Neuropsychopharmacology (2007) 32, 955–963

www.neuropsychopharmacology.org

Antagonism of CRF Receptors Prevents the Deficit in Brain Reward Function Associated with Precipitated Nicotine Withdrawal in Rats

Adrie W Bruijnzeel*[1], George Zislis[1], Carrie Wilson[1] and Mark S Gold[1,2,3,4]

[1]Department of Psychiatry, University of Florida, Gainesville, FL, USA; [2]Department of Neuroscience, University of Florida, Gainesville, FL, USA; [3]Department of Anesthesiology, University of Florida, Gainesville, FL, USA; [4]Department of Community Health and Family Medicine, University of Florida, Gainesville, FL, USA

Nicotine dependence is a chronic mental illness that is characterized by a negative affective state upon tobacco smoking cessation and relapse after periods of abstinence. It has been hypothesized that cessation of nicotine administration results in the activation of brain corticotropin-releasing factor (CRF) systems that leads to the negative affective state of withdrawal. The aim of our experiments was to investigate the role of brain CRF systems in the deficit in brain reward function associated with the cessation of nicotine administration in rats. The intracranial self-stimulation procedure was used to assess to negative affective aspects of nicotine withdrawal as this procedure can provide a quantitative measure of emotional distress in rats. In the first experiment, mecamylamine induced a dose-dependent elevation in brain reward thresholds in nicotine-treated rats. In the follow-up experiment, it was shown that pretreatment with the corticotropin-receptor antagonist D-Phe $CRF_{(12–41)}$ prevents the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. In the third experiment, the effect of D-Phe $CRF_{(12–41)}$ on the elevations in brain reward thresholds associated with spontaneous nicotine withdrawal was investigated. Administration of D-Phe $CRF_{(12–41)}$ 6 h after the explantation of the nicotine pumps, did not result in a lowering of the brain reward thresholds. These findings indicate that antagonism of CRF receptors prevents, but not reverses, the deficit in brain associated with nicotine withdrawal. These data provide support for the hypothesis that a hyperactivity of brain CRF systems may at least partly mediate the initiation of the negative affective aspects of nicotine withdrawal.
Neuropsychopharmacology (2007) **32,** 955–963. doi:10.1038/sj.npp.1301192; published online 30 August 2006

Keywords: nicotine; corticotropin-releasing factor; dependence; reward deficit; rats

INTRODUCTION

Nicotine dependence is a chronic mental illness that is characterized by loss of control over tobacco smoking, the appearance of withdrawal symptoms upon the cessation of tobacco smoking, and relapse after periods of abstinence (American Psychiatric Association, 2000; McLellan *et al*, 2000; O'Brien, 2003). Cessation of tobacco smoking in humans is typically associated with negative affective symptoms such as depressed mood, anxiety, irritability, and difficulty concentrating (American Psychiatric Association, 2000). It has been hypothesized that the negative affective aspects of drug withdrawal provide a powerful motivational force for the continuation of drug use (Koob *et al*, 1997; Markou *et al*, 1998).

Experimental evidence indicates that nicotine is one of the main components of tobacco smoke that leads to and maintains smoking behavior (Bardo *et al*, 1999; Crooks and Dwoskin, 1997; Stolerman and Jarvis, 1995). Nicotine mediates its postive reinforcing effects at least partly via the activation of neuronal nicotinic acetylcholine receptors (nAChR). This is supported by the observation that nAChR antagonists decrease nicotine self-administration in rats (Corrigall *et al*, 1994; Corrigall and Coen, 1989; Donny *et al*, 1999; Watkins *et al*, 1999). In addition, mice that lack the $\beta 2$ subunit of nAChR self-administer less nicotine than wild-type controls (Picciotto *et al*, 1998). Nicotine withdrawal is associated with a deficit in brain reward function and somatic withdrawal signs in rats (Bruijnzeel and Markou, 2004; Epping-Jordan *et al*, 1998; Harrison *et al*, 2001). Epping-Jordan and colleagues reported that systemic administration of the nAChR antagonist dihydro-β-erythroidine (DHβE) induces an elevation in brain reward thresholds (decrease in the reinforcing properties of

*Correspondence: Dr AW Bruijnzeel, Department of Psychiatry, McKnight Brain Institute, University of Florida, 100 S Newell Dr, PO Box 100256, Gainesville, FL 32610, USA, Tel: +1 352 294 0421, Fax: +1 352 392 8217, E-mail: awbruijn@psychiatry.ufl.edu
Received 2 December 2005; revised 14 June 2006; accepted 10 July 2006
Online publication: 20 July 2006 at http://www.acnp.org/citations/Npp07200605017/default.pdf

intracranial self-stimulation) and an increase in somatic withdrawal signs in nicotine dependent rats. Similarly, abrupt cessation of nicotine administration results in an elevation in brain reward thresholds and an increase in somatic withdrawal signs (Epping-Jordan *et al*, 1998). The administration of nicotine after the discontinuation of chronic nicotine administration has been shown to mitigate somatic nicotine withdrawal signs (Malin *et al*, 1992). Experimental evidence suggests that treatments that elevate brain serotonin levels may partially reverse the elevations in brain reward thresholds associated with spontaneous nicotine withdrawal (Harrison *et al*, 2001).

Different lines of experimental evidence suggest that there is a relationship between a hyperactivity of brain stress systems and a decrease in brain reward function, which is one of the core symptoms of drug withdrawal and depression (Barr and Markou, 2005; Bruijnzeel and Gold, 2005). First, acute administration of the stress-responsive neuropeptide corticotropin-releasing factor (CRF) induces an elevation in brain reward thresholds in rats (Macey *et al*, 2000). Second, clinical studies indicate that brain CRF systems are hyperactive in patients with depressive disorders (De Bellis *et al*, 1993; Merali *et al*, 2004; Nemeroff *et al*, 1984, 1991; Veith *et al*, 1993; Zobel *et al*, 2000).

