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RESEARCH PAPER

Cannabinoid CB_1 receptors mediate the effects of corticotropin-releasing factor on the reinstatement of cocaine seeking and expression of cocaine-induced behavioural sensitization

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BACKGROUND AND PURPOSE

The endocannabinoid and corticotropin-releasing factor (CRF) systems have been implicated in several long-lasting behavioural effects of prior cocaine experience. The present experiments were designed to probe functional interactions between endocannabinoids and CRF by testing the role of cannabinoid CB_1 receptors in cocaine-related behaviours induced or mediated by CRF.

EXPERIMENTAL APPROACH

In Experiment 1, rats trained to self-administer cocaine were pretreated with the CB_1 receptor antagonist, AM251 (0, 10, 100 or 200 μg, i.c.v.), before tests for reinstatement in response to CRF (0, 0.5 μg, i.c.v.), intermittent footshock stress (0, 0.9 mA) or cocaine (0, 10 mg·kg^{-1}, i.p.). In Experiment 2, rats pre-exposed to cocaine (15–30 mg·kg^{-1}, i.p.) or saline for 7 days were pretreated with AM251 (0, 10 or 100 μg, i.c.v.) before tests for locomotion in response to CRF (0.5 μg, i.c.v.), cocaine (15 mg·kg^{-1}, i.p.) or saline (i.c.v.).

KEY RESULTS

Pretreatment with AM251 selectively interfered with CRF-, but not footshock- or cocaine-induced reinstatement. AM251 blocked the expression of behavioural sensitization induced by challenge injections of both CRF and cocaine.

CONCLUSIONS AND IMPLICATIONS

These findings reveal a mediating role for CB_1 receptor transmission in the effects of CRF on cocaine-related behaviours.

Abbreviations

2-AG, 2-arachidonoylglycerol; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CPP, conditioned place preference; CRF, corticotropin-releasing factor; NAc, nucleus accumbens; PE, pre-exposure; PFC, prefrontal cortex; TCAP, teneurin C-terminal associated peptides; VTA, ventral tegmental area

Introduction

Central actions of the stress-related neuropeptide, corticotropin-releasing factor (CRF), have been implicated in several long-lasting behavioural effects of prior cocaine experience. For example, in an animal model of relapse known as the reinstatement procedure, CRF, acting within the bed nucleus of the stria terminalis (BNST; Erb and Stewart, 1999; Erb *et al.*, 2001) and ventral tegmental area (VTA; Wang *et al.*, 2005), has been found to mediate the reinstatement of cocaine seeking in response to acute footshock stress (Erb *et al.*, 1998). Moreover, central injections of CRF itself into the lateral ventricles (Erb *et al.*, 2006), BNST (Erb and Stewart, 1999), or VTA (Wang *et al.*, 2007), reinstate cocaine seeking. CRF has similarly been implicated in the expression of long-term behavioural sensitization to cocaine. Indeed, repeated injections of cocaine, according to a regimen known to produce long-lasting enhancement of locomotion in response to a subsequent cocaine challenge, have been found to induce a potentiated locomotor response to subsequent i.c.v. injections of CRF (Erb *et al.*, 2003). Furthermore, in cocaine-sensitized rats, i.c.v. pretreatment with the non-selective CRF receptor antagonist, D-Phe $CRF_{12–41}$, or systemic pretreatment with the selective CRF_1 receptor antagonist, CP-154526, blocks the expression of behavioural sensitization to a subsequent cocaine challenge (Przegaliński *et al.*, 2005; Erb and Brown, 2006).

In recent years, the endocannabinoids, primarily through their actions at cannabinoid CB_1 receptors (receptor nomenclature follows Alexander *et al.* 2011), have been implicated in the expression of some long-term behavioural effects of cocaine. For example, systemic pretreatment with CB_1 receptor antagonists, such as SR141716A, blocked the reinstatement of cocaine seeking induced by non-contingent administration of cocaine (De Vries *et al.*, 2001; Filip *et al.*, 2006; Xi *et al.*, 2006; but see Ward *et al.*, 2009), but not by footshock stress (De Vries *et al.*, 2001). Somewhat in contrast, pretreatment with the selective CB_1 receptor antagonist, AM251, was without effect on cocaine-induced reinstatement of an extinguished cocaine conditioned place preference (CPP) but blocked its reinstatement by forced-swim stress (Vaughn *et al.*, 2011). Although the role of CB_1 receptors in cocaine-induced behavioural sensitization has been the subject of fewer investigations, studies carried out to date indicate an antagonizing effect of acute SR141716A administration on the expression of cocaine-induced locomotor sensitization to a cocaine challenge (Filip *et al.*, 2006; Ramiro-Fuentes and Fernandez-Espejo, 2011).

Growing neuroanatomical and functional evidence suggests that endocannabinoid and CRF systems may interact to mediate various behaviours, including those unique to subjects with prior cocaine experience. For example, *in situ* hybridization studies reveal significant co-localization of the mRNA of CB_1 receptors and both CRF peptide and CRF_1 receptors in brain regions implicated in affective behaviours (Hermann and Lutz, 2005; Cota *et al.*, 2007). Moreover, recent work from our laboratory has demonstrated that pretreatment with the CB_1 receptor antagonist, AM251, blocked the anxiety-like behaviour induced by i.c.v. CRF administration and withdrawal from chronic cocaine administration (Kupferschmidt *et al.*, 2011c), an effect known to be mediated by CRF signalling (Sarnyai *et al.*, 1995; DeVries and Pert, 1998).

Accordingly, the present experiments were designed to further probe functional interactions between endocannabinoids and CRF by testing the role of CB_1 receptor transmission in cocaine-related behaviours induced or mediated by CRF. Overall, our findings reveal a mediating role for CB_1 receptors in the effects of CRF on the reinstatement of cocaine seeking and the expression of cocaine-induced behavioural sensitization.

