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ORIGINAL INVESTIGATION

CP-154,526, a CRF type-1 receptor antagonist, attenuates the cue-and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior in rats

M. C. Moffett · N. E. Goeders

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Abstract
Rationale Previous studies from our laboratory and others have indicated a role for the hypothalamo-pituitary-adrenal (HPA) axis in the extinction/reinstatement animal model of cocaine relapse
Objective This present study was designed to investigate the potential role for the HPA axis in the cue- and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior by determining the effects of ketoconazole and the corticotropin-releasing hormone (CRF) type 1 receptor antagonist, CP-154,526, on these behaviors.
Materials and methods Male Wistar rats were trained to self-administer methamphetamine (0.03 mg/kg/infusion). The delivery of methamphetamine was paired with the presentation of a tone and the illumination of a house light. Once stable responding was reached, the rats were placed into extinction. The effects of pretreatment with ketoconazole (25, 50, or 100 mg/kg, i.p.) or CP-154,526 (20 or 40 mg/kg, i.p.; 3 μg, i.c.v) on cue-induced reinstatement were then evaluated.
Results Cue-induced reinstatement was not significantly attenuated by pretreatment with peripherally administered CP-154,526 or by pretreatment with ketoconazole. However, centrally administered CP-154,526 (3 μg, i.c.v.) significantly attenuated cue-induced reinstatement. In a separate group of rats, CP-154,526 (20 mg/kg, i.p.) attenuated methamphetamine-induced reinstatement (0.12 mg/kg priming infusion); whereas a higher dose (40 mg/kg) was necessary to attenuate reinstatement induced by a priming infusion of 0.24 mg/kg/infusion. Ketoconazole (50 mg/kg) did not affect reinstatement induced by a 0.12 mg/kg priming infusion and, therefore, was not tested at the higher methamphetamine priming dose.
Conclusions These data suggest an important role for CRF in the cue- and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior.

Keywords Methamphetamine · Self-administration · Reinstatement · Cues · CRF · Relapse

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Introduction

The hypothalamo-pituitary-adrenal (HPA) axis has been suggested to play a role in psychostimulant reward. The acquisition of psychostimulant self-administration is facilitated in rats exposed to a stressor. Repeated tail pinch (Piazza et al. 1990), social stress (Haney et al. 1995; Miczek and Mutschler 1996; Tidey and Miczek 1997), social isolation (Howes et al. 2000; Kosten et al. 2000; Schenk et al. 1987), and electric footshock (Goeders and Guerin 1994) have all been found to accelerate the acquisition of psychostimulant self-administration. We have previously shown that ketoconazole, a corticosterone synthesis inhibitor, decreases low-dose cocaine self-administration (Goeders et al. 1998), whereas CP-154,526, a corticotropin-releasing hormone (CRF) type 1 receptor antagonist, decreases cocaine intake across several doses of cocaine (Goeders and Guerin 2000).

Drug craving has often been implicated as a significant factor in the relapse to cocaine-taking after a period of abstinence in humans (Dackis and O'Brien 2001) and has been shown to predict drug use by patients undergoing treatment for methamphetamine dependence (Hartz et al. 2001). The extinction/reinstatement model is often used as an animal model of relapse (Gerber and Stretch 1975; Shaham et al. 2003; Stewart et al. 1984). In this model, rats are trained to self-administer a drug until stable behavior is maintained over several consecutive days. Then, the rats are exposed to prolonged periods of extinction when no drug is delivered. Once responding on the lever previously associated with drug infusions has met the extinction criteria, drug-seeking behavior is reinstated by the presentation of specific stimuli. These stimuli include: acute exposure to a stressor (Erb et al. 1996; Shaham et al. 2000; Shaham and Stewart 1995), the presentation of stimuli previously paired with the drug (Fuchs et al. 1998; Mantsch and Goeders 1999; Meil and See 1996; Weiss et al. 2001), and the acute exposure to the self-administered drug (de Wit and Stewart 1981; Gerber and Stretch 1975).

In addition to the role the HPA axis in the acquisition and maintenance of psychostimulant self-administration, its potential role in relapse has also been demonstrated. Treatment with a CRF receptor antagonist has been shown to block stress-induced reinstatement in heroin- and cocaine-trained rats (Erb et al. 1998; Shaham et al. 1997). The pharmacological attenuation of the corticosterone, using ketoconazole, attenuates the stress-induced reinstatement of cocaine seeking (Mantsch and Goeders 1999). Pretreatment with CRF receptor antagonists (Erb et al. 1998) or ketoconazole (Mantsch and Goeders 1999) has no or only minimal effects on the cocaine-induced reinstatement of cocaine seeking. Erb et al. (1998) demonstrated a possible modulatory role of CRF and the HPA axis in cocaine-induced reinstatement. In these experiments, treatment with the CRF receptor antagonist, D-Phe $CRF_{12–41}$, or adrenalectomy only slightly attenuated reinstatement induced by priming injections of cocaine.

The purpose of this experiment was to investigate the potential role for the HPA axis in the ability of environmental stimuli and priming infusions of methamphetamine to stimulate extinguished methamphetamine seeking by using two drugs that act on different levels of the HPA axis. Ketoconazole inhibits 11β-hydroxylase, a crucial enzyme in the synthesis of corticosterone. Ketoconazole has been shown to partially attenuate increases in plasma corticosterone observed during the reinstatement of cocaine-seeking behavior (Goeders and Clampitt 2002), exposure to electric footshock (Mantsch and Goeders 1999), and cocaine pretreatment (Goeders et al. 1998). CP-154,526 is a centrally acting antagonist at CRF1 receptors. We have previously demonstrated the ability of ketoconazole and CP-154,526 to attenuate the cue-induced reinstatement of cocaine-seeking behavior (Goeders and Clampitt 2002). The ability of both of these drugs to impact cocaine self-administration and reinstatement, along with other evidence, has indicated a role for the HPA axis in cocaine reward. We hypothesized that these drugs would attenuate the cue- and methamphetamine-induced reinstatement of methamphetamine-seeking behavior in a manner similar to what we observed in cocaine-trained rats.