Studies that reported that CRF may play a role in negative mood states and studies showing that CRF increases anxiety-like behavior in animal models (Koob, 1999) has led researchers to explore the role of brain CRF systems in the negative affective aspects of drug withdrawal. These studies have provided strong evidence for a role of CRF in drug withdrawal-induced anxiety-like behavior in rodents. Spontaneous alcohol and cocaine withdrawal and precipitated cannabinoid withdrawal have been shown to elevate extracellular CRF levels in the central nucleus of the amygdala (CeA), a brain area implicated in anxiety disorders (Merlo Pich *et al*, 1995; Richter and Weiss, 1999; Rodriguez de Fonseca *et al*, 1997). Furthermore, antagonism of CRF receptors decreases anxiety-like behavior associated with withdrawal from alcohol, cocaine, and other drugs of abuse (Baldwin *et al*, 1991; Basso *et al*, 1999; Overstreet *et al*, 2004; Rassnick *et al*, 1993; Sarnyai *et al*, 1995).

Taken together, the above-discussed studies suggest that increased CRF transmission may be implicated in the etiology and maintenance of depressive disorders and drug withdrawal-induced anxiety. Although tobacco-smoking cessation has been associated with negative mood states, the role of CRF in decreased brain reward function associated with acute nicotine withdrawal has not been investigated yet. It is hypothesized here that antagonism of CRF receptors reverses the deficit in brain reward function associated with nicotine withdrawal. The aim of the present experiments was to investigate the effects of the CRF antagonist D-Phe $CRF_{(12-41)}$ on the deficit in brain reward function associated with precipitated (repeated mecamylamine-induced withdrawal starting 6 days after the implantation of the minipumps) and spontaneous nicotine withdrawal (discontinuation of nicotine administration after 14 days of exposure). The first experiment investigated the effects of precipitated nicotine withdrawal on brain reward thresholds using a discrete trial intracranial self-stimulation procedure. This procedure was used in all the experiments to assess the negative affective aspects of nicotine withdrawal as it provides a quantitative measure of the emotional aspects of drug withdrawal (Bruijnzeel *et al*, 2006; Schulteis *et al*, 1995; Wise and Munn, 1995). The second and the third experiment investigated the effects of D-Phe $CRF_{(12-41)}$ on the elevations in brain reward thresholds associated with precipitated and spontaneous nicotine withdrawal. Experiments that further our understanding about the neurobiological substrates underlying the affective aspects of nicotine withdrawal may contribute to the development of pharmacotherapies that reduce withdrawal symptomatology and thereby decrease relapse rates.

MATERIALS AND METHODS

Subjects

Male Wistar rats (Charles River, Raleigh, NC) weighing 250–300 g at the beginning of the experiments were used. Animals were group housed (two per cage) in a temperature- and humidity-controlled vivarium and maintained on a 12 h light–dark cycle (lights off at 1800 hours). All testing occurred at the beginning of the light cycle. Food and water were available ad libitum in the home cages. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the University of Florida Institutional Animal Care and Use Committee.

Drugs

Nicotine hydrogen tartrate salt, mecamylamine hydrochloride, and pentobarbital sodium salt were purchased from Sigma (Sigma-Aldrich, St Louis, MO, USA) and dissolved in sterile saline (0.9% sodium chloride). The CRF antagonist (D-Phe^{12}, Nle^{21,38}C$^{\alpha}$Me Leu^{37})r/h$CRF_{(12-41)}$ (D-Phe $CRF_{(12-41)}$) was synthesized by The Clayton Foundation Laboratories for Peptide Biology and kindly provided by Dr Jean Rivier (Salk Institute for Biological Studies, La Jolla, CA). The peptide was dissolved in distilled water and administered within 1 h after being dissolved.

Surgical Procedures

Electrode and cannula implantation. For experiment 1, rats were prepared with an electrode in the medial forebrain bundle. For experiments 2 and 3, rats were prepared with both an electrode in the medial forebrain bundle and a cannula above the lateral ventricle. At the beginning of all the intracranial surgeries, the rats were anesthetized with an isoflurane/oxygen vapor mixture (1–3% isoflurane) and placed in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set 3.3 mm below the interaural line (flat skull). The rats were prepared with a stainless steel 23 gauge cannula 11 mm in length located immediately above the lateral ventricle using the following flat skull coordinates: anterior posterior (AP) −0.9 mm, medial lateral (ML) ±1.4 mm, dorsal ventral (DV) −3.0 mm from skull (Paxinos and Watson, 1998). At the end of the surgery, removable 30 gauge wire stylets 11 mm in length

were inserted in the cannulae so that the cannula would maintain patency. For electrode implantation, the incisor bar was set 5 mm above the interaural line. The rats were prepared with stainless steel bipolar electrodes (model MS303/2 Plastics One, Roanoke, VA) 11 mm in length in the medial forebrain bundle at the level of the posterior lateral hypothalamus (AP −0.5 mm; ML ±1.7 mm; DV −8.3 mm from dura). The electrodes and cannulae were permanently secured to the skull using dental cement anchored with four skull screws.

Osmotic minipump implantation. Minipumps (Alzet model 2ML2 14 day pumps or 2ML4 28 day pumps, Alza Corporation, Palo Alto, CA) filled with either saline or nicotine hydrogen tartrate dissolved in saline, were implanted subcutaneously (s.c.) under isoflurane/oxygen (1–3% isoflurane) anesthesia. The nicotine concentration was adjusted to compensate for differences in body weight to deliver a dose of 9 mg/kg/day of nicotine tartrate (3.16 mg/kg/day nicotine base).