Methods

All animal care and experimental procedures were in accordance with Canadian Council of Animal Care guidelines and approved by the University of Toronto animal care committee. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010). A total of 122 animals were used in these experiments.

Experiment 1: effects of AM251 on reinstatement of cocaine seeking

Subjects. Male Long–Evans rats (Charles River, Montreal, QC, Canada; $n = 67$; 275–300 g initial weight) were used. Rats were individually housed in plastic cages in a temperature-(21°C) and humidity-controlled vivarium and maintained on a reverse light–dark schedule (lights on 1900–0700) with free access to water and standard laboratory rat chow.

Surgery. Under isoflurane anaesthesia (3–5% in O_2; Benson Medical, Markham, ON, Canada), rats were implanted with a 22-gauge cannula (Plastics One, Roanoke, VA). The cannula was aimed 1 mm above the right lateral ventricle, according to the following stereotaxic coordinates: A/P: –1.0 mm from bregma; M/L: –1.4 mm from bregma; D/V: –2.7 mm from dura (Paxinos and Watson, 1997). Rats were also implanted with a silastic i.v. catheter (Dow Corning, Midland, MI; inner diameter: 0.51 mm; outer diameter: 0.94 mm) into the right jugular vein. The catheter was a total length of 12 cm, with 3 cm inserted into and secured to the vein with silk sutures. The remaining 9 cm was passed s.c. to the skull surface, where it exited into a modified 22-gauge cannula (Plastics One) that, along with the i.c.v. cannula, was embedded in dental cement and anchored to the skull with jeweller's screws. At the end of the surgery, the catheter was flushed with 0.2 mL of a solution containing 50% heparin (1000 IU) and 50% dextrose (25 g/50 mL), and a plastic blocker was placed over the opening of the cannula to protect the catheter from external debris and maintain catheter patency. Likewise, a stainless steel dummy cannula was placed in the i.c.v. guide cannula. Rats were given a 7 day recovery period before commencing any behavioural procedures.

Materials. Cocaine HCl (Medisca Pharmaceuticals, St. Laurent, QC, Canada) was dissolved in saline at concentrations of 3.5 $mg \cdot mL^{-1}$ (injected in a volume of 65 µL; i.v.) or 10 $mg \cdot mL^{-1}$ (injected in a volume of 1 $mL \cdot kg^{-1}$; i.p.). AM251 [(N-(piperidin-1-yl)-5(4-iodophenyl)-1-(2,4-dichlorophenyl)-

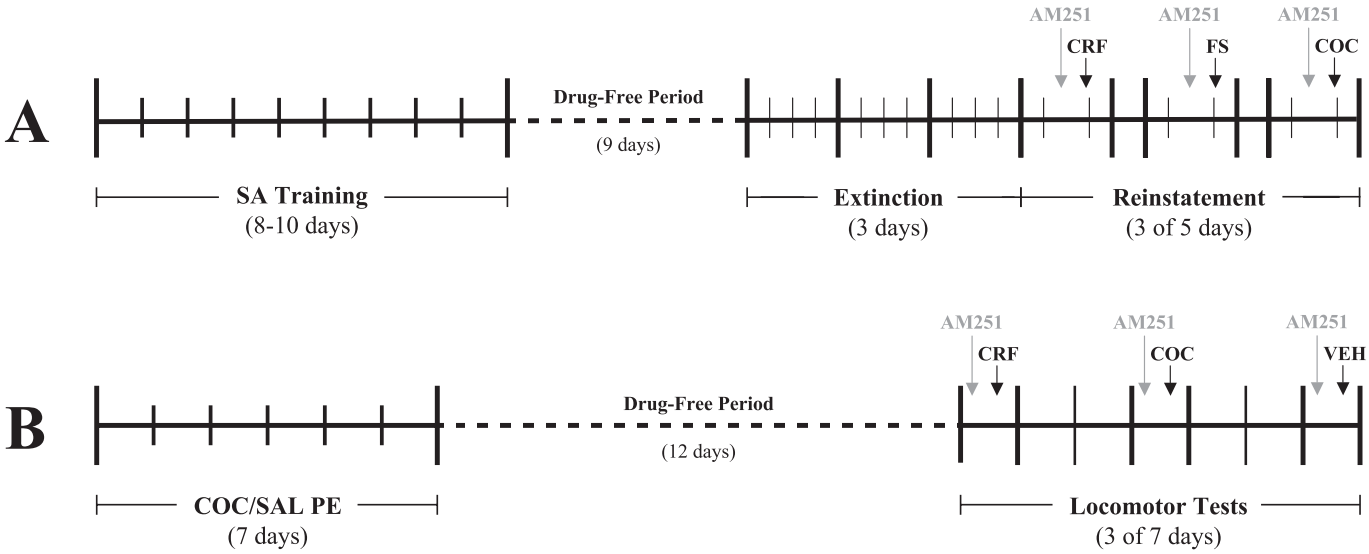

Figure 1

Procedural timelines for experiments 1 (A) and 2 (B). Footshock (FS); cocaine (COC); saline (SAL); pre-exposure (PE).

4-methyl-1*H*-pyrazole-3-carboxamide] (Tocris BioScience, Burlington, ON, Canada) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Oakville, ON, Canada) at concentrations of 2.5, 25 and 50 $\mu g \cdot \mu L^{-1}$ and injected in a volume of 4 µL (10, 100, 200 µg, i.c.v.); DMSO was injected as the vehicle, when no AM251 (0 µg AM251) was given. The dose range and vehicle used for AM251 were based on previous work (Sink *et al.*, 2009; Kupferschmidt *et al.*, 2011c). CRF (Sigma Aldrich) was dissolved in saline at a concentration of 0.125 $\mu g \cdot \mu L^{-1}$ and injected in a volume of 4 µL (0.5 µg, i.c.v.). AM251 and CRF were infused using 28-gauge stainless steel injectors that extended 1 mm below the tip of the cannula to the site of injection. Infusions took place over a 2 min period; injectors were left in place for an additional 2 min following infusion to prevent backflow.