Materials and methods

Subjects Eighty-five male Wistar rats (Harlan Sprague–Dawley, Indianapolis, IN) 80 to 100 days old at the start of the experiment were used. All rats were housed in individual cages equipped with a laminar flow unit and air filter in a temperature- and humidity-controlled AAALAC-accredited animal care facility on a reversed 12-h light–dark cycle (lights on at 7:00 P.M.). Rats were maintained at 85 to 90% of their free-feeding body weights by presentations of food pellets (P.J. Noyes, Lancaster, NH; 45 mg) during behavioral sessions when applicable and/or by supplemental feeding (Purina Rat Chow) and had access to water ad libitum. All procedures were approved by the LSUHSC-S Animal Care And Use Committee and were carried out in accordance with the National Institute of Health (NIH) "Guide to the Care and Use of Laboratory Animals (National Academy of Science 1996).

Venous catheterization and drug delivery Rats were implanted with chronic indwelling jugular catheters as described previously (Goeders et al. 1998). Briefly, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) with methylatropine nitrate (10 mg/kg, i.p.). The tip of the catheter assembly, composed of silicone tubing (0.3048 mm i.d.×0.635 mm, o.d.) connected to a 22-gauge cannula guide (Plastics One, Roanoke, VA), was inserted into the right posterior facial vein and pushed down into the jugular vein until it terminated outside the right atrium. The catheter assembly continued subcutaneously around the neck and was attached to the skull using stainless steel screws and dental acrylic. The rats were given an injection of a penicillin G procaine suspension (75,000 U, i.m.) immediately after surgery and were allowed a minimum of 4 days to recover before the start of operant testing. During testing, a stainless steel spring leash was attached to the catheter assembly and to a leak-proof fluid swivel suspended above the operant conditioning. The swivel was connected to a syringe in a motor-driven pump (Razel, St. Albans, VT) located outside the sound-attenuating chamber by tubing. At the conclusion of each session, the catheters were filled with streptokinase (816,000 IU) dissolved in heparinized

saline (0.1 ml) to prevent clot formation. Before the start of each session, the catheters were flushed with heparinized saline, and blood was drawn back. If blood was obtained via the catheter, it was judged to be patent. If not, then after the end of the session on Fridays, the rat was injected via the catheter with methohexital sodium (1.5 mg, i.v) with an immediate light anesthesia indicating that the catheter was functional.

Intracerebroventricular CP-154,526 infusions Rats receiving intracerebroventricular (i.c.v) infusions of CP-154,526 were implanted with a 22-gauge stainless steel guide cannula (Plastics One) into the right or left lateral cerebral ventricle under stereotaxic control (coordinates from bregma: anterior 0.8, lateral±1.3 and ventral −3.0) immediately after being implanted with the jugular catheter. Both the cannula guide and the catheter assembly were fixed to the top of the skull as described above.

Apparatus Standard plastic and stainless steel operant conditioning chambers contained within sound-attenuating enclosures (Med-Associates, St. Albans, VT) were used to run the operant experiments. Each operant conditioning chamber was equipped with two retractable response levers (Med-Associates) mounted on either side of the chamber with a stimulus light located above each lever. A house light was mounted on the wall opposite the levers with a tone source (66 dB) wired directly to the light located outside the chamber. The enclosures contained an exhaust fan that supplied ventilation and white noise to mask extraneous sounds. An IBM-compatible computer and interface system (Med-Associates) was used to program the procedures and collect the experimental data.

Self-administration training The rats were initially trained to self-administer methamphetamine (0.03 mg/kg/infusion) by pressing the appropriate lever under a continuous schedule of reinforcement during daily 2-h sessions 5 days a week. During these sessions, the two front levers, designated as the "active" lever and the "inactive" lever, were extended. The stimulus light above the methamphetamine lever was illuminated when methamphetamine was available for self-administration. Responses on the inactive lever were recorded, but had no programmed consequences. Responses on the methamphetamine lever resulted in an intravenous infusion of methamphetamine (200 μl over 5.6 s). During the infusion, the stimulus light was extinguished, the house light illuminated, and a tone (66 dB) presented. The stimulus light was once again illuminated after a 20-s timeout period during which responses on either lever had no programmed consequences. Once stable, responding on the methamphetamine lever occurred, the schedule of reinforcement was raised to fixed-ratio 2 (FR2) and to the final schedule, FR4. Self-administration training continued until three consecutive sessions with less than a 10% variation in active lever responses occurred. Once that criterion was met, the rats were placed into extinction.

Extinction During daily 2-h extinction sessions, the methamphetamine and inactive levers were extended into the operant conditioning chamber with the stimulus light above the methamphetamine lever illuminated. Responses on the methamphetamine lever resulted in a brief (0.6 s) darkening of the lever light, but no methamphetamine was delivered. Responses on both levers were recorded. Extinction sessions continued until the total number of responses on the former methamphetamine-associated lever were equal to or lower than 20% of the mean number of methamphetamine responses during self-administration. After extinction, the rats were tested for the reinstatement of extinguished methamphetamine seeking using the cues (i.e., the tone and house light) that were previously paired with drug delivery or priming infusions of methamphetamine as described below.

Cue-induced reinstatement The conditions during cue-induced reinstatement were similar to those used for self-administration, except methamphetamine was not delivered. Ketoconazole (25 [N=9], 50 [N=10], or 100 [N=7] mg/kg, 1 ml/kg, i.p.), CP-154,526 (20 or 40 mg/kg [N=8 per dose], 1 ml/kg, i.p.) or vehicle (5% emulphor in saline, 1 ml/kg [N=8]) was injected 30 min before the start of the test session while the rats were still in their home cages. After a single response on the methamphetamine lever, the house light was illuminated and a tone sounded for 5.6 s, but no methamphetamine was delivered. After reinstatement testing, the rats were returned to self-administration training.

Rats [N=11] implanted with i.c.v. cannulae underwent cue-induced reinstatement testing as described above. CP-154,526 (3 μg) or vehicle (0.9% acetic acid in distilled water) was injected 30 min before the session in a volume of 5 μl over 2 min. The cannula was left in place for an additional minute to ensure that the full dose was delivered. After testing, the rats were returned to self-administration training and eventually retested. Each rat received vehicle and CP-154,526 on separated occasions using a counterbalanced design.

