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β-Endorphin via the Delta Opioid Receptor is a Major Factor in the Incubation of Cocaine Craving

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Cue-induced cocaine craving intensifies, or 'incubates', during the first few weeks of abstinence and persists over extended periods of time. One important factor implicated in cocaine addiction is the endogenous opioid β-endorphin. In the present study, we examined the possible involvement of β-endorphin in the incubation of cocaine craving. Rats were trained to self-administer cocaine (0.75 mg/kg, 10 days, 6 h/day), followed by either a 1-day or a 30-day period of forced abstinence. Subsequent testing for cue-induced cocaine-seeking behavior (without cocaine reinforcement) was performed. Rats exposed to the drug-associated cue on day 1 of forced abstinence demonstrated minimal cue-induced cocaine-seeking behavior concurrently with a significant increase in β-endorphin release in the nucleus accumbens (NAc). Conversely, exposure to the cue on day 30 increased cocaine seeking, while β-endorphin levels remained unchanged. Intra-NAc infusion of an anti-β-endorphin antibody (4 μg) on day 1 increased cue-induced cocaine seeking, whereas infusion of a synthetic β-endorphin peptide (100 ng) on day 30 significantly decreased cue response. Both intra-NAc infusions of the δ opioid receptor antagonist naltrindole (1 μg) on day 1 and naltrindole together with β-endorphin on day 30 increased cue-induced cocaine-seeking behavior. Intra-NAc infusion of the μ opioid receptor antagonist CTAP (30 ng and 3 μg) had no behavioral effect. Altogether, these results demonstrate a novel role for β-endorphin and the δ opioid receptor in the development of the incubation of cocaine craving.

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INTRODUCTION

Cocaine addiction poses a serious problem to society. Countless deaths occur worldwide as a result of cocaine abuse, levels of which are on the rise (Watson, 2007). Cocaine craving, induced by drug-associated environmental cues, intensifies or 'incubates' over the first few weeks of abstinence and persists over extended periods of time. As a result, addicts are prone to relapse even after long durations of abstinence (Gawin and Kleber, 1986). Grimm *et al* (2001) first demonstrated an analogous incubation phenomenon in rats, in which onset of craving is delayed and craving does not decay but rather progressively increases over a prolonged period of forced abstinence. Many studies have been conducted to elucidate the nature of this process, and the main findings have been reviewed (Lu *et al*, 2004b; Pickens *et al*, 2011). The main findings implicate the glutamatergic system as a major contributor to the incubation process as activation of the AMPA receptor, and specifically the presentation of its subunit composition on the post-synaptic membrane, is associated with this incubation process, and furthermore, blockade of GluR2-lacking AMPA receptors attenuates the incubation of cocaine craving (Conrad *et al*, 2008). A possible role for other neuronal systems in this process, particularly the opioid system, has, however, remained relatively obscure thus far.

The opioid system consists of three classical opioid receptors, μ, κ, and δ, which are abundantly expressed in the mesolimbic system, including the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Le Merrer *et al*, 2009; Peng *et al*, 2012). Opioid receptors have a substantial role in motivational and reward processes (Pradhan *et al.*, 2011; Shippenberg *et al*, 2008). A single-nucleotide polymorphism in the gene coding for the μ opioid receptor results in a reduced response to natural reward in humans (Lee *et al*, 2011). Moreover, μ opioid receptor occupancy, measured by PET, predicted the treatment outcome in cocaine-abusing patients (Ghitza *et al*, 2010). In rats it has been shown that methadone, which binds to μ receptors, blocked cocaine-induced behavioral and neural adaptations

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(Kreek *et al*, 2009; Leri *et al*, 2009). Nonetheless, several other studies have demonstrated that blockade of these receptors had no effect on cocaine self-administration and reward (Badiani *et al*, 2011; Mello and Negus, 1996; Pettit *et al*, 1984).

There is also evidence for an involvement of the δ opioid receptor in reward and addiction processes, yet findings are equivocal. Non-peptidic δ receptor agonists elicit reward in the conditioned place-preference paradigm (Longoni *et al*, 1998), but other reports have shown negligible abuse-related effects in a monkey self-administration model (Negus *et al*, 1998) and in a rat model of intracranial self-stimulation (Do Carmo *et al*, 2009). Moreover, i.p. administration of the selective δ receptor antagonist naltrindole decreases responding for cocaine in rats, regardless of the schedule of reinforcement (Reid *et al*, 1995). However, others (de Vries *et al*, 1995) have reported that only a high dose of naltrindole (i.p.), which markedly depresses locomotor activity, results in a modest (16%) reduction of cocaine self-administration. Furthermore, intra-NAc infusion of naltrindole decreases cocaine self-administration, whereas intra-VTA infusion of this antagonist increases cocaine-maintained responses (Ward and Roberts, 2007).

