number of studies have investigated this 'gateway hypothesis,' testing effects of *in vivo* cannabinoid exposure on behavioral or biochemical measures of psychostimulant reward in animal models (Arnold, 2005; Panlilio *et al*, 2007; Ellgren *et al*, 2004). Few rodent studies have employed THC, however, and to our knowledge none have included CBD, a nonpsychoactive constituent in cannabis reported to modulate the psychomotor effects of THC alone (Petitet *et al*, 1998; Pertwee, 2005). CBD has relatively low affinity for the CB_1 receptor, yet recent studies have reported it as having potent inverse agonist activity (Thomas *et al*, 2007). In our limited set of studies, we failed to witness an effect of these phytocannabinoids on cocaine sensitization. These findings are fully consistent with a thorough study by Arnold *et al* (1998), investigating the THC analog CP-55 940 (see Arnold, 2005 for an overview of related studies).

Context-Specific Reversal of Cocaine Sensitization by Rimonabant

When cocaine-sensitized mice were given repeated daily rimonabant, coupled to the activity chambers where cocaine had been previously experienced, the expression of behavioral sensitization was disrupted. The context specificity of this effect was distinct, given that sensitization was not reversed when rimonabant was delivered in the very same room, but within subjects' home cages. It has been elegantly demonstrated that psychostimulant sensitization is sensitive to the environmental context of drug administration (Badiani and Robinson, 2004; Michel *et al*, 2003). For instance, home cage administration is less effective at inducing behavioral sensitization than when drug delivery is paired to a novel behavioral environment (Badiani and Robinson, 2004). Further, chronic cocaine administered within a test environment—but not within the home cage—leads to an enhanced glutamate release within the NAc in response to a subsequent cocaine challenge (Bell *et al*, 2000), and only in sensitized animals (Pierce *et al*, 1996). We speculate that such an effect may elicit eCB-mediated LTD of glutamatergic synapses in the NAc (Gerdeman *et al*, 2003; Kreitzer and Malenka, 2005; Robbe *et al*, 2002a, b), a form of presynaptic plasticity that is dynamically influenced by cocaine exposure (Fourgeaud *et al*, 2004). LTD or a similar eCB-signaling event cued by context-specific glutamate release (Bell *et al*, 2000) and NAc activation (Hollander and Carelli, 2007), could help to shape the maintenance of conditioned behaviors (eCB Signaling as an Occasion Setter).

To our knowledge, this is the first demonstration of a context-specific pharmacological reversal of cocaine sensitization. Most studies of the expression of behavioral sensitization have looked only at acute blocking effects of different neurotransmitter receptor antagonists delivered shortly prior to cocaine challenge (Karler *et al*, 1994; Vanderschuren and Kalivas, 2000). Reversal of cocaine sensitization has, however, been reported following repeated administration of 5-HT_3 receptor antagonists, but only when administered after cocaine or the mixed D_1/D_2 receptor agonist pergolide (Zhang *et al*, 2006; Li *et al*, 2000).

Importance of the CB_1 Receptor in Cocaine-Induced Behavioral Conditioning

Our findings help to reconcile a conflicting literature on CB_1 receptor involvement in the acute rewarding effects of cocaine (Arnold, 2005), and is consistent with a role of eCB signaling in the rewarding effects of other abused drugs (Maldonado *et al*, 2006). Yet, it is important to note that although locomotor sensitization is believed to have considerable mechanistic overlap with other models of cocaine addiction (Everitt and Wolf, 2002; Ferrario *et al*, 2005), there is also evidence to question its use as a proxy for drug seeking behaviors *per se* (Ben-Shahar *et al*, 2004; Kalivas and Hu, 2006). It is thus important to interpret our findings within the broader literature relating eCB signaling to cocaine reward. Regarding self-administration, Cossu *et al* (2001) found that genetic deletion of the CB_1 receptor did not prevent operant responding for a fixed cocaine reward in restrained mice. In rats, rimonabant was likewise ineffective at preventing self-administration of cocaine under fixed-ratio schedules (Caille and Parsons, 2006; De Vries *et al*, 2001). Yet, Soria *et al* (2005) showed that cocaine self-administration under a progressive ratio schedule was significantly attenuated in both CB_1 KO mice and wild-type

mice treated with rimonabant. This was especially evident as a reduction in the breakpoint of responding for cocaine, consistent with an eCB effect on the maintenance of the conditioned behavior (Soria *et al*, 2005).