Methamphetamine-induced reinstatement The conditions during methamphetamine-induced reinstatement were identical to those during extinction sessions. Ketoconazole (50 mg/kg, 1 ml/kg, i.p.), CP-154,526 (20 or 40 mg/kg [N=12, 0.24 mg/kg], 1 ml/kg, i.p.) or vehicle (5% emulphor in saline, 1 ml/kg) was injected 30 min before

the start of the test session while the rats were still in their home cages. Immediately before the start of the session, the rats received a single methamphetamine priming infusion (0.12–0.24 mg/kg, 0.2 ml, i.v.). The effects of ketoconazole 50 mg/kg (N=10) compared to vehicle (N=10) on responding after a 0.12−mg/kg priming infusion were examined. The ability of CP-154,526 20 mg/kg (N=8) versus vehicle (N=8) to attenuate methamphetamine-primed reinstatement at the 0.12-mg/kg dose was also tested. Finally, the effects of two doses of CP-154,526, 20 mg/kg (N=8) and 40 mg/kg (N=12) versus vehicle (N=9) on 0.24 mg/kg methamphetamine-induced reinstatement were examined. After reinstatement testing, the rats were returned to self-administration training.

Retesting The rats received a minimum of five self-administration retraining sessions before being placed back into extinction if the criteria for stable self-administration (i.e., three consecutive sessions with less than a 10% variation in active lever responses) were also met. The rats were tested for reinstatement a maximum of three times using only one stimulus (i.e., cues or methamphetamine); once after receiving vehicle and the other two after receiving different doses of the test drugs (CP-154,526, 20–40 mg/kg or ketoconazole 25–50 mg/kg). The order of testing was randomly assigned in a counterbalanced manner, and rats were only tested with one of the treatment compounds. There were no significant differences in the number of self-administration or extinction sessions between treatment groups within any reinstatement method (Table 1).

Food training Naïve male Wistar rats (N=17) were trained to respond under a fixed-interval (FI) schedule of food reinforcement during daily 1-h sessions. Initially, the rats were trained on a FI25 schedule of reinforcement where the stimulus light above the food lever was illuminated every 25 s, indicating food availability. Depression of the lever when the stimulus light was illuminated resulted in the presentation of a single food pellet (45 mg), and the stimulus light was extinguished, the houselight illuminated, and a tone (66 dB) presented for 5.6 s as in the self-administration experiments. The fixed interval was gradually raised from the initial schedule of FI25 to a FI50 schedule of food reinforcement. The FI50 schedule was used to produce rates of responding that were similar to those seen during methamphetamine self-administration. Food training continued until three consecutive sessions with less than a 10% variation in active lever responding occurred. Once that criterion was met, the rats were tested on the following day. The test session was identical to the training sessions (i.e., FI50 schedule of food reinforcement for 1 h). The rats were pretreated with either CP-154,526 (20–40 mg/kg, 1 ml/kg, i.p.) or vehicle (5% emulphor in saline) 30 min before the start of the test session while still in their home cages. The number of active lever responses during the test session was compared to the number of active lever responses made on the last day of training. After testing, the rats were returned to food training for a minimum of five sessions before being tested with a different drug or dose. The rats were tested a maximum of three times; once after receiving vehicle and the other two after receiving different doses of CP-154,526. Six of the rats used in this experiment were subsequently implanted with catheters and used in addition to naïve rats for the ketoconazole experiment. Approximately 3 months elapsed from the last food test session to the reinstatement test session.

Drugs Methamphetamine was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC) and was dissolved in bacteriostatic, heparinized 0.9% saline. Ketoconazole was purchased from RBI (Natick, MA) and was administered intraperitoneally as a suspension in 5% emulphor in saline in a volume of 1 ml/kg. Each rat was injected with ketoconazole (25 mg/kg) at least once before testing during reinstatement, as we have observed that the first experience with ketoconazole typically produces

Table 1 The mean number of self-administration (SA) and extinction (EXT) sessions conducted prior to cue- or methamphetamine-induced reinstatement

	Cue		Cue (i.c.v.)		Meth 0.12		Meth 0.24	
	SA	EXT	SA	EXT	SA	EXT	SA	EXT
Vehicle	13.0±1.9	7.2±1.3	13.2±2.0	10.5±2.0	8.9±1.2	11.4±1.2	12.5±1.7	8.0±1.1
CP20	19.1±4.3	6.7±0.9			12.5±1.9	9.6±1.3	13.3±1.5	5.5±0.7
CP40	14.9±5.9	7.4±1.5					10.9±0.9	7.9±0.9
CP 3 μg			10.8±1.2	8.0±1.4				
Keto25	12.3±2.5	8.3±1.0						
Keto50	14.7±1.5	7.9±1.4			18.2±3.4	8.7±1.6		
Keto100	13.0±2.4	12.0±2.8						

The data are presented for each treatment group as the mean±SEM.

remarkably different behavioral and neurochemical effects than all subsequent exposures (Goeders and Clampitt 2002). CP-154,526 was a generous gift from Dr. Robert Mansbach from Pfizer Central Research (Groton, CT) and was suspended in 5% emulphor in saline in a volume of 1 ml/kg. For experiments involving i.c.v. infusions, CP-154,526 (3 μg) was dissolved in a volume of 5 μl vehicle (0.9% acetic acid in distilled water).

Statistical analysis The significance of the differences in active and inactive lever responding between treatment groups during reinstatement was determined using a one-way analysis of variance (ANOVA) when three or more treatment groups were used. Although some rats underwent re-testing up to three times (as described above), there were not enough rats receiving the same three treatments to conduct within-subject comparisons of drug effects. Post-hoc analyses were preformed using Tukey's multiple comparison test. Unpaired two-tailed *t* tests were used to analyze active lever responding in vehicle versus drug treated animals. Data comparing i.c.v. vehicle and CP-154,526 treated animals were analyzed using paired two-tailed *t* test. Within-subject comparisons of active lever responding during extinction and reinstatement were made using a paired two-tailed *t* test. Food-reinforced responding was analyzed using a repeated measures ANOVA. All statistical analyses were performed using GraphPad Prism 4 for Windows (GraphPad Software, San Diego).

