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Attenuation of Cocaine-Induced Conditioned Place Preference and Motor Activity via Cannabinoid CB2 Receptor Agonism and CB1 Receptor Antagonism in Rats

Foteini Delis, PhD; Alexia Polissidis, PhD; Nafsika Poulia, MSc; Zuzana Justinova, PhD; George G. Nomikos, PhD; Steven R. Goldberg, PhD;[†] Katerina Antoniou, PhD

Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece (Dr Delis, Dr Polissidis, Ms Poulia, and Dr Anoniou); Laboratory of Neurodegenerative Diseases, Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation Academy of Athens, Athens, Greece (Dr Polissidis); Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, Intramural Research Program, National Institute on Drug Abuse, NIH, DHHS, Baltimore, MD (Drs Justinova and Goldberg); Global Clinical Science, Takeda Development Center Americas, Inc, Deerfield, IL (Dr Nomikos).

[†] In memoriam.

Correspondence: Katerina Antoniou, PhD, Associate Professor of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, 45110 Ioannina, Greece (kantoniu@cc.uoi.gr).

Abstract

Background: Studies have shown the involvement of cannabinoid (CB) receptors in the behavioral and neurobiological effects of psychostimulants. Most of these studies have focused on the role of CB1 receptors in the psychostimulant effects of cocaine, while very few have investigated the respective role of CB2 receptors. Further studies are warranted to elucidate the extent of CB receptor involvement in the expression of cocaine-induced effects.
Methods: The role of CB1 and CB2 receptors in the rewarding and motor properties of cocaine was assessed in conditioned place preference, conditioned motor activity, and open field activity in rats.
Results: The CB1 receptor antagonist rimonabant (3 mg/kg) decreased the acquisition and the expression of conditioned place preference induced by cocaine (20 mg/kg). Rimonabant inhibited cocaine-elicited conditioned motor activity when administered during the expression of cocaine-induced conditioned place preference. Rimonabant decreased ambulatory and vertical activity induced by cocaine. The CB2 receptor agonist JWH-133 (10 mg/kg) decreased the acquisition and the expression of cocaine-induced conditioned place preference. JWH-133 inhibited cocaine-elicited conditioned motor activity when administered during the acquisition and the expression of cocaine-induced conditioned place preference. JWH-133 decreased ambulatory activity and abolished vertical activity induced by cocaine. The effects of JWH-133 on cocaine conditioned and stimulated responses were abolished when the CB2 receptor antagonist/inverse agonist AM630 (5 mg/kg) was preadministered.
Conclusions: Cannabinoid CB1 and CB2 receptors modulate cocaine-induced rewarding behavior and appear to have opposite roles in the regulation of cocaine's reinforcing and psychomotor effects.

Keywords: cocaine, conditioned place preference, CB1, CB2, motor activity

Significance Statement

This pharmacological study directly compares the effects of the CB1 receptor antagonist, rimonabant, and the CB2 receptor agonist, JWH-133, in cocaine-induced conditioned place preference (CPP), conditioned motor activity, and hyperactivity in the open field. Both rimonabant and JWH-133 attenuated acquisition and expression of CPP, conditioned motor activity, and hyperactivity. AM630, a CB2 receptor antagonist, was able to reverse JWH-133's effects, confirming CB2-specific actions. CB1 and CB2 receptors appear to have opposite roles in the regulation of cocaine's reinforcing and psychomotor effects.

Introduction

Δ^9-Tetrahydrocannabinol, the main psychoactive component of *Cannabis sativa*, is a partial agonist of cannabinoid (CB) CB1 and CB2 receptors. Cannabinoid receptors are heterogeneously distributed in motor, limbic, and cognitive regions of the brain (Tsou et al., 1998; Gong et al., 2006), including–but not limited to—the caudate/putamen and cerebellum, hippocampus, rhinal cortices, and amygdala as well as the cerebral cortex. CB1 receptors are abundantly expressed at pre- and postsynaptic sites (Ong and Mackie 1999), while CB2 receptors are expressed in the postsynaptic somatodendritic area of the neuron and on glial cells of the brain at much lower levels than CB1 receptors (Onaivi et al., 2006).

Studies have shown the involvement of CB receptors in the behavioral and neurobiological effects of psychostimulants, such as amphetamine and cocaine (e.g., Chaperon et al., 1998; De Vries et al., 2001; Parker et al., 2004; Xi et al., 2006; Polissidis et al., 2009, 2014; Ward et al., 2009). The majority of these studies have investigated the complex role of CB1 receptors in the psychostimulant effects of cocaine, while few studies have focused on the respective role of CB2 receptors (Xi et al., 2011; Zhang et al., 2014). Therefore, further studies are needed to elucidate the extent of CB receptor involvement in the expression of reinforcing and psychostimulant properties of cocaine.

There is evidence for and against the involvement of CB receptors in cocaine-induced effects. The selective CB1 receptor antagonists/inverse agonists rimonabant and AM251 decrease cue- and drug priming-induced reinstatement of cocaine seeking (De Vries et al., 2001; Xi et al., 2006) as well as the acquisition and reinstatement of cocaine-induced conditioned place preference (CPP) (Chaperon et al., 1998; Yu et al., 2011; Vaughn et al., 2012). In an analogous manner, genetic elimination of CB1 receptors is associated with a decrease in cocaine self-administration (Soria et al., 2005), CPP (Miller et al., 2008), and basal and cocaine-induced motor activity (Li et al., 2009). On the other hand, Cossu and colleagues (2001) reported that cocaine is self-administered to the same extent by both wild-type and CB1-receptor knockout mice. In addition, studies have shown that cocaine-induced CPP is similar between CB1 knockout and wild-type mice (Martin et al., 2000; Houchi et al., 2005). Despite substantial evidence supporting the involvement of CB1 receptors in the effects of cocaine, inconsistent findings limit our understanding.