Apparatus. The self-administration (SA) chambers were equipped with two retractable levers (Medical Associates, St. Albans, VT) located 6 cm above a stainless steel rod floor. An infusion pump (Razel Scientific Instruments, Stamford, CN) was activated by responses on one lever (active lever). Each active lever response was automatically recorded using a computer interface operating Medical-IV software (Medical Associates). Responses on the second lever (inactive lever) were recorded but did not result in activation of the pump. Each chamber was equipped with a white stimulus light, located directly above the active lever, and a white house light. Each chamber was also fitted to deliver constant-current, intermittent, inescapable, electric footshock through a scrambler to the metal rod floor (Medical Associates). Footshock was delivered according to a variable time schedule at a mean interval of 40 s (10–70 s range). Each shock (0.9 mA) was 0.5 s in duration.

Procedures. Experiment 1 was conducted in four phases: (i) self-administration (SA) training; (ii) drug-free period; (iii) extinction and (iv) testing for reinstatement. A timeline of the experimental procedures is provided in Figure 1A.

SA training. Before the start of training, rats were habituated to the SA chambers during one 2 h session. Twenty-four to 48 h later, rats were trained to self-administer cocaine (0.23 mg per infusion, i.v.) on a fixed ratio-1 schedule of reinforcement, during once daily 3 h sessions, as described previously (Kupferschmidt *et al.*, 2011a). Briefly, the availability of cocaine was signalled by the introduction of the active lever into the chamber, illumination of the white house light (which remained lit throughout the session), and illumination of the white stimulus light above the active lever for 20 s. During the SA session, responses on the active lever resulted in a 3 s infusion of cocaine (in 65 mL saline) and 20 s illumination of the stimulus light, which signalled a 'timeout' period in which additional responses were recorded but not reinforced. SA training was conducted for 10 days. Rats exhibiting stable SA (less than 20% variance in number of infusions between the last 2 days of training) proceeded to subsequent phases of the experiment.

Drug-free period. Rats were given a drug-free period of 9 days, such that extinction and testing for reinstatement occurred outside of the initial cocaine withdrawal period. Rats were left undisturbed in their home cages during this time, with the exception of routine cleaning, feeding and monitoring of weight and health.

Extinction. Rats were given three consecutive days of extinction training. Days 1 and 2 consisted of four 60 min extinction sessions, during which all conditions present during SA training were maintained, except that lever presses were not reinforced by cocaine. Each 60 min session was initiated by the same events that occurred at the start of SA training sessions, and extinction sessions were separated by

30 min intervals, during which the active lever was retracted. On day 3 of extinction, conditions were the same as on days 1 and 2, with two exceptions: (i) rats were given one sham i.c.v and one i.p. saline injection, separated by 30 min, between the third and fourth extinction sessions; and (ii) the fourth session began 10 min after the i.p. saline injection. Sham injections were given to familiarize rats with the manipulations used in subsequent tests for reinstatement.

Testing for reinstatement. Following extinction, rats were given up to three days of reinstatement testing. At the start of each test day, rats were given three 60 min extinction sessions. Rats that exhibited 20 or fewer responses on the active lever during the second and third sessions (combined) were subsequently tested for reinstatement. Rats that did not reach this criterion were given an additional extinction session, and testing was delayed for 1 day. Immediately following the third extinction session, rats were pretreated acutely with AM251 (0, 10, 100 or 200 μg; i.c.v.); different groups of rats were tested with different doses. Thirty minutes later, rats were exposed to a reinstatement challenge [CRF (0.5 μg; i.c.v.); footshock (20 min; 0.9 mA); cocaine (10 mg·kg^{-1}; i.p.)] or its corresponding baseline condition [i.c.v. saline; no footshock; i.p. saline]. Thirty, 0 or 10 min following the CRF, footshock, or cocaine challenges (or corresponding baseline conditions), respectively, tests for reinstatement began, whereby the previously drug-reinforced (active) lever was extended into the chamber and responding was recorded for 60 min. Responding on the active lever following exposure to CRF, footshock or cocaine, relative to the baseline condition, was used as a measure of the reinstatement of cocaine seeking. Each rat was given up to three tests for reinstatement on three separate days; tests were separated by 48 h and administered in a counterbalanced order. Based on initial indications that the higher doses of AM251 (100 and 200 μg) completely blocked CRF-induced reinstatement, a lower dose (10 μg) was added in subsequent replications to achieve a meaningful dose–response profile.

Data analyses. The main dependent measures in Experiment 1 were the number of responses on the active and inactive levers in response to the test challenges (CRF, footshock and cocaine) and their baseline conditions (vehicle [VEH], No FS). Separate analyses were carried out for each test condition, using repeated measures ANOVA for the between-subjects factor of AM251 (0, 10, 100, 200 μg), and the within-subjects factor of test (VEH, CRF/footshock/cocaine). Significant interactions were followed by Fisher's LSD *post hoc* comparisons ($P < 0.05$), as appropriate.

Experiment 2: effects of AM251 on cocaine sensitization

Subjects. Male Wistar rats (Charles River; $n = 55$; 275–300 g initial weight) were used. Rats were maintained under conditions identical to those described in Experiment 1. Our research program for studies on psychostimulant sensitization has developed using the Wistar rats, in part because this albino strain offers a suitable contrast of a white object on a black apparatus background, which is ideal for the purposes of configuring our activity monitoring system (Ethovision by Noldus, Wageningen, the Netherlands). In addition, this strain has been used widely in studies of psychostimulant sensitization (Erb *et al.*, 2003; Przegaliński *et al.*, 2005; Ramiro-Fuentes and Fernandez-Espejo, 2011).