Results

Effects of CP-154,526 on cue-induced reinstatement Figure 1a illustrates the effects of cue-induced reinstatement in rats treated with peripherally administered CP-154,526. The presentation of cues reliably reinstated methamphetamine-seeking behavior in vehicle treated rats evidenced by an increase in active lever responding compared to those observed during extinction (two-tailed paired *t* test, $p<0.001$). Treatment with systemic CP-154,526 (20 or 40 mg/kg, i.p.) did not significantly effect cue-induced reinstatement compared to vehicle.

The effects of centrally administered CP-154,526 are shown in Fig. 1b. Once again, the contingent presentation of cues reliably reinstated active lever responding (within subjects, two-tailed paired *t* test, $p<0.01$) in animals treated with vehicle. Central administration of CP-154,526 (3 μg) attenuated cue-induced reinstatement (two-tailed paired *t* test, $p<0.05$); however, it failed to reduce active lever responding to levels seen during extinction (within-subject, two-tailed paired *t* test, $p<0.05$). Neither centrally nor systemically administered CP-154,526 significantly affected inactive lever responding during reinstatement (Table 2).

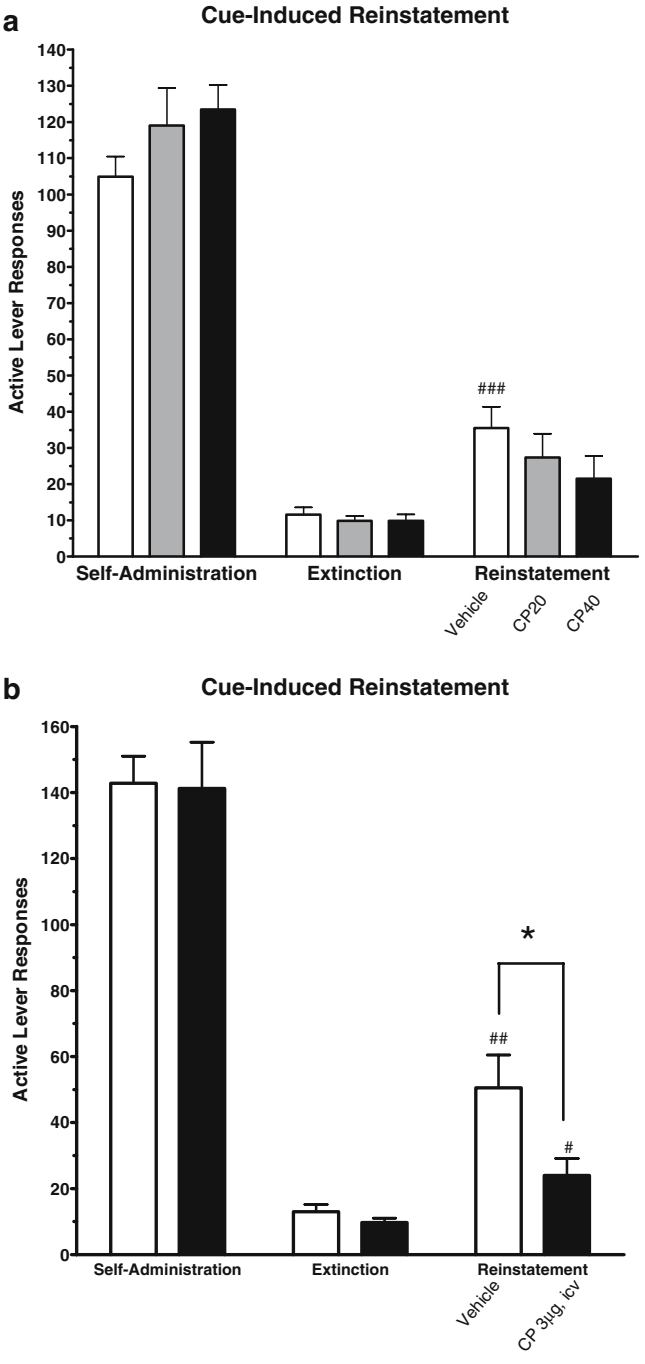

Fig. 1 CP-154,526 administered centrally, but not peripherally attenuates cue-induced reinstatement. **a** Active lever responding (means±SEM) for the last day of self-administration, the last day of extinction, and the reinstatement test session for rats in the vehicle (*white bars*), CP 20 (*gray bars*), or CP 40 (*black bars*) groups. Rats pretreated with CP-154,526 20 or 40 mg/kg, i.p ($N=8$ per dose) 30 min before the reinstatement test session showed a dose-dependent attenuation of cue-induced reinstatement compared to vehicle-treated animals ($N=8$). **b** The central administration of CP-154,526 attenuated reinstatement. Each rat ($N=11$) received vehicle (*white bars*) and CP-154,526 (*black bars*) on separate occasions in a counter-balanced manner. *Asterisk* indicates $p<0.05$ vehicle vs CP-154,526 $p<0.05$ (*number sign*), $p<0.01$ (*two number signs*), $p<0.001$ (*three number signs*) reinstatement vs extinction

Table 2 Inactive lever responding did not differ between treatment groups during self-administration, extinction, or reinstatement

		Self-Administration	Extinction	Reinstatement
Cue	Vehicle	16.3±4.8	9.1±2.0	9.3±1.6
	CP-154,526 20 mg/kg	14.4±7.6	8.1±2.9	11.6±5.3
	CP-154,526 40 mg/kg	27.4±12.8	8.6±2.9	7.5±3.2
	Ketoconazole 25 mg/kg	28.8±8.6	4.7±1.1	7.1±1.6
	Ketoconazole 50 mg/kg	17.4±6.4	4.9±1.4	6.8±1.8
	Ketoconazole 100 mg/kg	29.6±10.4	14.7±3.5	15.9±5.0
Cue	Vehicle (i.c.v.)	12.6±4.8	13.6±6.0	17.8±6.3
	CP-154,526 (3 μg, i.c.v.)	27.9±12.1	17.3±8.1	16.2±5.0
Methamphetamine 0.12 mg/kg (0.2 ml)	Vehicle (Ketoconazole)	19.3±8.1	11.5±2.9	19.9±8.1
	CP-154,526 20 mg/kg	9.0±2.5	9.6±4.0	8.5±3.8
	Vehicle	25.4±8.8	16.9±4.8	26.5±6.8
	Ketoconazole 50 mg/kg	21.6±7.3	20.4±5.8	26.0±6.5
Methamphetamine 0.24 mg/kg (0.2 ml)	Vehicle	13.6±3.3	8.1±1.9	17.6±3.8
	CP-154,526 20 mg/kg	13.0±5.5	5.4±1.1	13.5±5.1
	CP-154,526 40 mg/kg	12.8±2.6	8.1±1.6	8.2±2.9

The data are presented as the mean±SEM for the last day of self-administration, the last day of extinction, and the reinstatement test session.