Recent studies in mice have shown that CB2 receptors may also be critically involved in cocaine-induced behavioral effects. Treatment with the CB2-selective agonist JWH-133 inhibits cocaine self-administration and decreases cocaine-induced motor activity via dopamine-dependent pathways (Xi et al., 2011; Zhang et al., 2014). This is consistent with findings in CB2-overexpressing mice that showed decreased motor sensitization to cocaine, decreased cocaine-induced CPP, and also lower levels of cocaine self-administration (Aracil-Fernandez et al., 2012).

After taking into consideration the importance of the endocannabinoid system in the modulation of cocaine-induced effects and based on the aforementioned findings, we aimed to further investigate the roles of CB1 receptor antagonism and CB2 receptor agonism in different aspects of cocaine-induced behavioral profile under the same methodological conditions for purposes of direct comparison. In particular, we compared the effects of the CB1 receptor antagonist/inverse agonist rimonabant and the CB2 receptor agonist JWH-133 on the acquisition and expression of cocaine-induced CPP and motor activity expressed in the CPP apparatus. We also evaluated the effects of rimonabant and JWH-133 on cocaine-induced open field activity. Finally, to elucidate the specific role of CB2 receptors, we assessed the reversal of the effects of JWH-133 by using the CB2 receptor antagonist/inverse agonist AM630.

Materials and Methods

Animals

Male Sprague-Dawley rats were bred in the Animal Facility of the University of Ioannina. A total of 218 rats were used. The rats were 80 to 90 days old and weighed 250 to 300 g at the beginning of the experiments. They were housed in groups of 2 or 3 in plastic cages ($42.5 \times 26.6 \times 15.5$ cm) on a 12-h-light/-dark cycle, lights on at 7:00 AM, at $21 \pm 1°C$, and had unlimited access to food and water. Experiments were performed during the light cycle of the rats to compare findings with previously published results. Male rats were used in this study to avoid potential behavioral variation induced by females because of the stages of the estrus cycle. All experiments were reviewed and approved by the Institutional Animal Facility Committee of the University of Ioannina, and all studies were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011).

CPP

A total of 164 naïve rats were used for the CPP experiments. The rats were gently handled before the beginning of the experimental procedure and accustomed to the experimental room for 40 minutes prior to the experiment. CPP was performed in a rectangular Plexiglas box (ENV-013, MED Associates, Inc.) measuring $68 \times 21 \times 21$ cm with 3 distinct compartments (white/gray/black) separated by 2 guillotine doors. The black and white compartments ($21 \times 21 \times 28$ cm) serve as the conditioning chambers, while the gray compartment ($21 \times 21 \times 12$ cm) between them is designated as the neutral compartment. The white compartment is fitted with a textured stainless steel mesh floor, the black compartment with a stainless-steel grid rod floor, and the gray compartment with a smooth PVC floor. Low-level illumination (75 W, 125 V for each bulb in each compartment) was used

throughout the experiment. The place preference apparatus was placed in a quiet experimental room, well-protected from external environmental noise. The CPP procedure consisted of 3 phases: habituation, conditioning, and testing.

Habituation

On day 1, the rats were allowed to explore the apparatus for 15 minutes with both guillotine doors open. During this session, spontaneous individual preference was recorded, and efforts were made to obtain a cohort of rats with no significant preference or aversion to achieve an unbiased place conditioning procedure (Tzschentke, 2007). During this session, 3 rats were considered outliers and were excluded from the experiment, since they presented increased preference or aversion towards one of the compartments (time spent in compartment >65% of total session time).

Conditioning

Conditioning sessions were conducted once daily for the subsequent 8 days (days 2–9). On days 2, 4, 6, and 8, animals received vehicle treatment and were placed into one conditioning chamber with the guillotine door closed for 30 minutes. On days 3, 5, 7, and 9, rats received drug treatment and were placed into the opposite conditioning chamber with the guillotine door closed for 30 minutes. Drug-compartment pairings were counterbalanced, that is, compartment designation was evenly distributed.

Testing

On day 10 (test day), the rats were placed into the neutral compartment with free access to both sides for 15 minutes. The amount of time spent in each chamber was automatically recorded as an index of drug-seeking behavior. The data are presented as the difference between time spent in the drug-paired chamber and time spent in the vehicle-paired chamber.

Cocaine-Induced CPP Acquisition

Cannabinoid compounds, cocaine, and their respective vehicles (see below, "Drugs") were administered i.p.. On days 2, 4, 6, and 8, the rats were injected with vehicle 50, 20, and 10 minutes before being placed in the chamber. On days 3, 5, 7, and 9, the rats were injected with AM630 (5 mg/kg) or vehicle 50 minutes before being placed in the chamber, with vehicle, rimonabant (3 mg/kg), or JWH-133 (10 mg/kg) 20 minutes before being placed in the chamber, and with vehicle or cocaine (20 mg/kg) 10 minutes before being placed in the chamber. On test day, the rats were placed in the neutral compartment without any prior drug injection.

Cocaine-Induced CPP Expression

Cocaine conditioning was performed as described above. On test day, AM630 and/or JWH-133 was administered i.p. 50 and 20 minutes before the rats were placed in the neutral compartment, respectively. Rimonabant was administered i.p. 20 minutes before the rats were placed in the neutral compartment.

Conditioned Motor Activity

Motor activity in the CPP chambers was recorded on test day for all rats subjected to either cocaine-induced CPP acquisition or cocaine-induced CPP expression. Motor activity and time spent in CPP chambers were automatically recorded using software available from ENV-013, MED Associates, Inc.