Surgery. Under isoflurane anaesthesia (3–5% in O2; Benson Medical, Markham, ON, Canada), rats were implanted with a 22-gauge cannula in the right lateral ventricle, as described in Experiment 1. Rats were given a 7 day recovery period before commencing the experimental procedures.

Materials. Cocaine HCl (Medisca Pharmaceuticals) was dissolved in saline at concentrations of 15 and 30 mg·mL^{-1}, and injected in a volume of 1 mL·kg^{-1} (i.p.). AM251 (Tocris Bio-Science) and CRF (Sigma-Aldrich) were dissolved and injected as described in Experiment 1.

Apparatus. Locomotor testing was carried out in opaque Plexiglas chambers (40 × 25 × 20 cm) with wire mesh lids. An infrared camera positioned above the chambers recorded distance travelled (cm) using EthoVision software (version 3, Noldus).

Procedures. Experiment 2 was conducted in four phases: (i) habituation; (ii) cocaine pre-exposure; (iii) drug-free period and (iv) testing for sensitization. A timeline of the experimental procedures is provided in Figure 1B.

Habituation. Seven days after surgery, rats were transported to the room housing the behavioural testing apparatus, placed in the activity chambers for 60 min, administered an injection of saline (i.p.) and returned to the chambers for an additional 120 min. Activity was monitored both before and after the saline injection. Rats were assigned to cocaine or saline pre-exposure conditions such that each group was equated based on their average level of activity in the post-saline injection period.

Cocaine pre-exposure. Starting 48 h after habituation, rats were given once daily injections of cocaine or saline for 7 days. The first and last injections (15 mg·kg^{-1}, i.p.) were administered in the activity chambers, immediately after a 60 min habituation period. The five intervening injections were given in the home cages (30 mg·kg^{-1}, i.p.). This dosing regimen has been found previously to produce behavioural sensitization (Churchill *et al.*, 1999; Kupferschmidt *et al.*, 2011b).

Drug-free period. To parallel the drug-free period in Experiment 1, rats were given a drug-free period of 12 days between cocaine exposure and testing.

Locomotor testing. Following the drug-free period, rats were given three tests for locomotor sensitization. On each test day, rats were initially placed in the activity chambers for 60 min. Rats were then pretreated with AM251 (0, 10 or 100 μg; i.c.v.); different groups of rats were tested with different doses. Thirty minutes later, rats were given challenge injections of CRF (0.5 μg, i.c.v.), cocaine (15 mg·kg^{-1}, i.p.) or saline (i.c.v.), and returned to the chambers to assess locomo-

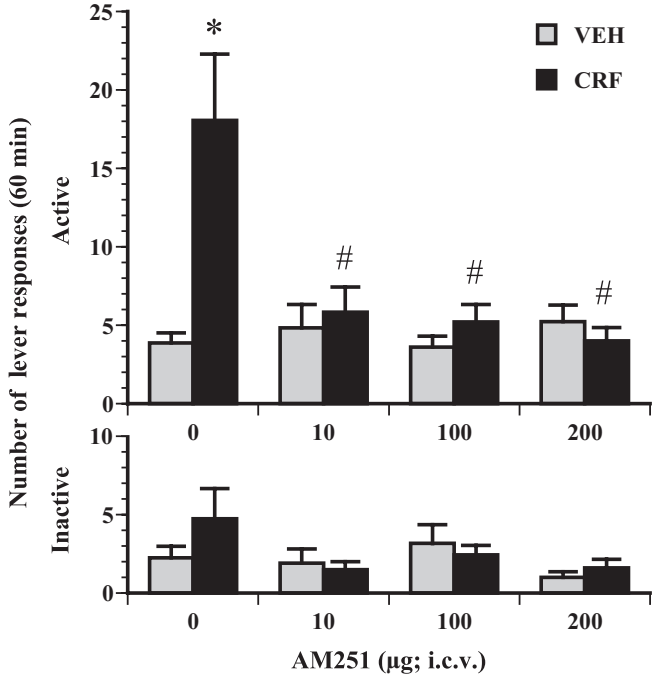

Figure 2

Mean (± SEM) number of responses on the active and inactive levers during 60 min reinstatement test sessions prior to which rats were pretreated with AM251 (0, 10, 100 or 200 µg; i.c.v.) and subsequently challenged with saline (VEH; i.c.v.) or CRF (0.5 µg; i.c.v.). In rats pretreated with 0 µg AM251, CRF induced a higher level of responding on the active lever than VEH (*$P < 0.005$). Rats pretreated with 10, 100 or 200 µg AM251 responded less on the active lever in response to CRF than those pretreated with 0 µg AM251 (#$P < 0.01$).