Apparatus

The experimental apparatus consisted of eight Plexiglas chambers (30.5 × 30 × 17 cm; Med Associates, Georgia, VT), each housed in a sound-attenuating melamine chambers (Med Associates, Georgia, VT). The operant chamber consisted of a metal grid floor and a metal wheel (5 cm wide) centered on a sidewall. A photobeam detector was attached next to the response wheel and recorded every 90 degrees of rotation. Brain stimulation was delivered using constant current stimulators (Model 1200C, Stimtek, Acton, MA). Subjects were connected to the stimulation circuit through bipolar leads (Plastics One, Roanoke, VA) attached to gold-contact swivel commutators (model SL2C Plastics One, Roanoke, VA). A computer controlled the stimulation parameters, data collection, and all test session functions.

Intracranial Self-Stimulation Procedure

The subjects initially were trained to turn the wheel on a fixed ratio 1 (FR1) schedule of reinforcement. Each quarter turn of the wheel resulted in the delivery of a 0.5 s train of 0.1 ms cathodal square-wave pulses at a frequency of 100 Hz. After the successful acquisition of responding for stimulation on this FR1 schedule, defined as 100 reinforcements within 10 min, the rats were trained gradually on a discrete-trial current-threshold procedure. The discrete-trial current-threshold procedure used was a modification of a task developed Kornetsky and Esposito (1979), and previously described in detail by Bruijnzeel and Markou (2004, 2005). Each trial began with the delivery of a noncontingent electrical stimulus, followed by a 7.5 s response window within which the animal can respond to receive a second contingent stimulus identical in all parameters to the initial noncontingent stimulus. A response during this 7.5 s response window was labeled a positive response, while the lack of a response was labeled a negative response. During a 2 s period immediately after a positive response, additional responses had no consequence. The intertrial interval (ITI) that followed either a positive response or the end of the response window (in the case of a negative response), had an average duration of 10 s (ranging from 7.5 to 12.5 s). Responses that occurred during the ITI resulted in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the ITI and delay periods induced by time-out responses were gradually increased until animals performed consistently at standard test parameters. The subjects subsequently were tested on the current-threshold procedure in which stimulation intensities varied according to the classical psychophysical method of limits. A test session consisted of four alternating series of descending and ascending current intensities starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the intensity was altered systematically between blocks of trials by 5 μA steps. The initial stimulus intensity was set 40 μA above the baseline current-threshold for each animal. Each test session typically lasted 30–40 min and provided two dependent variables for behavioral assessment: brain reward thresholds and response latencies.

Threshold. The current threshold for a descending series was defined as the midpoint between stimulation intensities that supported responding (ie positive responses on at least two of the three trials), and current intensities that failed to support responding (ie positive responses on fewer than two of the three trials for two consecutive blocks of trials). The threshold for an ascending series was defined as the midpoint between stimulation intensities that did not support responding and current intensities that supported responding for two consecutive blocks of trials. Thus, four threshold estimates were recorded, and the mean of these values was taken as the threshold for each subject on each test session.

Response latency. The time interval between the beginning of the noncontingent stimulus and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency on all trials during which a positive response occurred.

Drug Administration Procedure

For intracerebroventricular injections, 30 gauge stainless steel injectors projecting 2.5 mm beyond the guide cannula were used. All intracerebroventricular drug injections were made by gravity induced by raising the Hamilton syringe above the animal's head. Five μl of solution was administered over a 30–60 s period, and the injector was left in place for another 30 s to allow diffusion from the injector tip. Immediately after finishing drug administration, the dummy stylets were reinserted.

Experimental Design

Precipitated nicotine withdrawal. The aim of this experiment was to investigate the effects of precipitated nicotine withdrawal on brain reward thresholds in rats. Naïve rats were used for all the experiments. After recovery from the electrode implantations, the rats were trained on the ICSS procedure. When stable baseline brain reward thresholds were achieved (defined as less than 10% variation within a

5 day period), the rats were prepared with 14-day osmotic minipumps containing either saline ($n=9$) or nicotine ($n=11$) dissolved in saline. Brain reward thresholds and response latencies were assessed daily throughout the experiment between 0900 and 1200 hours. The nAChR antagonist mecamylamine was used to investigate the effects of precipitated withdrawal on brain reward thresholds and response latencies. Mecamylamine (1.3 mg/kg, s.c.) injections started at least 6 days after the implantation of the minipumps, so that nicotine dependence could develop. Mecamylamine was administered 5 min before the rats were placed in the ICSS test chambers. There was a 48-h interval between each mecamylamine injection. This time interval allowed the reestablishment/maintenance of nicotine dependence. The serum elimination half-life of mecamylamine is approximately 1 h (Debruyne *et al*, 2003).

D-Phe CRF$_{(12-41)}$ and precipitated withdrawal. The aim of this experiment was to investigate the effects of antagonism of CRF receptors on the elevations in brain reward thresholds associated with mecamylamine-precipitated nicotine withdrawal. The initial experimental procedures for this experiment were the same as those for the first experiment with the exception that 28-day osmotic minipumps containing either saline ($n=8$) or nicotine solution ($n=7$) were implanted. Mecamylamine (3 mg/kg, s.c.) injections started at least 6 days after the implantation of the minipumps to allow the development of nicotine dependence. The CRF antagonist D-Phe CRF$_{(12-41)}$ (1–20 μg, i.c.v.) was administered 15 min prior treatment with mecamylamine. The rats were placed in the ICSS test chambers 5 min after mecamylamine administration. It was ensured that the minimum time interval between the mecamylamine injections was at least 72 h to re-establish/maintain nicotine dependence. At the end of all the experiments the rats were killed using an overdose of pentobarbital (150 mg/kg, intraperitoneally (i.p.)), and cannulae placement were verified by administering 5 μl of a 0.5% aqueous methyl blue solution at the injections site.