Open Field Activity

Motor behavior was recorded with computerized activity monitoring (ENV515, Activity Monitor, v. 5; Med Associates) in a transparent open activity box (40 x 40 x 40 cm). All animals (n = 54) were acclimatized to the experimental room for 40 minutes prior to the experiment and for 30 minutes in the activity box. A subset of rats received one of the following treatment combinations via i.p. injections: vehicle/saline, vehicle/cocaine (20 mg/kg), rimonabant (3 mg/kg)/saline, rimonabant/cocaine, with a 10-minute interval between injections. Another subset of rats was injected with AM630 (5 mg/kg) or vehicle 50 minutes before motor activity recording, with vehicle or JWH-133 (10 mg/kg) 20 minutes before recording, while vehicle or cocaine (20 mg/kg) was administered 10 minutes later. Ten minutes following the last injection, horizontal and vertical motor activity (ambulatory distance, expressed in centimeters, and number of vertical counts, respectively) were recorded for 1 hour. Ambulatory distance is a measure of the animal's overall motor activity, while the number of vertical counts (rearings) is a measure of the animal's reactivity to a novel environment (Thiel et al., 1999).

Drugs

Cocaine hydrochloride (Ministry of Health, Greece) was dissolved in saline (0.9% NaCl, SAL). The cocaine dose (20 mg/kg) was chosen based on previous studies showing a clear cocaine-induced place preference (Spyraki et al., 1982; Polissidis et al., 2009; Zakharova et al., 2009). AM630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)-ethyl]-1H-indol-3-yl](4-methoxyphenyl)-methanon; synthesized in the laboratory of Dr. Alexandros Makriyannis, Center for Drug Discovery, Northeastern University, Boston, MA) was dissolved in vehicle solution (VEH 1) containing 10% dimethylsulfoxide and 10% Tween 80 in SAL. The AM630 dose (5 mg/kg) was chosen based on a previous study (Gamaleddin et al., 2012). JWH-133 (6aR,10aR)-3-(1,1-Dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; Tocris) and rimonabant (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide) (NIDA Drug Supply Program) were dissolved in a vehicle solution (VEH 2) containing 5% dimethylsulfoxide and 5% cremophor-EL in SAL. The JWH-133 doses (3 and 10 mg/kg) were tested in the open field, and the dose of 10 mg/kg was chosen based on a previous study (Xi et al., 2011) and our preliminary findings revealing robust effects on cocaine-induced behavioral responses. In particular, preadministration of 3 mg/kg JWH-133 produced a decrease in cocaine-induced stimulation of horizontal activity that did not reach statistical significance, while the respective decrease following the 10 mg/kg dose was statistically significant. Furthermore, preadministration of 10 mg/kg but not 3 mg/kg JWH-133 decreased cocaine-induced increased vertical counts. The rimonabant dose (3 mg/kg) was chosen based on previous work (Chaperon et al., 1998; De Vries et al., 2001; Soria et al., 2005; Polissidis et al., 2014) and on the behavioral effects measured during our pilot findings. Other doses of rimonabant (0.3 and 1 mg/kg) were used based on previously established protocols (Chaperon et al., 1998; De Vries et al., 2001; Soria et al., 2005; Polissidis et al., 2014).

Statistics

CPP scores are presented as the difference between times spent in drug-paired and unpaired chambers. Effects of rimonabant on CPP

scores were analyzed using 2-way ANOVA, with rimonabant (RIM) and cocaine (COC) as factors. Effects of JWH-133 were analyzed with 3-way ANOVA with AM630, JWH-133, and COC as factors. Effects of rimonabant on cocaine-conditioned motor activity were analyzed with 3-way ANOVA with RIM and COC as between-subjects factors and chamber as within-subject factor. Effects of JWH-133 on cocaine-conditioned motor activity were analyzed with 4-way repeated measures ANOVA with AM630, JWH-133, and COC as between-subjects factors and chamber as within-subject factor. Effects of rimonabant on open field ambulatory distance and vertical counts were analyzed with 2-way ANOVA with RIM and COC as factors. Effects of JWH-133 on open field ambulatory distance and vertical counts were analyzed with 3-way ANOVA with AM630, JWH-133, and COC as factors. When appropriate, ANOVA was followed by multiple pairwise comparisons using the Bonferroni correction. Overall level of significance was set at $P < .05$. Analysis was performed with the statistical package SPSS v.21.

Results

CPP

Effects of Rimonabant on Cocaine-Induced CPP Acquisition and Expression

Pre-pairing administration of rimonabant (3 mg/kg) decreased cocaine-induced CPP acquisition (Figure 1A). Two-way ANOVA with RIM and COC as factors showed a significant main effect of RIM ($F(1, 26) = 5.89$, $P = .02$), a significant main effect of COC ($F(1, 26) = 68.58$, $P < .001$), and a RIM x COC interaction that did not reach significance ($F(1, 26) = 3.222$, $P = .08$). Posthoc comparisons showed that rimonabant did not induce CPP, in contrast to cocaine that induced robust CPP ($P < .001$), compared with vehicle. Cocaine-induced CPP acquisition after rimonabant pre-pairing was significantly lower than cocaine-induced CPP in the absence of rimonabant ($P = .02$). Pre-pairing administration of rimonabant at lower doses (1 or 0.3 mg/kg) did not affect cocaine-induced CPP acquisition (data not shown).

Administration of rimonabant (3 mg/kg) on test day decreased cocaine-induced CPP expression (Figure 1B). Two-way ANOVA, with RIM and COC as factors, showed a significant RIM x COC interaction ($F(1, 29) = 5.39$, $P = .03$). Posthoc comparisons showed

that cocaine-induced CPP following rimonabant administration on test day was significantly lower than cocaine-induced CPP in the absence of rimonabant ($P < .001$). Rimonabant administration on test day at 1 mg/kg dose also decreased cocaine-induced CPP expression ($P = .007$), while the 0.3 mg/kg dose had no effect (data not shown).

Effects of JWH-133 on Cocaine-Induced CPP Acquisition and Expression

Pre-pairing administration of JWH-133 (10 mg/kg) decreased cocaine-induced CPP acquisition, an effect that was prevented when the CB2 receptor antagonist AM630 was administered prior to JWH-133 (Figure 2A). Three-way ANOVA with AM630, JWH-133, and COC as between-subjects factors showed a significant AM630 x JWH-133 x COC interaction ($F(1, 63) = 7.27$, $P = .009$). Posthoc comparisons showed that cocaine CPP was significantly higher compared with vehicle ($P < .001$), while JWH-133, AM630, and JWH-133+AM630 did not induce CPP or aversion. Cocaine-induced CPP significantly decreased by JWH-133 administration ($P = .003$) and was unaffected by AM630 and by AM630+JWH-133 preadministration.