Results from experiments measuring CPP to cocaine have also not clearly resolved an importance of CB_1 receptors. Cocaine CPP was reported in CB_1 KO mice (Martin *et al*, 2000; Houchi *et al*, 2005), yet it was disrupted by rimonabant in rats (Chaperon *et al*, 1998). Parker *et al* (2004) found that rimonabant alone, as well as either THC or CBD, 'potentiated the extinction' of cocaine place preference when repeatedly paired to the preferred test chamber—a protocol that more closely resembles our present findings in a sensitization model.

Investigators have failed to observe an effect of CB_1 receptor blockade on cocaine-induced rises in NAc dopamine using microdialysis (Caille and Parsons, 2006; Soria *et al*, 2005; Houchi *et al*, 2005), supporting the notion that the acutely rewarding properties of cocaine are mostly independent of the eCB system. Recently however, Cheer *et al* (2007) used higher-resolution voltammetry to reveal eCB influences on spontaneous, transient dopamine release in the NAc on a subsecond timescale. Rimonabant administration was shown to disrupt both the enhancement of spontaneous dopamine transients and psychomotor activation induced by cocaine (Cheer *et al*, 2007). Similar to our findings, however, rimonabant pretreatment did not fully prevent cocaine-induced hyperlocomotion, as compared to the activity level of saline-injected control animals (see online Supplementary information in Cheer *et al*, 2007). Perhaps context-evoked and eCB-mediated subsecond dopamine fluctuations may help to maintain established behavioral sensitization to cocaine.

eCB Signaling as an Occasion Setter

Our findings are consistent with a model in which eCB signaling acts as a facilitating occasion setter. A concept developed from Pavlovian learning theory, occasion-setting is an associative memory function that provides contextual information about a stimulus, and subsequently allows context to modulate the excitatory strength of the stimulus to drive behavior (Anagnostaras and Robinson, 1996). An occasion setter can hypothetically be either facilitating (acting within a drug-paired context to promote expression of sensitization) or inhibitory (acting within unpaired contexts to suppress or negate sensitized responses). Previously, Anagnostaras *et al* (2002) provided evidence for inhibitory occasion-setting as a factor influencing the context dependence of amphetamine sensitization. Based on our observation that rimonabant reversed sensitization to a cocaine stimulus only when administered within the cocaine-paired environment, we propose that the context specificity of cocaine sensitization involves a facilitating occasion setter, mediated by CB_1 receptors.

Possible Sites and Mechanism of Action

As both cocaine and rimonabant were administered systemically, we can only make informed speculations regarding neural mechanisms responsible for our findings. The neurobiological correlates of behavioral sensitization have been studied extensively, however, and a number of factors warrant that eCB signaling within the NAc or dorsal striatum be given particular consideration. Whereas the VTA is fundamentally important for the induction of behavioral sensitization, and an area where synaptic transmission is modulated by CB_1 receptors (Lupica and Riegel, 2005), it is not critical for its maintenance/expression (Vanderschuren and Kalivas, 2000; Wolf *et al*, 2004). The maintenance or lasting expression of behavioral sensitization to cocaine is, however, dependent upon the ventral striatum, and upon NAc glutamate transmission in particular (Bell *et al*, 2000; Pierce *et al*, 1996; Vanderschuren and Kalivas, 2000). Plasticity of NAc glutamatergic synapses has been associated with both cocaine sensitization (Brebner *et al*, 2005; Goto and Grace, 2005; Thomas *et al*, 2001; Yao *et al*, 2004) and cocaine-seeking behaviors (Martin *et al*, 2006; Schramm-Sapyta *et al*, 2006). CB_1 receptors are functionally expressed on glutamatergic axon terminals onto NAc output neurons (Robbe *et al*, 2001), and cocaine has been reported to alter the plasticity of these synapses (Fourgeaud *et al*, 2004). Moreover, CB_1 receptor antagonists can prevent cued reinstatement of cocaine seeking in rats when delivered either systemically (De Vries *et al*, 2001) or directly into either the NAc or dorsal striatum (Xi *et al*, 2006). Although GABAergic striatal synapses are also inhibited by eCBs (Freiman *et al*, 2006; Narushima *et al*, 2007)—a function that may be upregulated by cocaine (Centonze *et al*, 2004)—modulation of GABA release is not a likely mechanism for the effects of CB_1 antagonists on cocaine-motivated behaviors (Xi *et al*, 2006).