D-Phe CRF$_{(12-41)}$ and spontaneous withdrawal. The aim of this experiment was to investigate the effect of antagonism of CRF receptors on the elevations in brain reward thresholds associated with spontaneous nicotine withdrawal. Minipumps containing either saline ($n=17$) or nicotine ($n=19$) were implanted when the rats had been stabilized on the ICCS procedure. The minipumps were removed after 14 days to evaluate the effects of D-Phe CRF$_{(12-41)}$ on the affective aspects of spontaneous nicotine withdrawal. Brain reward thresholds and response latencies were assessed 3, 6, 12, 24 36, 48, 72, and 96 h after minipump explantation. D-Phe CRF$_{(12-41)}$ (20 μg, i.c.v.) was administered to rats chronically treated with saline ($n=9$) or nicotine ($n=9$), 15 min before the 6-h test session. The remainder of the rats (chronic saline, $n=8$; chronic nicotine, $n=10$) was injected with saline 15 min before the 6-h test session. At the end of all the experiments the rats were killed using an overdose of pentobarbital (150 mg/kg, i.p.), and cannulae placement were verified by administering 5 μl of a 0.5% aqueous methyl blue solution at the injections site.

RESULTS

Precipitated Nicotine Withdrawal

Mean (±SEM) absolute brain reward thresholds before pump-implantation for saline- and nicotine-treated rats were 110.14±17.32 and 101.40±8.19 μA ($t(18)=0.50$, NS), respectively. Mean (±SEM) absolute response latencies for saline- and nicotine-treated rats were 3.17±0.26 and 3.38±0.13 s ($t(18)=0.29$, NS), respectively. Systemic administration of the nAChR-receptor antagonist mecamylamine resulted in an elevation in brain reward thresholds in rats chronically treated with nicotine, but did not elevate the brain reward thresholds of the saline-treated controls (Figure 1a; dose × treatment interaction: $F2,36=7.864$, $P<0.0015$). Newman–Keuls *post hoc* comparisons revealed that the administration of mecamylamine resulted in a dose-dependent elevation in brain reward thresholds in nicotine-treated rats. Systemic administration of mecamylamine did increase the response latencies of both the nicotine- and saline-treated rats (Figure 1b; dose: $F2,36=6.633$, $P<0.0035$).

D-Phe CRF$_{(12-41)}$ and Precipitated Withdrawal

Mean (±SEM) absolute brain reward thresholds before pump-implantation for saline- and nicotine-treated rats were 126.25±15.67 and 117.26±20.34 μA ($t(13)=0.73$, NS), respectively. Mean (±SEM) absolute response latencies for saline- and nicotine-treated rats were 3.65±0.13 and 3.41±0.15 s ($t(13)=0.24$, NS), respectively. Figure 2a indicates that the administration of 3 mg/kg of mecamylamine to nicotine-treated rats induces a strong elevation, ~140%, in brain reward thresholds, which is in line with the results depicted in Figure 1a. Pretreatment with D-Phe CRF$_{(12-41)}$ reduced the threshold elevating effects of mecamylamine in nicotine dependent rats, but did not affect the brain reward thresholds of the rats that were chronically treated with saline and acutely treated with mecamylamine (Figure 2a; dose × treatment interaction: $F4,52=2.743$, $P<0.038$). Newman–Keuls *post hoc* comparisons indicated that 1–10 μg of D-Phe CRF$_{(12-41)}$ did not reverse the elevations in brain reward thresholds associated with mecamylamine-precipitated nicotine withdrawal. However, the 20 μg dose of D-Phe CRF$_{(12-41)}$ completely reversed the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. In addition, the brain reward thresholds of the rats chronically treated with nicotine and acutely treated with mecamylamine and 20 μg of D-Phe CRF$_{(12-41)}$ were lower than those of the rats chronically treated with nicotine and acutely treated with mecamylamine and vehicle (0 μg of D-Phe CRF$_{(12-41)}$). The administration of D-Phe CRF$_{(12-41)}$ did not alter the response latencies (Figure 2b; dose: $F4,52=1.979$, NS).

D-Phe CRF$_{(12-41)}$ and Spontaneous Withdrawal

Mean (±SEM) absolute brain reward thresholds before pump-implantation for saline- and nicotine-treated rats were 105.44±8.13 and 116.16±7.42 μA ($t(34)=0.98$, NS), respectively. Mean (±SEM) absolute response latencies for saline- and nicotine-treated rats were 3.31±0.11 and 3.28±0.11 s ($t(34)=0.18$, NS), respectively. Administration

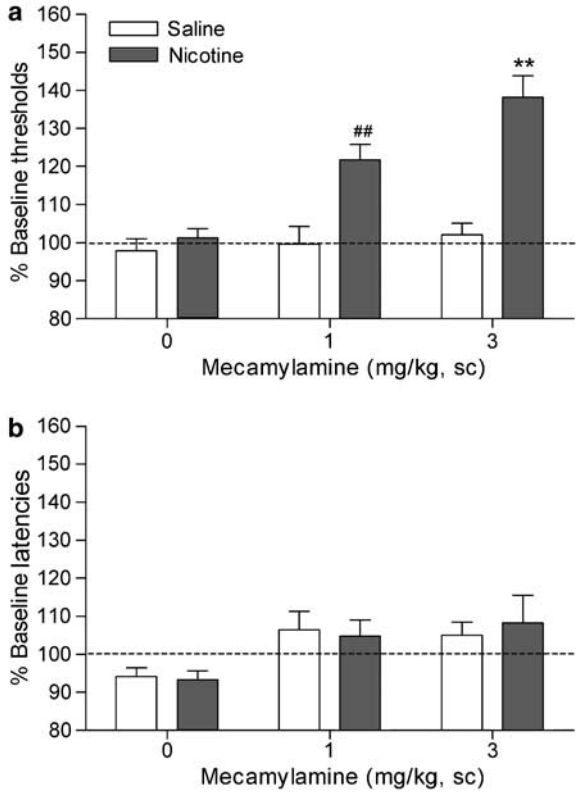

Figure 1 Effects of mecamylamine-precipitated nicotine withdrawal on brain reward thresholds (a; saline, $n=9$; nicotine, $n=11$) and response latencies (b; saline, $n=9$; nicotine, $n=11$) in rats. Brain reward thresholds are expressed as a percentage of the pretest day values. Asterisks ($^{**}P<0.01$) indicate elevations in brain reward thresholds compared to all other groups. Pound signs ($^{\#\#}P<0.01$) indicate elevations in brain reward thresholds compared to those after administration of vehicle to rats chronically treated with saline or nicotine, and compared to the corresponding saline-treated control group. Thus, mecamylamine dose dependently elevated brain reward thresholds in rats chronically treated with nicotine. Data are expressed as means ± SEM.