Administration of JWH-133 (10 mg/kg) on test day decreased cocaine-induced CPP expression, an effect that was prevented following the preadministration of the CB2 receptor antagonist AM630 (Figure 2B). Three-way ANOVA with AM630, JWH-133, and COC as between-subject factors showed a significant AM630 x JWH-133 x COC interaction ($F(1, 65) = 7.27$, $P = .001$). Cocaine-induced CPP significantly decreased by JWH-133 treatment ($P = .001$) and was unaffected by AM630 treatment and by AM630+JWH-133 preadministration.

Conditioned Motor Activity

Effects of Rimonabant on Cocaine-Induced Conditioned Motor Activity

Pre-pairing administration of rimonabant (3 mg/kg) did not affect cocaine-induced conditioned motor activity (Figure 3A). Three-way repeated measures ANONA with RIM and COC as between-subjects factors and chamber as within-subject factor showed a significant chamber x COC interaction ($F(1, 27) = 18.96$, $P < .001$). Motor activity in the cocaine-paired chambers was significantly higher than motor activity in cocaine-unpaired chambers ($P < .001$). Lower doses of rimonabant had similar effects (data
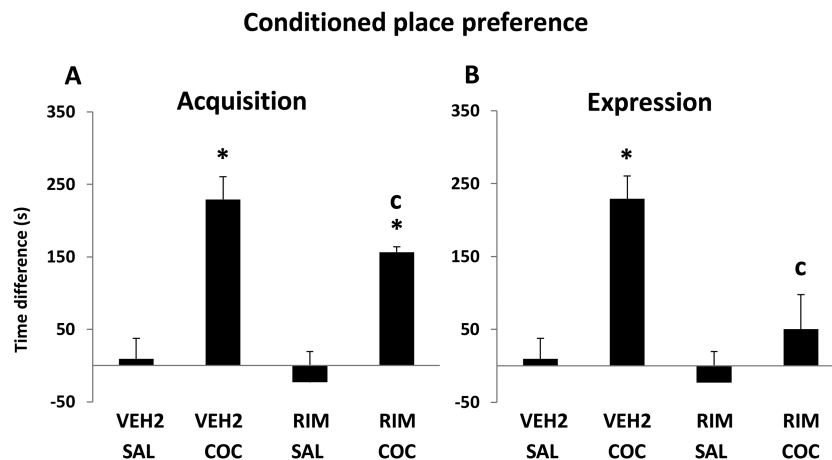
Conditioned place preference

Figure 1. Effects of rimonabant on cocaine-induced conditioned place preference (CPP) acquisition (A) and expression (B). The bars represent the mean + SEM of the difference between time spent in the drug-paired and vehicle-paired chambers on test day. COC, cocaine 20 mg/kg; RIM, rimonabant 3 mg/kg. *Compared with corresponding vehicle treatment, C compared with cocaine (VEH2+COC).
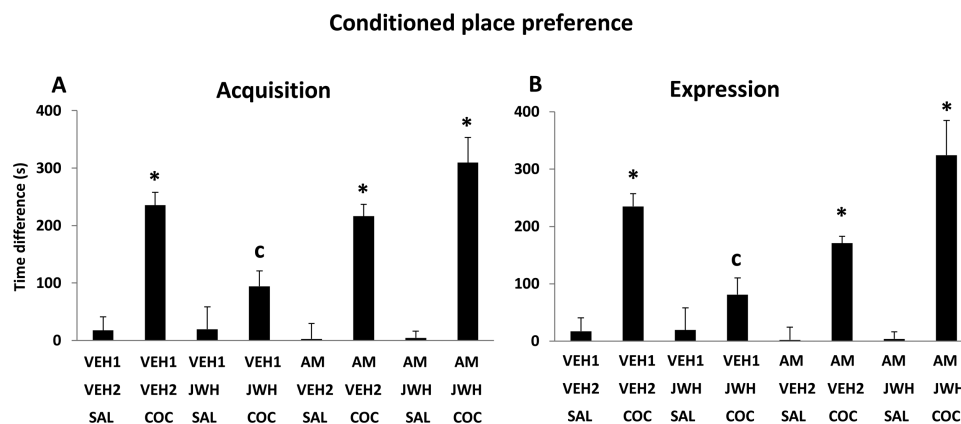
Conditioned place preference

Figure 2. Effects of JWH-133 on cocaine-induced conditioned place preference (CPP) acquisition (A) and expression (B). The bars represent the mean + SEM of the difference between time spent in drug-paired and vehicle-paired chambers on test day. COC, cocaine, 20 mg/kg; JWH, 10 mg/kg JWH-133; AM, 5 mg/kg AM630. * compared with corresponding vehicle treatment, C compared with cocaine (VEH1+VEH2+COC).
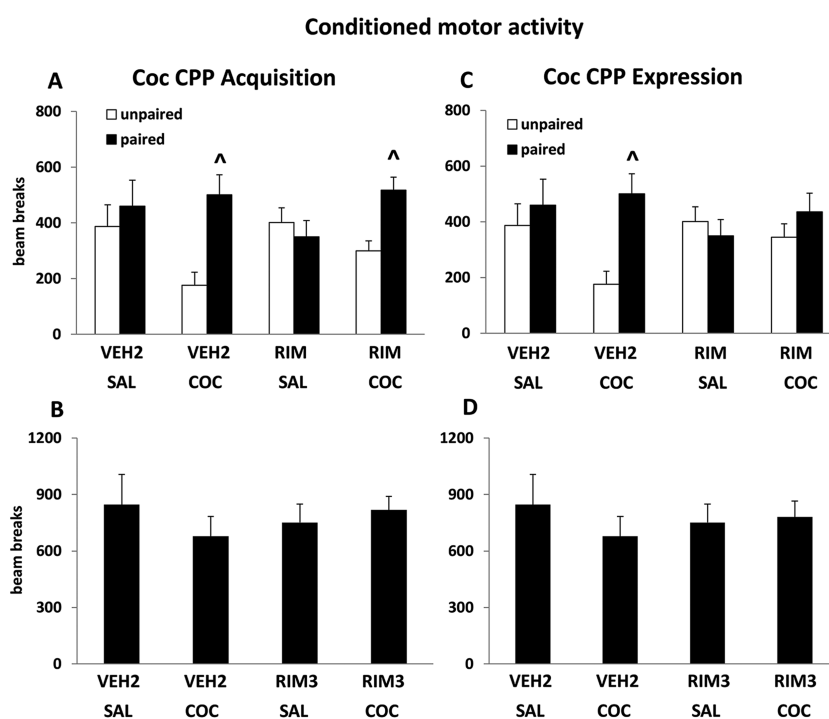
Conditioned motor activity