Effects of ketoconazole on cue-induced reinstatement Figure 2 illustrates the active lever responding during self-administration, extinction, and reinstatement in vehicle- and ketoconazole-treated rats. There were no significant effects of ketoconazole pretreatment on cue-induced reinstatement. All rats showed a significant reinstatement of methamphetamine-seeking behavior after presentation of the cues ($p<0.05$–$p<0.001$). There was a slight trend towards decreased responding after the 50-mg/kg dose of ketoconazole. To determine if ketoconazole was attenuating cue-induced reinstatement, a very high dose of ketoconazole was tested (100 mg/kg). Active lever responding after treatment with 100 mg/kg ketoconazole was similar to what was seen in vehicle-treated rats. Inactive lever responding during reinstatement did not differ between vehicle and ketoconazole treated rats.

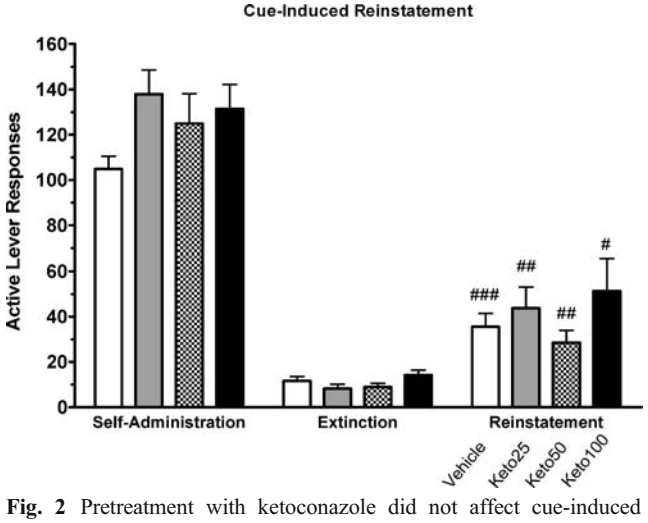

Fig. 2 Pretreatment with ketoconazole did not affect cue-induced reinstatement. The data (means±SEM) are presented as active lever responding for the last day of self-administration, the last day of extinction, and the reinstatement test session for rats in the vehicle (*white bars*), keto25 (*gray bars*), keto50 (*checkered bars*), or keto100 (*black bars*) groups. Reinstatement after pretreatment with ketoconazole (25 mg/kg, $N=9$; 50 mg/kg, $N=10$; 100 mg/kg, $N=7$) did not differ significantly from vehicle-treated rats ($N=8$). *Number sign* indicates $p<0.05$, *two number signs* indicate $p<0.01$, *three number signs* indicate $p<0.001$ reinstatement vs extinction

Effects of CP-154,526 on methamphetamine-induced reinstatement Methamphetamine (0.12 and 0.24 mg/kg/infusion, 200 μl) infusions delivered before a session during which the conditions were identical to those used in extinction will reliably reinstate methamphetamine seeking. Figure 3a and b illustrates the attenuation of methamphetamine-induced reinstatement by pretreatment with CP-154,526. Methamphetamine infusions (0.12 mg/kg/infusion) induced robust reinstatement in vehicle-treated animals (within-subject two-tailed paired t test, $p<0.01$). Pretreatment with CP-154,526 (20 mg/kg) significantly attenuated methamphetamine-induced reinstatement (two-tailed unpaired t test, $p<0.05$), reducing responding to levels not statistically different from those seen during extinction.

Vehicle-treated animals increased responding on the active lever after a methamphetamine (0.24 mg/kg/infusion) priming infusion (within-subject two-tailed paired t test, $p<0.001$). Figure 3b illustrates that CP-154,526 dose-dependently attenuated methamphetamine-induced reinstatement [one-way ANOVA, $F(2, 28)=4.628$, $p<0.05$], with the 40-mg/kg dose significantly attenuating responding compared to vehicle (Tukey's post hoc, $p<0.05$). As shown in Table 1, pretreatment with CP-154,526 before methamphetamine (0.12 and