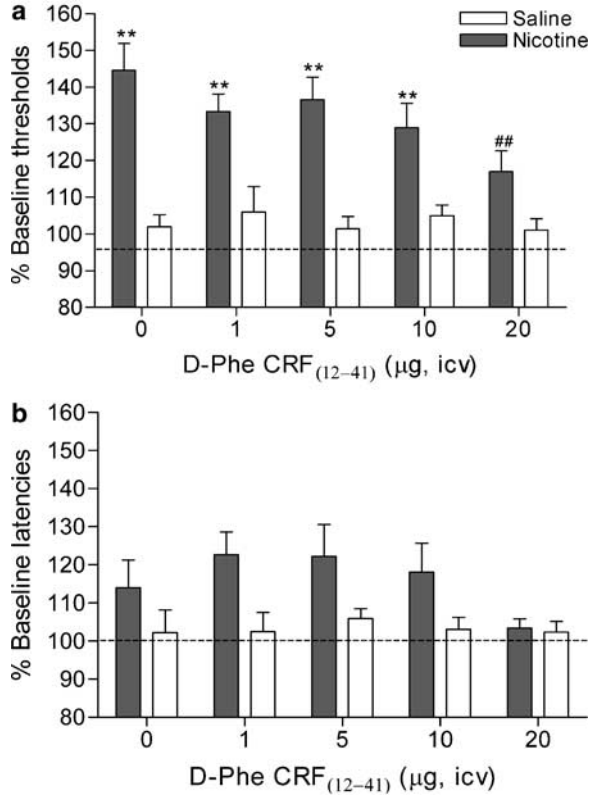

Figure 2 Effects of D-Phe $CRF_{(12-41)}$ (saline, $n=8$; nicotine, $n=7$) on the elevations in brain reward thresholds associated with mecamylamine-precipitated nicotine withdrawal (a). Effects of D-Phe $CRF_{(12-41)}$ on the response latencies of rats chronically treated with saline ($n=8$) or nicotine ($n=7$) and acutely treated with mecamylamine (b). Brain reward thresholds and response latencies are expressed as a percentage of the pre-test day values. Asterisks ($^{**}P<0.01$) indicate elevations in brain reward thresholds compared to those of the corresponding saline-treated control group. Pound signs ($^{\#\#}P<0.01$) indicate elevations in brain reward thresholds compared to those of rats chronically treated with nicotine and acutely treated with mecamylamine and vehicle (0 μg of D-Phe $CRF_{(12-41)}$).

of D-Phe $CRF_{(12-41)}$ 15 min before the 6-h time point did not affect the brain reward thresholds (Table 1; time: $F10,170=8.038$, $P<0.0001$; time × treatment interaction: $F10,170=0.771$, NS) nor response latencies (Table 1; time: $F10,170=6.608$, $P<0.0001$; time × treatment: $F10,170=0.793$, NS) of rats during nicotine withdrawal. The administration of D-Phe $CRF_{(12-41)}$ 15 min before the 6-h time point effected neither the brain reward thresholds (Table 2; time: $F10,150=4.023$, $P<0.0001$; time × treatment: $F10,150=0.502$, NS) nor response latencies (Table 2; time: $F10,150=3.671$, $P<0.0002$; time × treatment: $F10,150=1.256$, NS) of saline-treated control rats. To investigate the effects of spontaneous nicotine withdrawal on brain reward thresholds and response latencies, the chronic nicotine-treated rats that received saline or D-Phe $CRF_{(12-41)}$ before the 6 h time point were grouped together; the chronic saline-treated rats that received saline or D-Phe $CRF_{(12-41)}$ before the 6 h time point were also grouped together. Explantation of the minipumps resulted in a strong elevation in the brain reward thresholds (Data not shown; time: $F10,340=10.889$, $P<0.0001$; time × treatment: $F10,340=2.486$, $P<0.0069$) and response latencies (data not shown; time: $F10,340=8.192$, $P<0.0001$; time × treatment: $F10,340=2.742$, $P<0.0029$) in the rats chronically treated with nicotine. *Post hoc* analysis indicated that the brain reward thresholds of chronic nicotine-treated rats were elevated: 3, 6, 12, 24, 36, 48, 72, and 96 h postminipump explantation. Latencies of the chronic nicotine-treated rats were increased 3, 6, 12, 24, 36, 48, 72, and 144 h post minipump explantation.