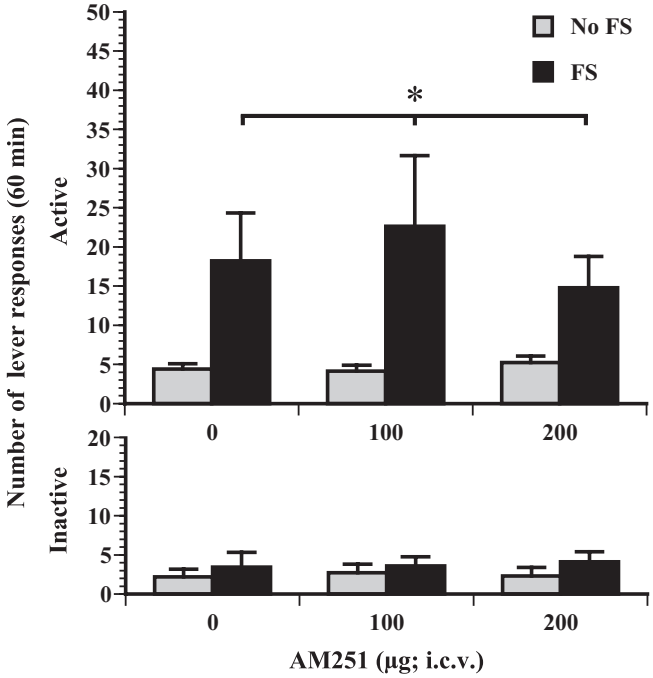

Figure 3

Mean (±SEM) number of responses on the active and inactive levers during 60 min reinstatement test sessions prior to which rats were pretreated with AM251 (0, 100 or 200 µg; i.c.v.) and subsequently exposed to intermittent footshock (FS; 20 min; 0.9 mA) or no footshock (No FS). Irrespective of AM251 pretreatment, footshock induced a higher level of responding on the active lever than No FS (*$P < 0.005$).

tor activity for an additional 120 min. Rats were tested in response to each challenge on separate days in a counterbalanced order, and tests were separated by 48 h.

Data analyses. The main dependent measure in Experiment 2 was the distance travelled (cm) during the CRF, cocaine and vehicle tests for locomotion. Separate analyses were carried out for each test condition, using two-way ANOVA for the between-subjects factors of cocaine pre-exposure (cocaine, saline) and AM251 (0, 10, 100, 200 µg). Significant interactions were followed by Fisher's LSD *post hoc* comparisons ($P < 0.05$), as appropriate.

Results

Experiment 1: effects of AM251 on reinstatement of cocaine seeking

Figure 2 shows the mean ± SEM number of responses on the active (A) and inactive (B) levers during 60 min reinstatement test sessions prior to which rats were pretreated with AM251 (0, 10, 100 or 200 µg; i.c.v.) and subsequently challenged with saline (VEH; i.c.v.) or CRF (0.5 µg; i.c.v.). It can be seen that, across the dose range tested, AM251 blocked CRF-induced reinstatement of cocaine seeking. Indeed, a repeated measures ANOVA of total responses on the active lever revealed significant main effects of CRF [$F(1,55) = 8.59$, $P < 0.005$] and AM251 [$F(3,55) = 5.62$, $P < 0.005$], and a significant CRF × AM251 interaction [$F(3,55) = 7.30$, $P < 0.001$]. In the 0 µg AM251 group, CRF, relative to VEH, induced a higher level of responding on the active lever ($P < 0.005$); in each of the 10, 100 and 200 µg AM251 groups, this difference between CRF and VEH tests was non-significant. In addition, rats in the 0 µg AM251 group, relative to all other dose groups, responded significantly more on the active lever following CRF treatment ($P < 0.01$).

Figure 3 shows the mean ± SEM number of responses on the active (A) and inactive (B) levers during 60 min reinstatement test sessions prior to which rats were pretreated with AM251 (0, 100 or 200 µg; i.c.v.) and subsequently exposed to intermittent footshock (FS: 20 min; 0.9 mA) or no footshock (No FS). In this case, a repeated-measures ANOVA of responses on the active lever revealed only a significant main effect of footshock [$F(1,25) = 9.24$, $P < 0.005$]. Indeed, irrespective of the dose of AM251 administered, rats responded significantly more under the FS than No FS test condition. Thus, whereas AM251 pretreatment blocked the effect of i.c.v. CRF on reinstatement of cocaine seeking, it was without effect on reinstatement induced by footshock stress.

Figure 4 shows the mean ± SEM number of responses on the active (A) and inactive (B) levers during 60 min reinstatement test sessions prior to which rats were pretreated with

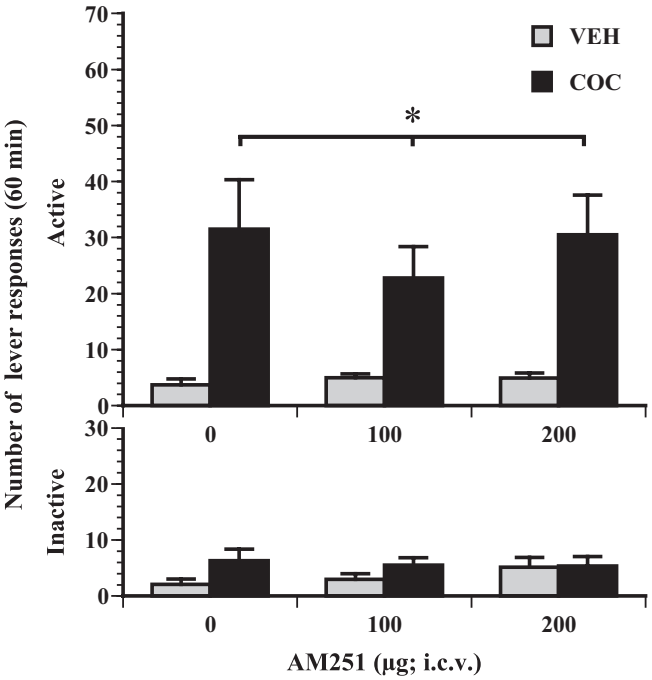

Figure 4

Mean (± SEM) number of responses on the active (A) and inactive (B) levers during 60 min reinstatement test sessions prior to which rats were pretreated with AM251 (0, 100 or 200 µg; i.c.v.) and subsequently challenged with saline (VEH; i.p.) or cocaine (15 mg·kg^{-1}; i.p.). Irrespective of AM251 pretreatment, cocaine induced a higher level of responding on the active lever than VEH (*P < 0.001).