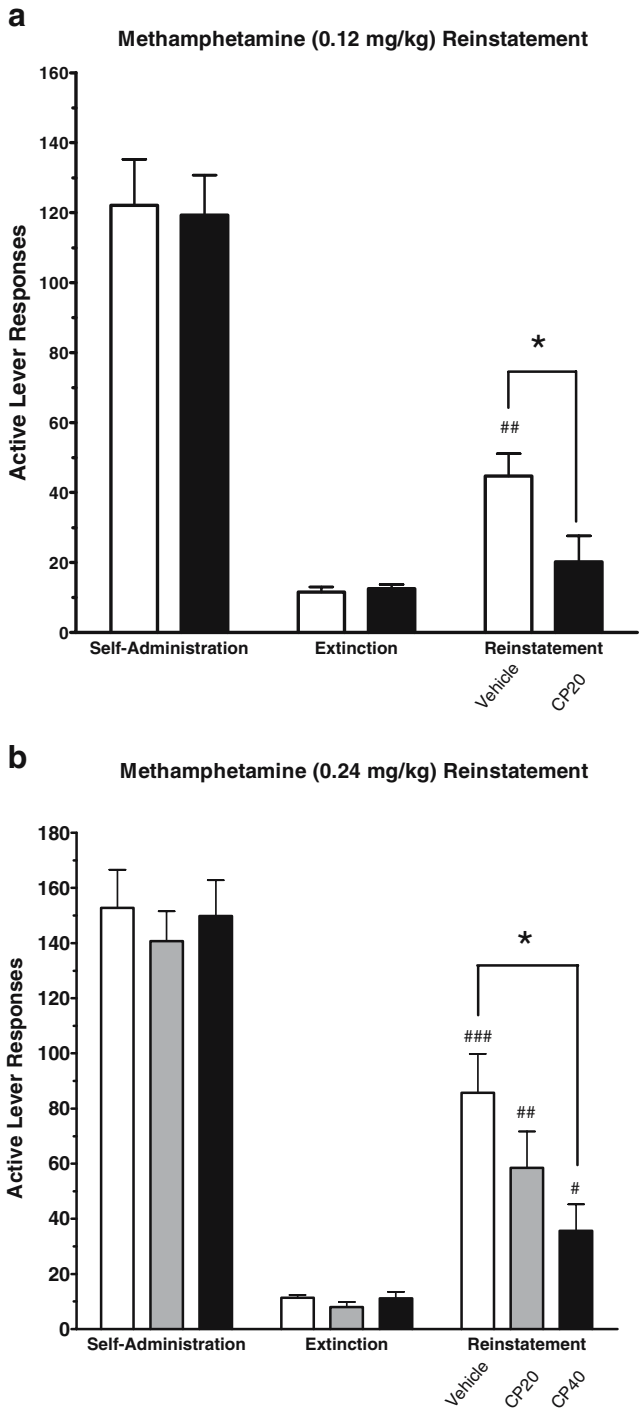

Fig. 3 CP-154,526 attenuates methamphetamine-induced reinstate using **a** 0.12 mg/kg/infusion or **b** 0.24 mg/kg/infusion priming doses. The data (means±SEM) are presented as active lever responding for the last day of self-administration, the last extinction session, and the reinstatement test session for rats in the vehicle (*white bars*), CP20 (*gray bars*), or CP40 (*black bars*) groups. *Asterisk* indicates $p<0.05$, $p<0.05$ (*number sign*), $p<0.01$ (*two number signs*), $p<0.001$ (*three number signs*) reinstatement vs extinction

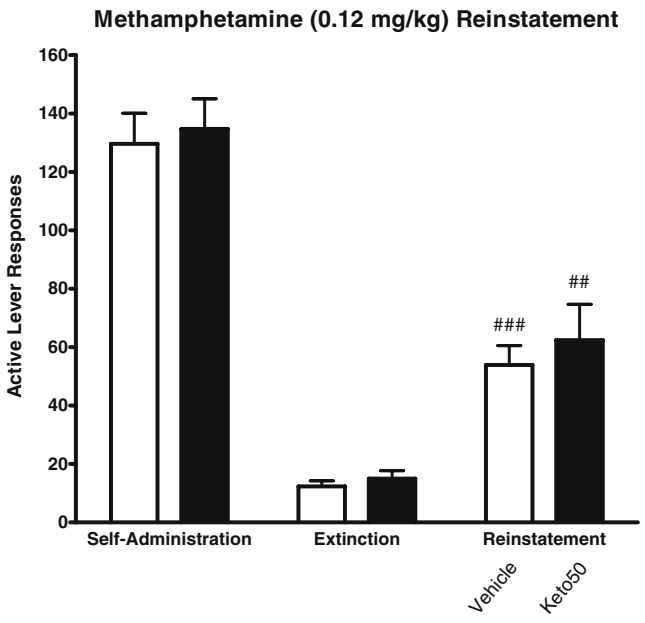

Fig. 4 Effects of ketoconazole on methamphetamine (0.12 mg/kg)-induced reinstatement. Data (means±SEM) are presented as active lever responding during the last day of self-administration, the last extinction session, and the reinstatement test session for rats in the vehicle (*white bars*) or keto50 (*black bars*) groups. *Asterisk* indicates $p<0.05$, $p<0.01$ (*two number signs*), $p<0.001$ (*three number signs*) reinstatement vs extinction

0.24 mg/kg/infusion)-induced reinstatement did not significantly affect inactive lever responding.

Effects of ketoconazole on methamphetamine-induced reinstatement To determine if ketoconazole would attenuate methamphetamine-induced reinstatement, we tested the effects of the 50-mg/kg dose (Fig. 4). Ketoconazole did not attenuate methamphetamine-induced (0.12 mg/kg/infusion) reinstatement compared to vehicle (two-tailed unpaired *t* test, $p=0.55$). Inactive lever responding was also unaffected (Table 1). Because 50 mg/kg ketoconazole did not affect methamphetamine-induced reinstatement using the 0.12 mg/kg priming infusion, the effects of ketoconazole on 0.24 mg/kg/infusion methamphetamine-induced reinstatement were not determined.

Effects of CP-154,526 on food-reinforced responding The administration of CP-154,526 does not affect food-reinforced responding (Table 3). The total food reinforcers received and the mean lever responses were unaffected by pretreatment with vehicle, 20- or 40-mg/kg dose of CP-154,526. These data suggest that CP-154,526 is not attenuating cue-induced reinstatement by general suppression of lever pressing activity.

Table 3 CP-154,526 does not affect food-reinforced lever pressing

Baseline		Test day		
Treatment	Foods	Responses	Foods	Responses
Vehicle (N=10)	129.4±1.75	144.1±3.93	130.1±1.22	141.2±2.27
CP20 (N=11)	129.8±1.90	141.7±2.16	131.2±1.20	141.0±3.67
CP40 (N=10)	131.3±0.75	136.1±1.54	131.4±0.90	134.2±1.09

Data are presented as means±SEM for the baseline number of food reinforcers received and total responses followed by the number of food reinforcers and total responses after vehicle or CP-154,526 (20 or 40 mg/kg, i.p.) pretreatment.

Discussion

In the present experiments, we examined the potential role for the HPA axis in the cue-induced reinstatement of extinguished methamphetamine-seeking behavior. Previously, we found that both ketoconazole, an inhibitor of corticosterone synthesis, and CP-154,526, a CRF1 receptor antagonist, attenuated cue-induced reinstatement in cocaine-trained rats (Goeders and Clampitt 2002). Using similar methods, we tested the same drugs in rats trained to self-administer methamphetamine. Although both ketoconazole and CP-154,526 reversed the cue-induced reinstatement of cocaine-seeking behavior, only CP-154,526 attenuated the cue-induced reinstatement of methamphetamine seeking. Interestingly, CP-154,526 also attenuated methamphetamine-induced reinstatement.