DISCUSSION

The present results showed that the nAChR antagonist mecamylamine dose-dependently elevates brain reward thresholds in rats that are chronically treated with nicotine. Pretreatment with the CRF-receptor antagonist D-Phe $CRF_{(12-41)}$ prevented the mecamylamine-induced elevations in brain reward thresholds in the nicotine dependent rats. In contrast, the same dose of D-Phe $CRF_{(12-41)}$ (20 μg, i.c.v.) that prevented precipitated nicotine withdrawal did not lower the brain reward thresholds of the nicotine-treated rats during spontaneous withdrawal. The results presented here are in line with a previous study showing that precipitated and spontaneous nicotine withdrawal are associated with an elevation in brain reward thresholds,

Table 1 Effects of D-Phe $CRF_{(12–41)}$ on the Elevations in Brain Reward Thresholds and Response Latencies Associated with Spontaneous Nicotine Withdrawal

	% Baseline thresholds		% Baseline latencies	
Time	**Saline**	**D-Phe $CRF_{(12–41)}$**	**Saline**	**D-Phe $CRF_{(12–41)}$**
3	122.9±8.7	135.3±6.2	119.3±3.3	113.9±2.5
6	138.9±14.9	134.8±3.9	124.3±3.9	113.1±3.0
12	141.8±10.8	137.1±4.9	117.3±3.0	115.2±5.2
24	127.0±8.6	131.3±5.5	112.3±4.5	111.8±3.7
36	121.8±5.7	131.8±6.8	111.3±3.1	110.7±3.4
48	126.8±6.1	118.8±5.4	111.8±4.8	111.0±4.1
72	114.8±7.3	119.6±7.3	110.1±4.0	109.5±3.7
96	108.7±7.0	114.3±4.1	103.8±4.3	105.1±2.6
120	109.7±7.3	114.4±7.4	105.0±3.3	100.9±3.0
144	108.0±8.7	117.9±3.9	108.4±3.7	103.5±2.8
168	108.2±6.7	106.4±4.3	104.3±2.4	105.7±3.7

All rats were chronically (14-days) exposed to nicotine (s.c.). D-Phe $CRF_{(12–41)}$ (20-μg, i.c.v., $n=10$) or saline (i.c.v., $n=10$) was administered 15 min before the 6-h time point (postminipump explantation). Data are expressed as means±SEM.

Table 2 Effects of D-Phe $CRF_{(12–41)}$ on the Brain Reward Thresholds and Response Latencies of Saline-Treated Control Rats

	% Baseline thresholds		% Baseline latencies	
Time	**Saline**	**D-Phe $CRF_{(12–41)}$**	**Saline**	**D-Phe $CRF_{(12–41)}$**
3	108.3±5.6	106.8±6.0	105.7±4.1	106.9±2.2
6	102.7±5.3	103.7±4.8	103.0±3.0	105.1±1.6
12	109.5±7.0	116.3±5.3	101.1±5.3	103.0±1.8
24	99.3±7.2	99.2±6.4	96.6±5.2	106.4±4.0
36	103.6±5.4	102.1±7.9	96.9±5.3	99.6±2.9
48	101.7±4.6	100.7±6.6	97.8±3.3	101.3±1.9
72	95.7±6.2	98.6±8.6	96.5±3.8	96.1±2.3
96	97.1±5.5	94.0±4.7	100.1±4.8	102.8±3.4
120	101.0±8.4	96.3±5.9	97.6±3.5	102.7±2.5
144	98.4±6.2	96.9±5.8	96.4±3.2	96.2±3.4
168	101.8±5.1	95.3±7.2	102.3±4.6	97.9±1.7

All rats were chronically (14-days) exposed to saline (s.c.). D-Phe $CRF_{(12–41)}$ (20-μg, i.c.v., $n=9$) or saline (i.c.v., $n=8$) was administered 15 min before to the 6-h time point (postminipump explantation). Data are expressed as means±SEM.

which is indicative of a deficit in brain reward function (Epping-Jordan *et al*, 1998). Our findings extend and corroborate previous findings by demonstrating that antagonism of CRF receptors before precipitating withdrawal prevents the negative affective state of nicotine withdrawal, but antagonism of CRF receptors during spontaneous withdrawal does not reverse the negative affective aspects of nicotine withdrawal.

The results presented here indicate that antagonism of CRF receptors prevents the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. To our knowledge, this is the first study to show that CRF-receptor antagonism could be an efficacious treatment for the decrease in brain reward function associated with tobacco smoking cessation. Although the role of CRF in negative mood states associated with nicotine withdrawal had not been investigated, experimental evidence indicates that a hyperactivity of brain CRF systems may play a role in negative emotional states associated with withdrawal from other drugs of abuse. For example, antagonism of CRF receptors has been shown to prevent the development of opioid withdrawal-induced conditioned place aversion (Heinrichs *et al*, 1995; Stinus *et al*, 2005). It has been suggested that an increased release of CRF in extrahypothalamic brain sites mediates the negative affective state of drug withdrawal (Koob and Le Moal, 2005). Support for a role of CRF in the CeA in drug withdrawal symptomatology is provided by microdialysis experiments that indicate that abrupt cessation of drug administration causes an elevation in CRF levels in the CeA (Merlo Pich *et al*, 1995; Richter and

Weiss, 1999; Rodriguez de Fonseca *et al*, 1997). Another CRF-containing brain site that could possibly mediate the affective aspects of drug withdrawal is the bed nucleus of stria terminalis (Olive *et al*, 2002; Stout *et al*, 2000).

The present data showed that antagonism of CRF receptors prevents the elevations in brain reward thresholds associated with precipitated nicotine withdrawal, but does not reverse the elevations in brain reward thresholds associated with spontaneous nicotine withdrawal. It is unlikely that this discrepancy can be attributed to the doses of D-Phe $CRF_{(12-41)}$ used. The same dose of D-Phe $CRF_{(12-41)}$ (20 μg, i.c.v.) that prevented the elevations in brain reward thresholds associated with precipitated nicotine withdrawal did not reverse the elevations in brain reward thresholds associated with spontaneous nicotine withdrawal. In addition, a large number of studies have reported that doses of D-Phe $CRF_{(12-41)}$ that are lower than the 20 μg (i.c.v.) dose used in the present experiment are efficacious in reversing the neurobehavioral effects of stressors or drugs of abuse (Basso *et al*, 1999; Le *et al*, 2000; Valdez *et al*, 2003). A possible explantation for the observation that D-Phe $CRF_{(12-41)}$ reduced precipitated, but not spontaneous, nicotine withdrawal could be that there was a difference in the severity of the precipitated and the spontaneous withdrawal syndrome. However, this explanation is unlikely as both methods of withdrawal induction, administration of a nAChR antagonist or discontinuation of nicotine administration, induced a similar 40% elevation in brain reward thresholds.