AM251 (0, 100 or 200 µg; i.c.v.) and subsequently challenged with saline (VEH; i.p.) or cocaine (COC: 15 mg·kg^{-1}; i.p.). Similar to the footshock test, a repeated-measures ANOVA of responses on the active lever revealed only a significant main effect of cocaine [$F(1,33) = 32.56$, $P < 0.001$]. Again, irrespective of AM251 pretreatment, cocaine induced a higher level of responding on the active lever than VEH. Thus, AM251 pretreatment had no effect on cocaine-induced reinstatement.

All repeated-measures ANOVA of responses on the inactive lever, irrespective of test condition, were non-significant. As can be seen in the corresponding figures (Figures 2–4), responses on the inactive lever were consistently very low (less than 25% of responses on the active lever) across all pretreatment and test conditions.

Experiment 2: effects of AM251 on cocaine sensitization

Figure 5 (left panel) shows the percent ± SEM distance travelled by cocaine, relative to saline, pre-exposed rats in response to a CRF challenge (0.5 µg; i.c.v.), administered 30 min after acute AM251 pretreatment (0, 10 or 100 µg; i.c.v.) and 12 days after the last cocaine pre-exposure (0 or 15–30 mg·kg^{-1}; i.p). Although the test sessions were 120 min in duration, initial analyses of the raw data revealed that the acute activational effects of CRF occurred in the first 60 min. Thus, subsequent analyses (and presented data) were based on activity monitored during this period. Two-way ANOVA of the mean distance (cm) travelled in the first 60 min revealed a significant interaction of cocaine pre-exposure × AM251 pretreatment [$F(2,48) = 3.36$, $P < 0.05$]. Subsequent analyses of this interaction revealed that in rats pretreated with 0 µg

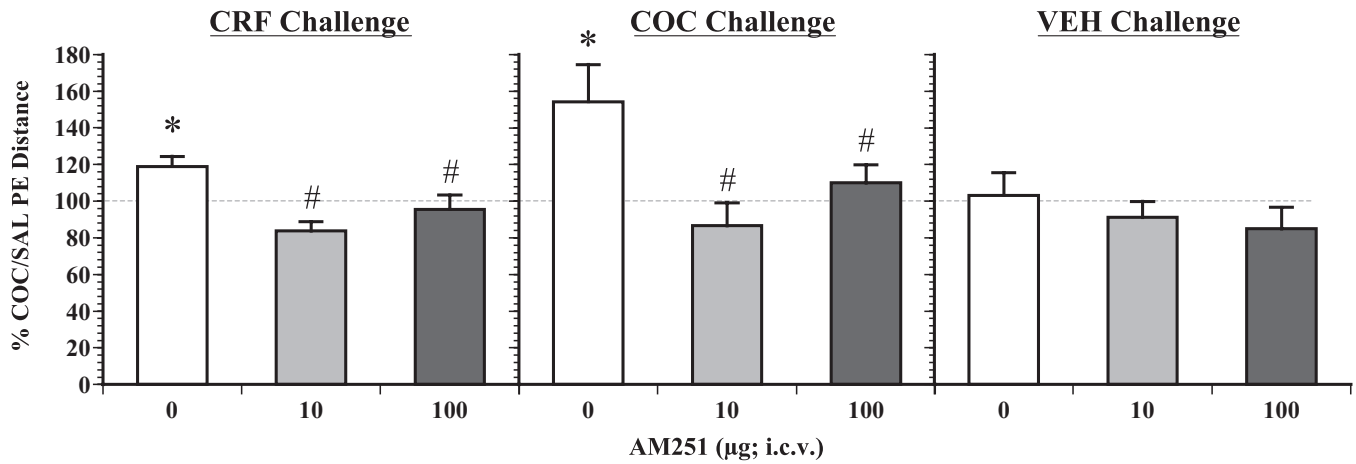

Figure 5

Distance travelled by cocaine (COC) pre-exposed, relative to saline pre-exposed (SAL PE) rats, shown as percent (± SEM), in response to a CRF (0.5 µg; i.c.v.), cocaine (COC; 15 mg·kg^{-1}; i.p.) or saline (VEH; i.c.v.) challenge, administered 30 min after acute AM251 pretreatment (0, 10 or 100 µg; i.c.v.) and 12 days after the last cocaine pre-exposure (0 or 15–30 mg·kg^{-1}; i.p). Statistical analyses were conducted on the mean distance travelled (cm) in response to each challenge. In rats pretreated with 0 µg AM251, CRF and cocaine challenges induced sensitized locomotor responses in COC PE, relative to SAL PE, rats (*P < 0.05). In COC PE rats, pretreatment with 10 and 100 µg AM251 blocked the cocaine-sensitized locomotor responses to CRF and cocaine challenges seen following pretreatment with 0 µg AM251 (#P < 0.05).

AM251, CRF induced a sensitized locomotor response in cocaine, relative to saline, pre-exposed rats, and pretreatment with 10 or 100 μg AM251 blocked this effect of CRF ($P < 0.05$). Moreover, in cocaine pre-exposed rats, 10 and 100 μg AM251, relative to 0 μg AM251, reduced CRF-induced activity ($P < 0.05$). Thus, cocaine pre-exposure resulted in a sensitized response to a CRF challenge that was blocked by pretreatment with AM251.