Figure 3. Effects of rimonabant on cocaine-elicited conditioned motor activity. (A) Motor activity in drug-paired and unpaired chambers on test day, after pre-pairing administration of rimonabant. (B) Total motor activity (drug-paired + unpaired chambers) on test day, after pre-pairing administration of rimonabant. (C) Motor activity in drug-paired and unpaired chambers on test day, after rimonabant administration on test day. (D) Total motor activity (drug-paired + unpaired chambers) on test day, after rimonabant administration on test day. The bars represent mean + SEM; COC, 20 mg/kg cocaine; RIM, 3 mg/kg rimonabant. ^ compared with unpaired chamber.

not shown). Total motor activity (activity in paired+unpaired chambers) was unaffected by treatments (Figure 3B).

Administration of rimonabant (3 mg/kg) on test day abolished the difference in motor activity between paired and unpaired chambers induced by cocaine (Figure 3C). Three-way repeated-measures ANOVA with RIM and COC as between-subjects factors and chamber as within-subject factor showed a significant chamber x RIM interaction ($F(1, 29) = 6.53$, $P = .02$) and a significant chamber x COC interaction ($F(1, 29) = 7.85$, $P = .009$). Only the cocaine-treated group showed a significant difference between motor activities in the paired and unpaired chambers ($P < .001$). Lower doses of rimonabant had similar effects (data not shown). Total motor activity (sum of activities in paired and unpaired chambers) was unaffected by treatments (Figure 3D).

Effects of JWH-133 on Cocaine-Induced Conditioned Motor Activity

Pre-pairing administration of JWH-133 (10 mg/kg) abolished the difference between motor activities in the cocaine-paired and unpaired chambers. This effect was counteracted when AM630 (5 mg/kg) was administered before JWH-133. Administration of AM630 alone did not affect cocaine-induced conditioned motor activity (Figure 4A). Four-way repeated-measures ANOVA with AM630, JWH-133, and COC as between-subjects factors and chamber as within-subject factor showed a significant AM630 x JWH-133 x COC x chamber interaction ($F(1,63) = 7.40$, $P = .008$). Activity in the paired chamber was significantly higher than in the unpaired chamber in the cocaine-, AM630+cocaine-, and JWH-133 + cocaine-treated groups ($P < .001$). The sum of activities in the paired and unpaired chambers was not affected by the treatments (Figure 4B).

Administration of JWH-133 (10 mg/kg) on test day abolished the difference in motor activity between paired and unpaired chambers induced by cocaine. This effect was counteracted when AM630 (5 mg/kg) was administered before JWH-133. Administration of AM630 alone did not affect cocaine-induced conditioned motor activity (Figure 4C). Four-way repeated-measures ANOVA with AM630, JWH-133, and COC as between-subjects factors and chamber as within-subjects factor showed a significant AM630 x JWH-133 x COC x chamber interaction ($F_{(1,65)} = 11.11$, $P = .001$). Activity in the paired chamber was significantly higher than in the unpaired chamber in the cocaine- ($P < .001$), AM630 + cocaine- ($P = .024$), and AM630 + JWH-133 + cocaine- ($P = .012$) treated groups. The sum of activities in the

paired and unpaired chambers was not affected by the treatments (Figure 4D).

Open Field Activity

Effects of Rimonabant on Cocaine-Induced Motor Activity

Rimonabant preadministration moderated cocaine-induced increase in horizontal motor activity (Figure 5A). Two-way ANOVA for ambulatory distance in the open field with RIM and COC as factors showed a significant RIM x COC interaction ($F_{(1, 20)} = 10.48$, $P = .004$). Cocaine- and cocaine + rimonabant-treated rats travelled significantly more than their corresponding controls ($P < .001$). Rimonabant alone had no effect on
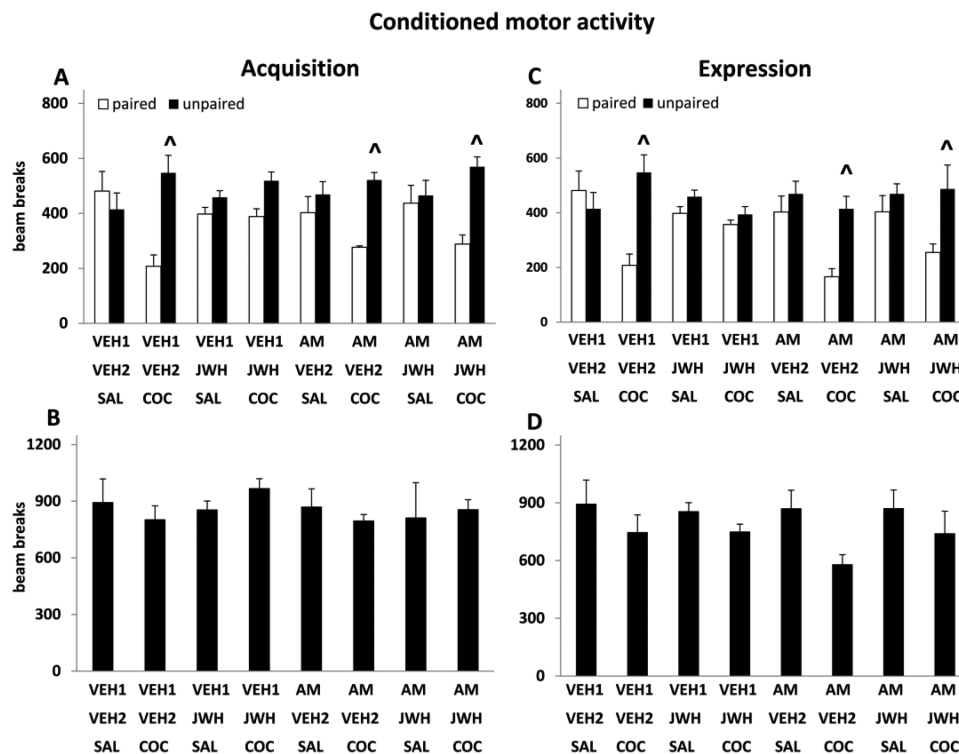
Conditioned motor activity