Another possible explanation for the dissimilar effects of D-Phe $CRF_{(12-41)}$ on precipitated and spontaneous nicotine withdrawal might be that in the precipitated withdrawal experiment D-Phe $CRF_{(12-41)}$ was administered before the onset of withdrawal and in the spontaneous withdrawal experiment D-Phe $CRF_{(12-41)}$ was administered after the onset of withdrawal. It is speculated here that CRF may play a critical role in the initiation of stress responses such as the one caused by acute cessation of drug administration, while it is of lesser importance for the continuity of a stress response. This would explain the observation that D-Phe $CRF_{(12-41)}$ administered before the onset of withdrawal prevents the elevations in brain reward thresholds while the administration of D-Phe $CRF_{(12-41)}$ during the withdrawal phase does not result in a lowering of the brain reward thresholds. We suggest that activation of brain CRF systems may initiate a cascade of neurochemical events that result in the elevations in brain reward thresholds. For example, it has been shown that central administration of CRF results in brain site-specific changes in norepinephrine and serotonin levels (De Groote *et al*, 2005; Lavicky and Dunn, 1993; Price and Lucki, 2001; Shimizu and Bray, 1989; Zhang *et al*, 1998). It is argued here that CRF-induced alterations in the state of these monoamine systems or in other brain systems may induce a negative emotional state. After onset, a decrease in brain CRF levels may not result an immediate diminution of the dysphoric state. Chronic treatment with CRF antagonists could possibly reduce the duration of the drug withdrawal syndrome as it has been hypothesized that CRF may be part of a feed forward loop that causes the activation of brain stress systems (Dunn *et al*, 2004).

The present findings indicate that acute antagonism of CRF receptors prevents the elevations in brain reward thresholds associated with precipitated nicotine withdrawal but does not reverse the elevations in brain reward thresholds associated with spontaneous nicotine withdrawal. Experiments that investigate the effects of different CRF-based treatment regimens on the affective aspects of nicotine withdrawal are warranted as treatments that counteract the acute and protracted negative affective aspects of nicotine withdrawal may help to prevent relapse to smoking behavior.

ACKNOWLEDGEMENTS

This work was funded by a National Institute on Drug Abuse grant (RO3 DA020502-01) to Adrie Bruijnzeel. Carrie Wilson received salary support from The University of Florida's College of Veterinary Medicine Merck-Merial Veterinary Scholars Program. We would like to thank Dr Jean Rivier (The Clayton Foundation Laboratories for Peptide Biology, the Salk Institute for Biological Studies, San Diego, CA) for generously providing D-Phe $CRF_{(12-41)}$.

REFERENCES

American Psychiatric Association (2000). *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Press: Washington, DC.

Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT (1991). CRF antagonist reverses the 'anxiogenic' response to ethanol withdrawal in the rat. *Psychopharmacology (Berlin)* **103**: 227–232.

Bardo MT, Green TA, Crooks PA, Dwoskin LP (1999). Nornicotine is self-administered intravenously by rats. *Psychopharmacology (Berlin)* **146**: 290–296.

Barr AM, Markou A (2005). Psychostimulant withdrawal as an inducing condition in animal models of depression. *Neurosci Biobehav Rev* **29**: 675–706.

Basso AM, Spina M, Rivier J, Vale W, Koob GF (1999). Corticotropin-releasing factor antagonist attenuates the 'anxiogenic-like' effect in the defensive burying paradigm but not in the elevated plus-maze following chronic cocaine in rats. *Psychopharmacology (Berlin)* **145**: 21–30.

Bruijnzeel AW, Gold MS (2005). The role of corticotropin-releasing factor-like peptides in cannabis, nicotine, and alcohol dependence. *Brain Res Brain Res Rev* **49**: 505–528.

Bruijnzeel AW, Lewis B, Bajpai LK, Morey TE, Dennis DM, Gold M (2006). Severe deficit in brain reward function associated with fentanyl withdrawal in rats. *Biol Psychiatry* **59**: 477–480.

Bruijnzeel AW, Markou A (2004). Adaptations in cholinergic transmission in the ventral tegmental area associated with the affective signs of nicotine withdrawal in rats. *Neuropharmacology* **47**: 572–579.

Bruijnzeel AW, Markou A (2005). Decreased sensitivity to the effects of dopamine D1-like, but not D2-like, receptor antagonism in the posterior hypothalamic region/anterior ventral tegmental area on brain reward function during chronic exposure to nicotine in rats. *Brain Res* **1058**: 91–100.

Corrigall WA, Coen KM (1989). Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacology (Berlin)* **99**: 473–478.

Corrigall WA, Coen KM, Adamson KL (1994). Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res* **653**: 278–284.

Crooks PA, Dwoskin LP (1997). Contribution of CNS nicotine metabolites to the neuropharmacological effects of nicotine and tobacco smoking. *Biochem Pharmacol* **54**: 743–753.

De Bellis MD, Gold PW, Geracioti Jr TD, Listwak SJ, Kling MA (1993). Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am J Psychiatry* **150**: 656–657.

De Groote L, Penalva RG, Flachskamm C, Reul JM, Linthorst AC (2005). Differential monoaminergic, neuroendocrine and behavioural responses after central administration of corticotropin-releasing factor receptor type 1 and type 2 agonists. *J Neurochem* **94**: 45–56.

Debruyne D, Sobrio F, Hinschberger A, Camsonne R, Coquerel A, Barre L (2003). Short-term pharmacokinetics and brain distribution of mecamylamine as a preliminary to carbon-11 labeling for nicotinic receptor investigation. *J Pharm Sci* **92**: 1051–1057.