Conclusions

CB_1 receptors are expressed throughout the cortico-basal ganglia circuitry implicated in addiction (Maldonado *et al*, 2006), and may contribute to the development of drug-directed behaviors through influences on synaptic plasticity (Gerdeman *et al*, 2003; Lupica and Riegel, 2005; Chevaleyre *et al*, 2006). Here, specifically using a mouse model of cocaine sensitization that has been shown to modulate NAc LTD (Thomas *et al*, 2001), we report a context-specific reversal of established cocaine sensitization by rimonabant, at a concentration that also blocks conditioned cocaine seeking (De Vries and Schoffelmeer, 2005) via alterations of striatal glutamate signaling (Xi *et al*, 2006). Although we have not directly resolved a mechanism to our present findings, they suggest that eCB signaling acts as a facilitating occasion setter, allowing the maintenance of cocaine sensitization upon the reactivation of contextual memories (Lee *et al*, 2006; Miller and Marshall, 2005). These findings add to the evidence that motivational effects of drug-paired environments involve activation of the eCB system (Cohen *et al*, 2005; De Vries and Schoffelmeer, 2005; Maldonado *et al*, 2006), and add nuance to the consideration of CB_1 receptors as therapeutic targets for preventing cocaine relapse. In accordance with other recent studies (Parker *et al*, 2006), such approaches may be more successful when paired to drug-conditioned environments.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Anagnostaras SG, Robinson TE (1996). Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. *Behav Neurosci* **110**: 1397–1414.

Anagnostaras SG, Schallert T, Robinson TE (2002). Memory processes governing amphetamine-induced psychomotor sensitization. *Neuropsychopharmacology* **26**: 703–715.

Anggadiredja K, Nakamichi M, Hiranita T, Tanaka H, Shoyama Y, Watanabe S *et al* (2004). Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. *Neuropsychopharmacology* **29**: 1470–1478.

Arnold JC (2005). The role of endocannabinoid transmission in cocaine addiction. *Pharmacol Biochem Behav* **81**: 396–406.

Arnold JC, Topple AN, Hunt GE, McGregor IS (1998). Effects of pre-exposure and co-administration of the cannabinoid receptor agonist CP 55 940 on behavioral sensitization to cocaine. *Eur J Pharmacol* **354**: 9–16.

Badiani A, Robinson TE (2004). Drug-induced neurobehavioral plasticity: the role of environmental context. *Behav Pharmacol* **15**: 327–339.

Bell K, Duffy P, Kalivas PW (2000). Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology* **23**: 335–344.

Ben-Shahar O, Ahmed SH, Koob GF, Ettenberg A (2004). The transition from controlled to compulsive drug use is associated with a loss of sensitization. *Brain Res* **995**: 46–54.

Boudreau AC, Wolf ME (2005). Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci* **25**: 9144–9151.

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S *et al* (2005). Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science* **310**: 1340–1343.

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

Carai MA, Colombo G, Gessa GL (2005). Rimonabant: the first therapeutically relevant cannabinoid antagonist. *Life Sci* **77**: 2339–2350.

Centonze D, Battista N, Rossi S, Mercuri NB, Finazzi-Agro A, Bernardi G *et al* (2004). A critical interaction between dopamine D2 receptors and endocannabinoids mediates the effects of cocaine on striatal gabaergic transmission. *Neuropsychopharmacology* **29**: 1488–1497.

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

Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ *et al* (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J Neurosci* **27**: 791–795.

Chevaleyre V, Takahashi KA, Castillo PE (2006). Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci* **29**: 37–76.

Ciccocioppo R, Martin-Fardon R, Weiss F (2004). Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. *Nat Neurosci* **7**: 495–496.

Cohen C, Perrault G, Griebel G, Soubrie P (2005). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). *Neuropsychopharmacology* **30**: 145–155.

Corbillé AG, Valjent E, Marsicano G, Ledent C, Lutz B, Herve D *et al* (2007). Role of cannabinoid type I receptors in locomotor activity and striatal signaling in response to psychostimulants. *J Neurosci* **27**: 6937–6947.

Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M *et al* (2001). Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res* **118**: 61–65.

De Vries TJ, de Vries W, Janssen MC, Schoffelmeer AN (2005). Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. *Behav Brain Res* **161**: 164–168.

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

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

Ellgren M, Hurd YL, Franck J (2004). Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence. *Eur J Pharmacol* **497**: 205–213.

Everitt BJ, Robbins TW (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* **8**: 1481–1489.

Everitt BJ, Wolf ME (2002). Psychomotor stimulant addiction: a neural systems perspective. *J Neurosci* **22**: 3312–3320.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005). Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biol Psychiatry* **58**: 751–759.

Fourgeaud L, Mato S, Bouchet D, Hemar A, Worley PF, Manzoni OJ (2004). A single *in vivo* exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J Neurosci* **24**: 6939–6945.

Freiman I, Anton A, Monyer H, Urbanski MJ, Szabo B (2006). Analysis of the effects of cannabinoids on identified synaptic connections in the caudate-putamen by paired recordings in transgenic mice. *J Physiol* **575**: 789–806.

Gerdeman G, Lovinger DM (2001). CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J Neurophysiol* **85**: 468–471.

Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM (2003). It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci* **26**: 184–192.

Gerdeman GL, Ronesi J, Lovinger DM (2002). Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci* **5**: 446–451.

Goto Y, Grace AA (2005). Dopamine-dependent interactions between limbic and prefrontal cortical plasticity in the nucleus accumbens: disruption by cocaine sensitization. *Neuron* **47**: 255–266.

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF (2002). The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur J Neurosci* **16**: 1395–1398.

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.

Hollander JA, Carelli RM (2007). Cocaine-associated stimuli increase cocaine seeking and activate accumbens core neurons after abstinence. *J Neurosci* **27**: 3535–3539.

Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M (2005). CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacology* **30**: 339–349.

Kalivas PW, Hu XT (2006). Exciting inhibition in psychostimulant addiction. *Trends Neurosci* **29**: 610–616.

Kalivas PW, Volkow N, Seamans J (2005). Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron* **45**: 647–650.

Kandel D (1975). Stages in adolescent involvement in drug use. *Science* **190**: 912–914.

Kandel DB, Yamaguchi K, Klein LC (2006). Testing the gateway hypothesis. *Addiction* **101**: 470–472, discussion 474–476.

Karler R, Calder LD, Bedingfield JB (1994). Cocaine behavioral sensitization and the excitatory amino acids. *Psychopharmacology (Berl)* **115**: 305–310.

Kreitzer AC, Malenka RC (2005). Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. *J Neurosci* **25**: 10537–10545.

Lee JL, Milton AL, Everitt BJ (2006). Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. *J Neurosci* **26**: 5881–5887.

Lesscher HM, Hoogveld E, Burbach JP, van Ree JM, Gerrits MA (2005). Endogenous cannabinoids are not involved in cocaine reinforcement and development of cocaine-induced behavioural sensitization. *Eur Neuropsychopharmacol* **15**: 31–37.

Li Y, White FJ, Wolf ME (2000). Pharmacological reversal of behavioral and cellular indices of cocaine sensitization in the rat. *Psychopharmacology (Berl)* **151**: 175–183.

Lupica CR, Riegel AC (2005). Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacology* **48**: 1105–1116.

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

Martin M, Chen BT, Hopf FW, Bowers MS, Bonci A (2006). Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nat Neurosci* **9**: 868–869.

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.

Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE, Manzoni OJ (2004). A single *in-vivo* exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. *Nat Neurosci* **7**: 585–586.

Mato S, Robbe D, Puente N, Grandes P, Manzoni OJ (2005). Presynaptic homeostatic plasticity rescues long-term depression after chronic Delta 9-tetrahydrocannabinol exposure. *J Neurosci* **25**: 11619–11627.

Michel A, Tambour S, Tirelli E (2003). The magnitude and the extinction duration of the cocaine-induced conditioned locomotion-activated response are related to the number of cocaine injections paired with the testing context in C57BL/6J mice. *Behav Brain Res* **145**: 113–123.

Michel A, Tirelli E (2002). Conditioned hyperkinesia induced by cocaine in mice is dose-dependent but not correlated with the unconditioned response or the contextually-sensitized response. *Behav Pharmacol* **13**: 59–71.