Figure 5 (middle panel) shows the percent ± SEM distance travelled by cocaine, relative to saline, pre-exposed rats in response to a cocaine challenge (15 mg·kg^{-1}; i.p.), administered 30 min after acute AM251 pretreatment (0, 10 or 100 μg; i.c.v.) and 12 days after the last cocaine pre-exposure (0 or 15–30 mg·kg^{-1}; i.p.). Here, the first 30 min of the 120 min locomotor test were analysed and graphed, corresponding to the time interval in which the greatest activational effects of the cocaine challenge were observed. Analyses of these data revealed a pattern of results that were very similar to those described for the CRF test condition. Two-way ANOVA of mean distance (cm) travelled in the first 30 min revealed a significant interaction of cocaine pre-exposure × AM251 pretreatment [$F(2,49) = 3.27$, $P < 0.05$]. Subsequent analyses of this interaction revealed that in rats pretreated with 0 μg AM251, the cocaine challenge induced a sensitized locomotor response in cocaine, relative to saline, pre-exposed rats and pretreatment with 10 or 100 μg AM251 blocked this effect ($P < 0.05$). Moreover, in cocaine pre-exposed rats, 10 and 100 μg AM251, relative to 0 μg AM251, reduced cocaine-induced activity ($P < 0.05$). Thus, cocaine pre-exposure resulted in a sensitized response to a cocaine challenge that was blocked by pretreatment with AM251.

Figure 5 (right panel) shows the percent ± SEM distance travelled by cocaine, relative to saline, pre-exposed rats in response to a saline challenge (i.c.v.), administered 30 min after acute AM251 pretreatment (0, 10 or 100 μg; i.c.v.) and 12 days after the last cocaine pre-exposure (0 or 15–30 mg·kg^{-1}; i.p.). Although the data for the first 30 min of the test session are presented in the right panel of Figure 5, two-way ANOVA of the mean distance (cm) travelled in either the first 30 or 60 min of the session revealed no significant main effects or interactions of cocaine pre-exposure and AM251 pretreatment. Thus, AM251 alone did not alter locomotor activity, nor did it differentially alter activity in cocaine, relative to saline, pre-exposed rats.

Discussion and conclusions

The present findings are among the first to reveal a role for CB_1 receptors in the effects of CRF on cocaine-related behaviours. Specifically, central administration of the CB_1 receptor antagonist, AM251, blocked the reinstatement of cocaine seeking induced by CRF, but was without effect on reinstatement induced by footshock or cocaine. AM251 further blocked the expression of locomotor sensitization in response to CRF and cocaine challenges. These findings extend upon recent work from our laboratory showing that AM251 blocked the behavioural anxiety induced by i.c.v. CRF and withdrawal from chronic cocaine (Kupferschmidt *et al.*, 2011c), an effect mediated by CRF (DeVries and Pert, 1998).

The CB_1 receptor antagonist, AM251, blocked CRF-induced reinstatement of cocaine seeking but was without effect on footshock- or cocaine-induced reinstatement of cocaine seeking

One striking aspect of our findings is that AM251 potently inhibited CRF-induced reinstatement of cocaine seeking, but did not alter reinstatement by footshock stress, an effect known to be mediated by CRF (Erb *et al.*, 1998; 2001; Erb and Stewart, 1999; Wang *et al.*, 2005). The negative effect of AM251 on footshock-induced reinstatement of cocaine seeking is in fact consistent with work by De Vries *et al.* (2001) involving systemic administration of another CB_1 receptor antagonist, SR141716A. Moreover, it is consistent with other reported dissociations between the effects of footshock and CRF on the reinstatement of cocaine seeking (Brown *et al.*, 2009; Kupferschmidt *et al.*, 2011b). For example, we recently reported that repeated administration of the novel peptide, teneurin C-terminal associated peptide-1 (TCAP-1), completely blocked CRF-induced reinstatement of cocaine seeking over a wide dose range, without having any effect on footshock-induced reinstatement (Kupferschmidt *et al.*, 2011b). Several factors likely contributed to the differential regulation of CRF- and footshock-induced reinstatement by AM251, including the nature of the stressors (pharmacological vs. physical/environmental), the brain systems they engage (Imaki *et al.*, 1993; Dunn, 2000) and the onset and duration of their neurochemical effects (Matsuzaki *et al.*, 1989; Kalivas and Duffy, 1995; Galvez *et al.*, 1996; Erb *et al.*, 2000; de Groote *et al.*, 2005).

Unlike the negative effect of AM251 on footshock-induced reinstatement, its negative effect on cocaine-induced reinstatement was surprising. Indeed, the latter finding contrasts with reports of a mediating role for CB_1 receptors in cocaine-induced reinstatement of cocaine seeking in rats (De Vries *et al.*, 2001; Filip *et al.*, 2006; Xi *et al.*, 2006). Most notably, Xi *et al.* (2006) reported that systemic and intra-striatal administration of AM251 blocked and attenuated this behaviour. Although the discrepancies between these findings are unclear, they may owe to differences in the dose and regional distribution of AM251 resulting from different routes of administration (systemic/intra-striatal vs. i.c.v.). It is also possible that the discrepant findings are related to strain differences. This seems unlikely, however, given that positive effects of CB_1 receptor antagonists on cocaine-induced reinstatement of cocaine seeking have been reported previously in both Long Evans rats (Xi *et al.*, 2006), the strain used in the present reinstatement experiments, as well as in Wistar rats (De Vries *et al.*, 2001; Filip *et al.*, 2006), the strain used in the present sensitization experiments.

It is important to note that our failure to demonstrate a role for CB_1 receptors in cocaine-induced reinstatement of cocaine seeking in rats is in fact consistent with similar studies using mice or employing the CPP procedure. For example, Ward *et al.* (2009) reported that systemic pretreatment of mice with the CB_1 receptor antagonist, SR141716A, had no effect on cocaine-induced reinstatement of cocaine seeking, and Vaughn *et al.* (2011) reported that systemic pretreatment of rats with AM251 failed to alter the reinstatement of extinguished cocaine CPP by a priming injection of the

drug. Thus, clarification of the role of CB_1 receptors in the reinstatement of cocaine-associated behaviours by the drug itself represents an important objective for future research.