The ability of ketoconazole to inhibit steroidogenesis has been well documented both in vitro (Eckhoff et al. 1988; Morishita et al. 2001) and in vivo (Burrin et al. 1986). The CRF1 receptor antagonist, CP-154,526, attenuates the airpuff startle-induced rise in plasma corticosterone and adrenocorticotropic hormone (ACTH) when given acutely (Arborelius et al. 2000). Despite the evidence that both ketoconazole and CP-154,526 reduce plasma corticosterone, only CP-154,526 attenuated cue- and methamphetamine-induced reinstatement, indicating that CRF, but not corticosterone, plays a crucial role in the ability of environmental cues and priming injections to stimulate methamphetamine seeking. Others have demonstrated the ability of CRF antagonists to attenuate footshock-induced reinstatement of heroin-, ethanol-, and cocaine-seeking behavior in rats (Erb et al. 1998; Le et al. 2000; Shaham et al. 1997, 1998), whereas surgical or pharmacological manipulation of corticosterone levels had little effect (Erb et al. 1998; Le et al. 2000; Shaham et al. 1997).

Although very few studies have looked at the relationship between methamphetamine and the HPA axis, there is evidence for a methamphetamine-induced rise in plasma cortisol in humans (Besser et al. 1969; Fehm et al. 1984; Harris et al. 2003) and plasma corticosterone in rats (Asano and Moroji 1974; Szumlinski et al. 2001). Although some studies also suggested a potential role for corticosterone in the acquisition of D-amphetamine self-administration (Piazza et al. 1989, 1990), the HPA axis may play less of a role in the acquisition of methamphetamine self-administration (Moffett and Goeders 2005). Plasma corticosteroid levels appear to play little role in mediating the subjective effects of methamphetamine and D-amphetamine. Neither the augmentation of the methamphetamine-induced rise in plasma cortisol with hydrocortisone nor the attenuation of this response with metyrapone, a corticosteroid synthesis inhibitor, alters the pleasurable effects of methamphetamine in humans (Harris et al. 2003). Treatment with hydrocortisone also fails to alter the physiological, behavioral, or subjective effects of D-amphetamine in humans (Wachtel et al. 2001). As the ketoconazole-induced reduction of plasma corticosterone failed to attenuate cue-induced reinstatement in the present experiment, CP-154,526 may produce its effects on reinstatement by blocking CRF receptors at extrahypothalamic sites.

CRF is a 41-residue peptide (Vale et al. 1981) primarily released from the paraventricular nucleus of the hypothalamus that plays a critical role in the body's response to stress. CRF is a potent stimulator of ACTH release from the anterior pituitary through its actions on CRF1 receptors (Rivier et al. 2003), which in turn stimulates the release of corticosterone. The distribution of CRF in the brain is broad, including extrahypothalamic sites important in the behavioral responses to stressors (Sawchenko et al. 1993). In this current experiment, the cues that were paired with self-administered methamphetamine as well as the priming infusions of methamphetamine may have acted as stressors during the reinstatement test session. Cocaine-dependent individuals show a heightened activation of the HPA axis when exposed to drug cues compared to neutral imagery (Sinha et al. 2003). Previous investigations of the cue-induced reinstatement of cocaine seeking have reported a significant rise in post-session plasma corticosterone in response to the contingent presentation of drug-paired cues compared to post-session plasma corticosterone measured during extinction (Goeders and Clampitt 2002). Contextual stimuli associated with cocaine have also been shown to increase plasma corticosterone (DeVries and Pert 1998) and to induce anxiogenic-like behavior (DeVries and Pert 1998)

in the absence of drug, effects believed to be mediated by CRF. In this experiment, the cues may have functioned as stressors, thus, stimulating the release of CRF to increase plasma corticosterone. This increase in plasma corticosterone may simply be an indicator of increased CRH activity, as opposed to being a crucial element in the cue-induced reinstatement of extinguished methamphetamine-seeking behavior.

Interestingly, it has been demonstrated that intracranial injections of CP-154,526 into the amygdala or nucleus accumbens attenuate the morphine-primed reinstatement of morphine-induced conditioned place preference (CPP), whereas injections in the bed nucleus of the stria terminalis inhibit the footshock-induced reinstatement of morphine CPP (Wang et al. 2006). Footshock stress increases the release of CRF in the ventral tegmental area, which in turn increases local glutamate and dopamine release in rats trained to self-administer cocaine (Wang et al. 2005). Although different reinstatement procedures were used in the current experiments, these studies support a role for extrahypothalamic CRF in the reinstatement of drug seeking.

In the current experiment, we demonstrated that CP-154,526, but not ketoconazole, reversed the cue-induced reinstatement of methamphetamine-seeking behavior. The effects of CP-154,526 are believed to be primarily due to the antagonism of CRH-1 receptors. This study demonstrates the potential value of the development of CRH receptor antagonists as aids in preventing relapse in drug-dependent individuals.

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References

Arborelius L, Skelton KH, Thrivikraman KV, Plotsky PM, Schulz DW, Owens MJ (2000) Chronic administration of the selective corticotropin-releasing factor 1 receptor antagonist CP-154,526: behavioral, endocrine and neurochemical effects in the rat. J Pharmacol Exp Ther 294:588–597

Asano Y, Moroji T (1974) Effects of methamphetamine on daily rhythms of hypothalamic norepinephrine, serotonin and plasma corticosterone levels in the rat. Life Sci 14:1463–1472

Besser GM, Butler PW, Landon J, Rees L (1969) Influence of amphetamines on plasma corticosteroid and growth hormone levels in man. Br Med J 4:528–530

Burrin JM, Yeo TH, Ashby MJ, Bloom SR (1986) Effect of ketoconazole on adrenocorticotrophic hormone secretion in vitro and in vivo. J Endocrinol 108:37–41

Dackis CA, O'Brien CP (2001) Cocaine dependence: a disease of the brain's reward centers. J Subst Abuse Treat 21:111–117