Figure 4. Effects of JWH-133 on cocaine-elicited conditioned motor activity. (A) Motor activity in drug-paired and unpaired chambers on test day, after pre-pairing administration of JWH-133 and/or AM630 during conditioning. (B) Total motor activity (drug-paired + unpaired chambers) on test day, after pre-pairing administration of JWH-133 and/or AM630 during conditioning. (C) Motor activity in drug-paired and unpaired chambers on test day, after JWH-133 and/or AM630 administration on test day. (D) Total motor activity (drug-paired + unpaired chambers) on test day, after JWH-133 and/or AM630 administration on test day. COC, 20 mg/kg cocaine; JWH, 10 mg/kg JWH-133; AM630, 5 mg/kg AM. ^ compared with unpaired chamber.
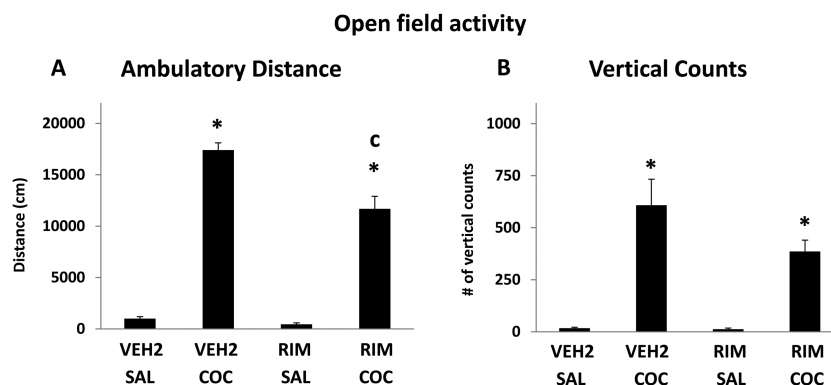
Open field activity

Figure 5. Effects of rimonabant on cocaine-stimulated motor activity. (A) Horizontal motor activity. The bars represent mean + SEM of the ambulatory distance (cm). (B) Vertical motor activity. The bars represent mean + SEM of the number of vertical counts. COC, 20 mg/kg cocaine; RIM, 3 mg/kg rimonabant. *Compared with respective vehicle treatment, C compared with cocaine (VEH2 + COC).

ambulatory distance, while cocaine + rimonabant treatment decreased ambulatory distance compared with cocaine treatment ($P < .001$).

Rimonabant preadministration had no statistically significant effect on cocaine-stimulated vertical motor activity ($P = .1$) (Figure 5B). Two-way ANOVA for vertical activity in the open field with RIM and COC as factors showed a significant main effect of COC (F(1, 20) = 45.79, $P < .001$).

Effects of JWH-133 on Cocaine-Induced Motor Activity

JWH-133 preadministration at 10 mg/kg decreased cocaine-induced increase in ambulatory distance by approximately 70%, while it blocked cocaine-induced stimulation of vertical activity (Figure 6). JWH-133 preadministration decreased cocaine-induced increase in ambulatory distance, and this effect was abolished following preadministration of AM630 (Figure 6A). Three-way ANOVA for ambulatory distance with JWH-133, AM630, and COC as factors showed a significant JWH x COC interaction (F(1, 37) = 83.05, $P < .001$). Subsequent analyses revealed that JWH-133 alone did not affect ambulatory distance, but when it was administered prior to cocaine it significantly decreased cocaine-induced ambulatory distance ($P < .001$). Preadministration of AM630 prevented the inhibitory effect of JWH-133 on cocaine-induced horizontal motor activity ($P < .001$).

JWH-133 preadministration blocked cocaine-induced stimulation of vertical activity (Figure 6B). Three-way ANOVA for ambulatory distance with JWH-133, AM630, and COC as factors showed a significant JWH x COC interaction (F(1, 37) = 34.22, $P < .001$). Subsequent analyses revealed that JWH-133 alone did not affect vertical counts, but when it was administered prior to cocaine it significantly decreased cocaine-induced vertical activity ($P < .001$). Cocaine-induced vertical activity after preadministration of AM630 followed by JWH13 was higher than cocaine-induced activity after JWH-133 administration alone ($P = .009$) and lower than cocaine-induced activity after vehicle administration ($P = .03$).