The CB_1 receptor antagonist, AM251, blocked the expression of cocaine-induced locomotor sensitization by both a CRF and cocaine challenge

In contrast to our findings that AM251 differentially affects various forms of reinstatement of cocaine seeking, we found that AM251 blocks the cocaine-sensitized locomotor response induced by both CRF and cocaine challenges. These results parallel the inhibitory effects of AM251 we have shown on CRF-induced reinstatement in the present report and on CRF-induced anxiety in another recent study (Kupferschmidt *et al.*, 2011c). They are also consistent with other reports demonstrating a mediating role for CB_1 (Filip *et al.*, 2006; Ramiro-Fuentes and Fernandez-Espejo, 2011) and CRF receptors (Przegaliński *et al.*, 2005; Erb and Brown, 2006) in cocaine-induced expression of behavioural sensitization. Taken together, this evidence suggests that CB_1 receptors may mediate the expression of cocaine-induced locomotor sensitization through a CRF-dependent mechanism.

Potential cellular mechanism of endocannabinoid–CRF interaction

The mechanisms by which endocannabinoids and CRF interact to mediate the reinstatement of cocaine seeking and expression of cocaine-induced sensitization are not known. One possibility is that CB_1 receptor transmission modulates the cellular action of CRF by altering the balance of excitatory and inhibitory tone on CRF receptor-expressing neurons. Endocannabinoids, through their action at CB_1 receptors, are critical regulators of glutamate and GABA release (Freund *et al.*, 2003). CRF, on the other hand, acts primarily as a neuromodulator, whereby it alters the excitability of the post-synaptic membrane so as to enhance or suppress the effects of neurotransmitters like glutamate and GABA (Aldenhoff *et al.*, 1983; Bishop and King, 1992; Haug and Storm, 2000; Gallagher *et al.*, 2008). For example, CRF increases the intrinsic excitability of basolateral amygdala (BLA) projection neurons (Giesbrecht *et al.*, 2010) and VTA dopaminergic neurons (Wanat *et al.*, 2008), and potentiates their response to excitatory input (Ugolini *et al.*, 2008; Hahn *et al.*, 2009). Thus, the cellular actions of CRF may be critically sensitive to endocannabinoid-mediated regulation of excitatory and inhibitory transmission.

Potential brain loci of endocannabinoid –CRF interaction: reinstatement of cocaine seeking

In speculating on possible loci in which endocannabinoids and CRF interact to mediate the reinstatement of cocaine seeking, the BNST and VTA seem reasonable candidates. Indeed, local CRF infusions into either region reinstate cocaine seeking in rats (Erb and Stewart, 1999; Wang *et al.*, 2005). Moreover, both regions express CB_1 receptors (Herkenham *et al.*, 1991; Mátyás *et al.*, 2008) that act to inhibit the local release of glutamate and GABA (Szabo *et al.*, 2002; Melis *et al.*, 2004; Riegel and Lupica, 2004; Puente *et al.*, 2010). Thus, it is conceivable that AM251 modulates excitatory and inhibitory transmission in the BNST and/or VTA to functionally block the effects of CRF on reinstatement behaviour. Problematic for this view, however, are findings that CRF signalling in both the BNST and VTA mediate footshock-induced reinstatement of cocaine seeking (Erb and Stewart, 1999; Wang *et al.*, 2005), but that antagonism of CB_1 receptors fails to interfere with this reinstatement (Experiment 1; De Vries *et al.*, 2001).

Another region of possible interest is the BLA, which expresses CRF_1 and CB_1 receptors more densely than the BNST or VTA (Herkenham *et al.*, 1991; Chalmers *et al.*, 1995; Marsicano and Lutz, 1999; Bittencourt and Sawchenko, 2000). Although functional inactivation of this nucleus is without effect on footshock-induced reinstatement (McFarland *et al.*, 2004), its activation by electrical stimulation and local NMDA infusion reinstates cocaine seeking (Hayes *et al.*, 2003). Moreover, BLA projection neurons, known to innervate many areas implicated in reinstatement [e.g. central nucleus of the amygdala (CeA), BNST, NAc and PFC] (Dong *et al.*, 2001; Sah *et al.*, 2003), are activated by CRF (Bittencourt and Sawchenko, 2000; Ugolini *et al.*, 2008; Giesbrecht *et al.*, 2010) and indirectly regulated by local CB_1 receptor transmission (Katona *et al.*, 2001; Azad *et al.*, 2004; Zhu and Lovinger, 2005). Thus, modulation of transmission within the BLA could help to account for the unique regulation of CRF-induced reinstatement of cocaine seeking by AM251.

Potential brain loci of endocannabinoid-CRF interaction: expression of cocaine-induced sensitization

Two regions of interest in the effects of AM251 on CRF- and cocaine-induced expression of sensitization are the NAc and VTA. The role of both nuclei in behavioural sensitization is well established (Steketee and Kalivas, 2011), and there is recent evidence that intra-NAc administration of the CB_1 receptor antagonist, SR141716A, blocks the expression of cocaine-induced sensitization (Ramiro-Fuentes and Fernandez-Espejo, 2011). With respect to the VTA, repeated exposures to cocaine are known to sensitize VTA dopamine neurons to the activational effects of both cocaine and CRF (Bonci and Williams, 1996; Kalivas and Duffy, 1998; Hahn *et al.*, 2009). In addition, endocannabinoids, released from VTA dopamine neurons under conditions of heightened activity, act on CB_1 receptors to regulate the balance of excitatory and inhibitory input to these same neurons (Riegel and Lupica, 2004; Pan *et al.*, 2008).

In conclusion, although the specific cellular and regional mechanisms are unclear, the present work demonstrates for the first time that CB_1 receptor transmission mediates the effects of CRF on the reinstatement of cocaine seeking and expression of cocaine-induced locomotor sensitization. These findings broaden our understanding of the role of endocannabinoids in cocaine-related behaviours and contribute to an emerging field of study into the functional interactions between the endocannabinoid and CRF systems.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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