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 75:134–143

DeVries AC, Pert A (1998) Conditioned increases in anxiogenic-like behavior following exposure to contextual stimuli associated with cocaine are mediated by corticotropin-releasing factor. Psychopharmacology (Berl) 137:333–340

Eckhoff C, Oelkers W, Bahr V (1988) Effects of two oral antimycotics, ketoconazole and fluconazole, upon steroidogenesis in rat adrenal cells in vitro. J Steroid Biochem 31:819–823

Erb S, Shaham Y, Stewart J (1996) Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology (Berl) 128:408–412

Erb S, Shaham Y, Stewart J (1998) The role of corticotropin-releasing factor and corticosterone in stress-and cocaine-induced relapse to cocaine seeking in rats. J Neurosci 18:5529–5536

Fehm HL, Holl R, Steiner K, Klein E, Voigt KH (1984) Evidence for ACTH-unrelated mechanisms in the regulation of cortisol secretion in man. Klin Wochenschr 62:19–24

Fuchs RA, Tran-Nguyen LT, Specio SE, Groff RS, Neisewander JL (1998) Predictive validity of the extinction/reinstatement model of drug craving. Psychopharmacology (Berl) 135:151–160

Gerber GJ, Stretch R (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 3:1055–1061

Goeders NE, Clampitt DM (2002) Potential role for the hypothalamo-pituitary-adrenal axis in the conditioned reinforcer-induced reinstatement of extinguished cocaine seeking in rats. Psychopharmacology (Berl) 161:222–232

Goeders NE, Guerin GF (1994) Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology (Berl) 114:63–70

Goeders NE, Guerin GF (2000) Effects of the CRH receptor antagonist CP-154,526 on intravenous cocaine self-administration in rats. Neuropsychopharmacology 23:577–586

Goeders NE, Peltier RL, Guerin GF (1998) Ketoconazole reduces low dose cocaine self-administration in rats. Drug Alcohol Depend 53:67–77

Haney M, Maccari S, Le Moal M, Simon H, Piazza PV (1995) Social stress increases the acquisition of cocaine self-administration in male and female rats. Brain Res 698:46–52

Harris DS, Reus VI, Wolkowitz OM, Mendelson JE, Jones RT (2003) Altering cortisol level does not change the pleasurable effects of methamphetamine in humans. Neuropsychopharmacology 28:1677–1684

Hartz DT, Frederick-Osborne SL, Galloway GP (2001) Craving predicts use during treatment for methamphetamine dependence: a prospective, repeated-measures, within-subject analysis. Drug Alcohol Depend 63:269–276

Howes SR, Dalley JW, Morrison CH, Robbins TW, Everitt BJ (2000) Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression. Psychopharmacology (Berl) 151:55–63

Kosten TA, Miserendino MJ, Kehoe P (2000) Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. Brain Res 875:44–50

Le AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y (2000) The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. Psychopharmacology (Berl) 150:317–324

Mantsch JR, Goeders NE (1999) Ketoconazole does not block cocaine discrimination or the cocaine-induced reinstatement of cocaine-seeking behavior. Pharmacol Biochem Behav 64:65–73

Meil WM, See RE (1996) Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. Behav Pharmacol 7:754–763

Miczek KA, Mutschler NH (1996) Activational effects of social stress on IV cocaine self-administration in rats. Psychopharmacology (Berl) 128:256–264

Moffett MC, Goeders NE (2005) Neither non-contingent electric footshock nor administered corticosterone facilitate the acquisition of methamphetamine self-administration. Pharmacol Biochem Behav 80:333–339

Morishita K, Okumura H, Ito N, Takahashi N (2001) Primary culture system of adrenocortical cells from dogs to evaluate direct effects of chemicals on steroidogenesis. Toxicology 165:171–178

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511–1513

Piazza PV, Deminiere JM, le Moal M, Simon H (1990) Stress-and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res 514:22–26

Rivier CL, Grigoriadis DE, Rivier JE (2003) Role of corticotropin-releasing factor receptors type 1 and 2 in modulating the rat adrenocorticotropin response to stressors. Endocrinology 144: 2396–2403

Sawchenko PE, Imaki T, Potter E, Kovacs K, Imaki J, Vale W (1993) The functional neuroanatomy of corticotropin-releasing factor. Ciba Found Symp 172:5–21; discussion 21–9

Schenk S, Lacelle G, Gorman K, Amit Z (1987) Cocaine self-administration in rats influenced by environmental conditions: implications for the etiology of drug abuse. Neurosci Lett 81: 227–231

Shaham Y, Stewart J (1995) Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology (Berl) 119:334–341

Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J (1997) Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 17:2605–2614

Shaham Y, Erb S, Leung S, Buczek Y, Stewart J (1998) CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine-and heroin-trained rats. Psychopharmacology (Berl) 137:184–190

Shaham Y, Erb S, Stewart J (2000) Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev 33:13–33

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 168:3–20

Sinha R, Talih M, Malison R, Cooney N, Anderson GM, Kreek MJ (2003) Hypothalamic-pituitary-adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. Psychopharmacology (Berl) 170:62–72

Stewart J, de Wit H, Eikelboom R (1984) Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 91:251–268

Szumlinski KK, Haskew RE, Balogun MY, Maisonneuve IM, Glick SD (2001) Iboga compounds reverse the behavioural disinhibiting and corticosterone effects of acute methamphetamine: Implications for their antiaddictive properties. Pharmacol Biochem Behav 69:485–491

Tidey JW, Miczek KA (1997) Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. Psychopharmacology (Berl) 130:203–212

Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397

Wachtel SR, Charnot A, de Wit H (2001) Acute hydrocortisone administration does not affect subjective responses to d-amphetamine in humans. Psychopharmacology (Berl) 153: 380–388

Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB (2005) Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. J Neurosci 25: 5389–5396

Wang J, Fang Q, Liu Z, Lu L (2006) Region-specific effects of brain corticotropin-releasing factor receptor type 1 blockade on foot-shock-stress-or drug-priming-induced reinstatement of morphine conditioned place preference in rats. Psychopharmacology (Berl) 185:19–28

Weiss F, Martin-Fardon R, Ciccocioppo R, Kerr TM, Smith DL, Ben-Shahar O (2001) Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues. Neuropsychopharmacology 25:361–372