Discussion

Here we show that blockade of cannabinoid CB1 receptors and stimulation of CB2 receptors modulate in a similar manner cocaine-induced CPP, conditioned motor activity, and the motor stimulatory effects of cocaine. The CB1 receptor antagonist rimonabant attenuated cocaine-induced CPP acquisition and abolished cocaine-induced CPP expression. The CB2 receptor agonist JWH-133 impaired cocaine-induced CPP acquisition and expression. These effects of JWH-133 were prevented by preadministration of the CB2 receptor antagonist AM630. Rimonabant reduced the expression of cocaine-induced conditioned motor activity, while JWH-133 abolished both the acquisition and expression of cocaine-induced conditioned motor activity. The effects of JWH-133 were prevented by preadministration of the CB2 receptor antagonist AM630. Regarding open field activity, rimonabant decreased cocaine-stimulated motor activity and JWH-133 decreased cocaine-stimulated horizontal and vertical motor activity. Neither drug had significant effects on motor activity when administered alone. The effects of JWH-133 on cocaine-stimulated motor activity were prevented by AM630 preadministration.

As previously shown (e.g., Spyraki et al., 1987; Mueller and Stewart 2000; Harris and Aston-Jones 2003; Krasnova et al., 2008), cocaine produced a reliable preference for the drug-paired compartment in the CPP paradigm in the current study. In contrast, neither rimonabant nor JWH-133 nor AM630 had primary motivational properties, as they did not elicit preference or aversion for the paired CPP chambers. This is in agreement with previous studies showing that rimonabant does not produce CPP in rats and mice (Chaperon et al., 1998) and that JWH-133 does not induce CPP in mice (Xi et al., 2011). This suggests that CB1 receptor antagonism and CB2 receptor agonism do not have a central role in hedonic processing that underlies place conditioning in the CPP procedure.

In this study, cocaine-induced CPP acquisition and expression were decreased by rimonabant treatment. Our findings are consistent with previous studies showing that CB1 receptor antagonism decreases or abolishes cocaine CPP acquisition (Chaperon et al., 1998; Yu et al., 2011) and enhances cocaine CPP extinction (Parker et al., 2004) and show, for the first time, a significant inhibitory effect of rimonabant on the expression of cocaine CPP. Rimonabant's inhibitory effects on cocaine CPP expression were stronger than its effects on cocaine CPP acquisition. In the "expression" experiment, rimonabant abolished both chamber preference and cocaine-conditioned motor activity, while in the "acquisition" experiment chamber preference was lower while
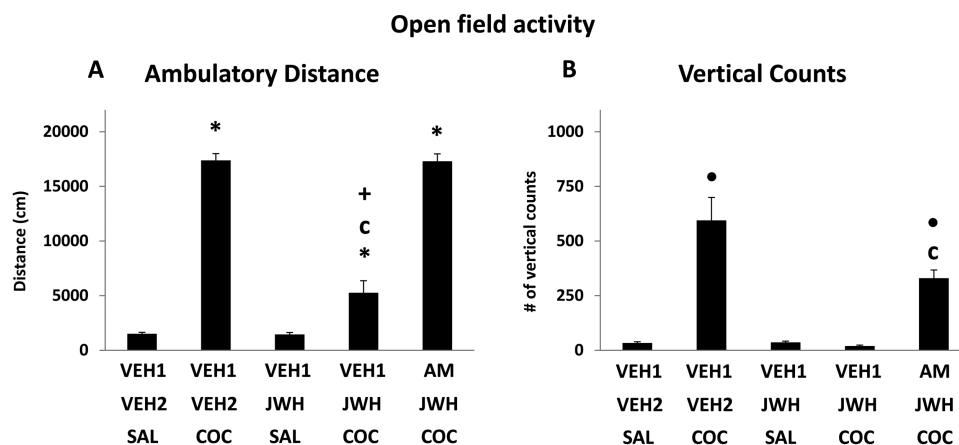

Open field activity

A **Ambulatory Distance**

B **Vertical Counts**

Figure 6. Effects of JWH-133 on cocaine-stimulated motor activity. (A) Horizontal motor activity. The bars represent mean + SEM of the ambulatory distance (cm). (B) Vertical motor activity. The bars represent mean + SEM of the number of vertical counts. COC, 20 mg/kg cocaine; JWH, 10 mg/kg JWH-133; AM, 5 mg/kg AM630. *Compared with vehicle (VEH1+VEH2+SAL) and with JWH (VEH1+JWH+SAL) treatments, C compared with cocaine (VEH1+VEH2+COC), + compared with AM+JWH+COC treatment, ●compared with vehicle (VEH1+VEH2+SAL), JWH (VEH1+JWH+SAL), and VEH1+JWH+COC treatments.

cocaine-conditioned motor activity remained unaffected by rimonabant pre-pairings. We should note that total motor activity during test days, in both experiments, remained unaffected, which excludes the possibility of an unspecific inhibitory effect in animal activity. Our findings related to CPP acquisition (Chaperon et al., 1998; Yu et al., 2011) and expression, together with our previous findings (Polissidis et al., 2009, 2013), further support and strengthen the concept that pharmacological blockade of CB1 receptors diminishes the motivational value of cocaine. Our data are in line with previous studies showing that CB1 receptor antagonism decreases cocaine-induced reinstatement of cocaine seeking in rodents (De Vries et al., 2001; Xi et al., 2006; Adamczyk et al., 2012) and cue-induced reinstatement of cocaine seeking in rodents and non-human primates (De Vries et al., 2001; Schindler et al., 2016). Lack of CB1 receptors in knockout mice has also been shown to impair cocaine self-administration (Soria et al., 2005; Ward et al., 2009) and decrease the breaking point during a progressive-ratio schedule (Ward and Walker 2009). On the other hand, findings excluding the role of CB1 receptors in the rewarding effects of cocaine based on data with CB1 receptor knockout mice have also been reported (Martin et al., 2000; Cossu et al., 2001; Houchi et al., 2005). Moreover, pharmacological blockade of CB1 receptor had no effect on cocaine self-administration and cocaine-induced reinstatement in nonhuman primates (Tanda et al., 2000; Justinova et al., 2005). Discrepancies in the results of these studies could be attributed to strain differences and protocol variations and show that the involvement of endocannabinoid signaling in cocaine dependence warrants further investigation (Wiskerke et al., 2008).