Miller CA, Marshall JF (2005). Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron* **47**: 873–884.

Narushima M, Uchigashima M, Fukaya M, Matsui M, Manabe T, Hashimoto K *et al* (2007). Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. *J Neurosci* **27**: 496–506.

Panlilio LV, Solinas M, Matthews SA, Goldberg SR (2007). Previous exposure to THC alters the reinforcing efficacy and anxiety-related effects of cocaine in rats. *Neuropsychopharmacology* **32**: 646–657.

Parker LA, Burton P, Sorge RE, Yakiwchuk C, Mechoulam R (2004). Effect of low doses of delta9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology (Berl)* **175**: 360–366.

Parker LA, Limebeer CL, Slomke J (2006). Renewal effect: context-dependent extinction of a cocaine- and a morphine-induced conditioned floor preference. *Psychopharmacology (Berl)* **187**: 133–137.

Pertwee RG (2005). Pharmacological actions of cannabinoids. *Handb Exp Pharmacol* **168**: 1–51.

Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC (1998). Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci* **63**: PL1–PL6.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996). Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J Neurosci* **16**: 1550–1560.

Robbe D, Alonso G, Duchamp F, Bockaert J, Manzoni OJ (2001). Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J Neurosci* **21**: 109–116.

Robbe D, Bockaert J, Manzoni OJ (2002a). Metabotropic glutamate receptor 2/3-dependent long-term depression in the nucleus accumbens is blocked in morphine-withdrawn mice. *Eur J Neurosci* **16**: 2231–2235.

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

Robinson TE, Berridge KC (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* **18**: 247–291.

Schramm-Sapyta NL, Olsen CM, Winder DG (2006). Cocaine self-administration reduces excitatory responses in the mouse nucleus accumbens shell. *Neuropsychopharmacology* **31**: 1444–1451.

Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M *et al* (2005). Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* **30**: 1670–1680.

Steiner H, Bonner TI, Zimmer AM, Kitai ST, Zimmer A (1999). Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. *Proc Natl Acad Sci USA* **96**: 5786–5790.

Tarter RE, Vanyukov M, Kirisci L, Reynolds M, Clark DB (2006). Predictors of marijuana use in adolescents before and after licit drug use: examination of the gateway hypothesis. *Am J Psychiatry* **163**: 2134–2140.

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB(1) and CB(2) receptor agonists *in vitro*. *Br J Pharmacol* **150**: 613–623.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat Neurosci* **4**: 1217–1223.

Tirelli E, Michel A, Brabant C (2005). Cocaine-conditioned activity persists for a longer time than cocaine-sensitized activity in mice: implications for the theories using Pavlovian excitatory conditioning to explain the context-specificity of sensitization. *Behav Brain Res* **165**: 18–25.

Vanderschuren LJ, Everitt BJ (2005). Behavioral and neural mechanisms of compulsive drug seeking. *Eur J Pharmacol* **526**: 77–88.

Vanderschuren LJ, Kalivas PW (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)* **151**: 99–120.

Vlachou S, Nomikos GG, Panagis G (2003). WIN 55 212-2 decreases the reinforcing actions of cocaine through CB1 cannabinoid receptor stimulation. *Behav Brain Res* **141**: 215–222.

Wolf ME, Sun X, Mangiavacchi S, Chao SZ (2004). Psychomotor stimulants and neuronal plasticity. *Neuropharmacology* **47** (Suppl 1): 61–79.

Wu X, French ED (2000). Effects of chronic delta9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. *Neuropharmacology* **39**: 391–398.

Xi ZX, Gilbert JG, Peng XQ, Pak AC, Li X, Gardner EL (2006). Cannabinoid CB1 receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens. *J Neurosci* **26**: 8531–8536.

Yao WD, Gainetdinov RR, Arbuckle MI, Sotnikova TD, Cyr M, Beaulieu JM *et al* (2004). Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. *Neuron* **41**: 625–638.

Zhang X, Lee TH, Davidson C, Lazarus C, Wetsel WC, Ellinwood EH (2006). Reversal of cocaine-induced behavioral sensitization and associated phosphorylation of the NR2B and GluR1 subunits of the NMDA and AMPA receptors. *Neuropsychopharmacology* **32**: 377–387.