Donny EC, Caggiula AR, Mielke MM, Booth S, Gharib MA, Hoffman A *et al* (1999). Nicotine self-administration in rats on a progressive ratio schedule of reinforcement. *Psychopharmacology (Berlin)* **147**: 135–142.

Dunn AJ, Swiergiel AH, Palamarchouk V (2004). Brain circuits involved in corticotropin-releasing factor-norepinephrine interactions during stress. *Ann NY Acad Sci* **1018**: 25–34.

Epping-Jordan MP, Watkins SS, Koob GF, Markou A (1998). Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* **393**: 76–79.

Harrison AA, Liem YT, Markou A (2001). Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. *Neuropsychopharmacology* **25**: 55–71.

Heinrichs SC, Menzaghi F, Schulteis G, Koob GF, Stinus L (1995). Suppression of corticotropin-releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. *Behav Pharmacol* **6**: 74–80.

Koob GF (1999). Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry* **46**: 1167–1180.

Koob GF, Caine SB, Parsons L, Markou A, Weiss F (1997). Opponent process model and psychostimulant addiction. *Pharmacol Biochem Behav* **57**: 513–521.

Koob GF, Le Moal M (2005). Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. *Nat Neurosci* **8**: 1442–1444.

Kornetsky C, Esposito RU (1979). Euphorigenic drugs: effects on the reward pathways of the brain. *Fed Proc* **38**: 2473–2476.

Lavicky J, Dunn AJ (1993). Corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. *J Neurochem* **60**: 602–612.

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 (Berlin)* **150**: 317–324.

Macey DJ, Koob GF, Markou A (2000). CRF and urocortin decreased brain stimulation reward in the rat: reversal by a CRF receptor antagonist. *Brain Res* **866**: 82–91.

Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA *et al* (1992). Rodent model of nicotine abstinence syndrome. *Pharmacol Biochem Behav* **43**: 779–784.

Markou A, Kosten TR, Koob GF (1998). Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology* **18**: 135–174.

McLellan AT, Lewis DC, O'Brien CP, Kleber HD (2000). Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *JAMA* **284**: 1689–1695.

Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO *et al* (2004). Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. *J Neurosci* **24**: 1478–1485.

Merlo Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF *et al* (1995). Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci* **15**: 5439–5447.

Nemeroff CB, Bissette G, Akil H, Fink M (1991). Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy. Corticotrophin-releasing factor, beta-endorphin and somatostatin. *Br J Psychiatry* **158**: 59–63.

Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K *et al* (1984). Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* **226**: 1342–1344.

O'Brien CP (2003). Research advances in the understanding and treatment of addiction. *Am J Addict* **12**(Suppl 2): S36–S47.

Olive MF, Koenig HN, Nannini MA, Hodge CW (2002). Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake. *Pharmacol Biochem Behav* **72**: 213–220.

Overstreet DH, Knapp DJ, Breese GR (2004). Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF1 receptors. *Pharmacol Biochem Behav* **77**: 405–413.

Paxinos G, Watson C (1998). *The Rat Brain in Stereotaxic Coordinates*. Academic Press: San Diego.

Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Merlo Pich E *et al* (1998). Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* **391**: 173–177.

Price ML, Lucki I (2001). Regulation of serotonin release in the lateral septum and striatum by corticotropin-releasing factor. *J Neurosci* **21**: 2833–2841.

Rassnick S, Heinrichs SC, Britton KT, Koob GF (1993). Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* **605**: 25–32.

Richter RM, Weiss F (1999). *In vivo* CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. *Synapse* **32**: 254–261.

Rodriguez de Fonseca F, Carrera MR, Navarro M, Koob GF, Weiss F (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* **276**: 2050–2054.

Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G (1995). Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. *Brain Res* **675**: 89–97.

Schulteis G, Markou A, Cole M, Koob GF (1995). Decreased brain reward produced by ethanol withdrawal. *Proc Natl Acad Sci USA* **92**: 5880–5884.

Shimizu H, Bray GA (1989). Modulation by corticotropin-releasing factor of monoamine metabolism in the lateral hypothalamus. *Neurosci Lett* **103**: 74–80.

Stinus L, Cador M, Zorrilla EP, Koob GF (2005). Buprenorphine and a CRF1 antagonist block the acquisition of opiate withdrawal-induced conditioned place aversion in rats. *Neuropsychopharmacology* **30**: 90–98.

Stolerman IP, Jarvis MJ (1995). The scientific case that nicotine is addictive. *Psychopharmacology (Berlin)* **117**: 2–10.

Stout SC, Mortas P, Owens MJ, Nemeroff CB, Moreau J (2000). Increased corticotropin-releasing factor concentrations in the bed nucleus of the stria terminalis of anhedonic rats. *Eur J Pharmacol* **401**: 39–46.

Valdez GR, Zorrilla EP, Roberts AJ, Koob GF (2003). Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. *Alcohol* **29**: 55–60.

Veith RC, Lewis N, Langohr JI, Murburg MM, Ashleigh EA, Castillo S *et al* (1993). Effect of desipramine on cerebrospinal fluid concentrations of corticotropin-releasing factor in human subjects. *Psychiatry Res* **46**: 1–8.

Watkins SS, Epping-Jordan MP, Koob GF, Markou A (1999). Blockade of nicotine self-administration with nicotinic antagonists in rats. *Pharmacol Biochem Behav* **62**: 743–751.

Wise RA, Munn E (1995). Withdrawal from chronic amphetamine elevates baseline intracranial self-stimulation thresholds. *Psychopharmacology (Berlin)* **117**: 130–136.

Zhang JJ, Swiergiel AH, Palamarchouk VS, Dunn AJ (1998). Intracerebroventricular infusion of CRF increases extracellular concentrations of norepinephrine in the hippocampus and cortex as determined by *in vivo* voltammetry. *Brain Res Bull* **47**: 277–284.

Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M *et al* (2000). Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res* **34**: 171–181.