Our findings related to CB2 receptor agonism clearly show for the first time that preconditioning administration of JWH-133 prevented cocaine-evoked CPP acquisition in rats. These results are consistent with previous findings in mice showing that O-1966, a CB2 receptor agonist, decreases cocaine CPP (Ignatowska-Jankowska et al., 2013), JWH-133 decreases cocaine self-administration (Xi et al., 2011; Zhang et al., 2016), and CB2 receptor overexpression also decreases cocaine self-administration (Aracil-Fernandez et al., 2012). In the current study, JWH-133 also decreased the expression of cocaine CPP. These findings show that apart from CB1, CB2 receptors are also involved in the rewarding effects of cocaine and more specifically suggest that the establishment of the primary rewarding effects of cocaine (acquisition phase), but also the manifestation of reinforcement-related preference (expression phase), are negatively modulated by CB2 receptor agonism, similarly to CB1 receptor antagonism.

The decrease in cocaine-induced place preference after pre-pairing or pre-test administration of JWH-133 could not be attributed to an unspecific effect on motor activity, since total motor activity on test day was not affected. However, both JWH-133 treatments decreased cocaine-conditioned motor activity on test day, which could be directly related to the respective decreases in preference for the cocaine-paired chamber. Our results also suggest that acute JWH-133 administration modifies psychomotor aspects of behavior similarly to subchronic JWH-133 administration. These findings further support our CPP data and highlight that apart from CB1, CB2 receptors exert a powerful action on rewarding properties of cocaine despite their relatively low expression levels.

Cocaine-stimulated motor activity in a familiar environment was decreased when rimonabant was administered prior to cocaine, in contrast to basal motor activity that was not affected by rimonabant. Our results are consistent with a previous study showing that CB1 receptor antagonism had no effects on mouse basal motor activity (Lesscher et al., 2005). On the other hand,

CB1 receptor knockout mice presented lower basal motor activity compared with wild-type animals (Martin et al., 2000; Li et al., 2009), an effect that was not supported by other studies (Soria et al., 2005). Previous studies in mice have also shown that CB1 receptor antagonism or knockout decrease cocaine-induced motor activity (Martin et al., 2000; Corbille et al., 2007; Gerdeman et al., 2008; Li et al., 2009), which is in contrast to other studies showing no effect of CB1 receptor deletion on cocaine-stimulated motor activity (Lesscher et al., 2005; Miller et al., 2008). Overall, the current study suggests that while CB1 receptors are not involved in the regulation of basal motor activity, they are significantly involved in cocaine-stimulated motor activity.

Similar to rimonabant, JWH-133 treatment had no effects on basal horizontal motor activity in the present study, in agreement with previous studies in mice (Xi et al., 2011) or on basal vertical motor activity, which rules out the possibility of JWH-133-induced side-effects on motor- and exploratory-related behaviors. The substantial decrease in cocaine-induced horizontal motor activity and the abolishment of cocaine-induced vertical motor activity, which are completely or largely prevented by preadministration with AM630, a CB2 receptor antagonist, illustrate the powerful modulatory role of CB2 receptors in response to psychostimulants.

The fact that antagonism at CB1 receptors and agonism at CB2 receptors produce similar effects on cocaine-induced behaviors may be indicative of a dynamic, functional crosstalk between CB1 and CB2 receptors that can form functional CB1-CB2 heteromers in the nucleus accumbens characterized by bidirectional antagonism (Callen et al., 2012). Alternatively, CB1 and CB2 receptors could have independent effects on the regulation of dopamine neuron function in response to cocaine. It has been recently shown that cocaine mobilizes the production of endocannabinoids in the ventral tegmental area that, in turn, inhibit local GABA release via presynaptic CB1 receptors, thus leading to disinhibition of dopamine neurons and increased dopamine release in the nucleus accumbens (Wang et al., 2015). These effects are blocked by CB1 receptor antagonists, consistent with the inhibitory effects of CB1 receptor antagonism or knockout on cocaine-induced dopamine release (Li et al., 2009; Mereu et al., 2015). On the other hand, activation of CB2 receptors that are localized postsynaptically (Brusco et al., 2008), decreases ventral tegmental neuronal firing (Zhang et al., 2014, 2016) as well as basal and cocaine-induced dopamine release in the nucleus accumbens (Xi et al., 2011). Therefore, regulation of dopamine neuron function could be under the indirect stimulatory effect of CB1 receptors that are located on ventral tegmental area afferents and under the direct inhibitory effect of CB2 receptors that are located on the dopaminergic neurons.

Our findings also show that acute rimonabant or JWH-133 administrations decrease both cocaine-stimulated and cocaine-elicited conditioned motor activity. These results suggest that motor activity in response to a psychostimulant and activity associated with the manifestation of reinforcement-related preference (during CPP expression) are similarly modulated by CB1 receptor antagonism and CB2 receptor agonism. The results further imply that comparable or overlapping brain mechanisms could be engaged in response to CB receptor modulation of cocaine-stimulated and cocaine-elicited conditioned motor activity.

In summary, the present results extend our previous findings showing that cannabinoid CB1 receptors negatively modulate behavioral effects of amphetamine (Polissidis et al., 2014) to cocaine. Here we show, for the first time and under directly comparable and validated methodological conditions, that CB2 receptor agonism exerts analogous behavioral effects with

CB1 receptor antagonism in acquisition and expression of cocaine-induced CPP as well as hyperactivity in rats. This study demonstrates that the cannabinoid system modulates cocaine-induced behavioral effects via both types of CB receptors and suggests that CB1 and CB2 receptors have differential, and perhaps opposing, roles in the regulation of cocaine's rewarding and motor effects. This functional interaction in endocannabinoid signaling provides novel information regarding the neurobiological underpinnings of cocaine addiction and its potential for pharmacological interventions.

Acknowledgments

Statement of Interest

None.

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