One of the prominent endogenous opioid receptor ligands is β-endorphin. This opioid peptide is produced, in part, in the arcuate nucleus of the hypothalamus and released in various brain regions, including reward-related areas (Roth-Deri *et al*, 2003; 2008). β-Endorphin was shown not only to attenuate acquisition as well as maintenance of cocaine self-administration (Roth-Deri *et al*, 2008) but also to reinstate cocaine seeking in rats after injections into the nucleus accumbens (Simmons and Self, 2009).

β-Endorphin binds at high affinity to both μ and δ receptors (Mansour *et al*, 1995). It was suggested that cocaine seeking is reinstated through β-endorphin's interaction with the μ, but not δ, receptor in the NAc (Simmons and Self, 2009).

To date, only few reports have addressed a possible role for the opioid system in the incubation of craving. It was found that heroin priming–induced reinstatement of cocaine craving incubates over time, while cocaine priming–induced reinstatement does not (Lu *et al*, 2004a). The authors hypothesized that the difference between the drugs' ability to induce craving after abstinence may be related to time-dependent neuroadaptations in the opioid system, which are modified by chronic cocaine exposure (Lu *et al*, 2004b; Turchan *et al*, 1999). It has also been demonstrated that naloxone, an opioid antagonist, attenuates incubation of sucrose craving in rats (Grimm *et al*, 2007). Bearing in mind the difference between sucrose and cocaine craving, this latter report is consistent with time-dependent changes in the opioid system following forced reward abstinence.

In the present study, we examined the role of β-endorphin in the incubation of cocaine craving. In this animal model, rats respond for the presentation of a cue that was previously associated with self-administration of cocaine. The extent of responding is considered as a measure of reward seeking and serves to evaluate craving. Thus, rats underwent 10 days of intensive (6 h/day) cocaine intake. We then examined the cue-induced cocaine-seeking behavior after short-term (1 day) and long-term (30 days) periods of forced abstinence and concurrent changes in NAc β-endorphin release. In addition, we examined the behavioral impact of β-endorphin on cocaine craving by evaluating cue-induced cocaine seeking after intra-NAc infusion of an anti-β-endorphin antibody (day 1) or exogenous β-endorphin peptide (day 30). Next, we examined which opioid receptor may mediate the observed effect of β-endorphin on craving via intra-NAc infusions of μ and δ receptor antagonists.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, Israel) weighing 250–350 g were maintained on a 12-h light/12-h dark reversed cycle, with food and water available *ad libitum*. Rats were housed two per cage with a metal-perforated divider between them. Experiments were conducted during the dark cycle. All experimental procedures were approved by the University Animal Care and Use Committees and were performed in accordance with the National Institutes of Health guidelines.

Guide Cannula Implantation

Rats were anesthetized with xylazine and ketamine (10 and 100 mg/kg, respectively). A 20-gauge guide cannula was then unilaterally implanted either into the NAc (AP, +1.4 mm; LM, +1.2 mm to bregma and DV, −5.6 mm) or dorsal striatum (AP +1.4 mm; LM +2.4 mm to bregma and DV, −4.4) of rats with the aid of a stereotactic device (David-Kopf Instruments, CA, USA).

Jugular Vein Catheterization

Immediately after cannula implantation, rats were implanted with intravenous silastic catheters (ID 0.55 mm, OD 0.94 mm, Dow Corning, MI) into the right jugular vein. The catheter was secured to the vein with silk sutures and was passed subcutaneously to the top of the skull, where it exited into a connector (a modified 22-gauge cannula; Plastics One, VA, USA) mounted to the skull with MX-80 screws (Small Parts, FL, USA) and dental cement (Yates and Bird, IL, USA).

Self-Administration Training Sessions

The self-administration chambers (Med-Associates; St Albans, VT, USA) had two levers, one active and one inactive. An active lever press generated a cocaine infusion (0.75 mg/kg, 0.13 ml, 5 s/infusion; cocaine obtained from the National Institutes on Drug Abuse, MD, USA) through the i.v. catheter, and also activated a light located above the lever, which was lit for 5 s. Active lever presses during the next 35 s after the light cue did not result in additional cocaine infusion. Presses on the inactive lever did not activate the infusion pump and light. The number of active lever responses, infusions, and inactive lever responses were recorded. Rats were returned to their home cages at the end of the daily session.

Forced Abstinence

Immediately after the 10-day period of self-administration training sessions, rats were subjected to either a 1-day or a 30-day period of forced abstinence. During abstinence, rats were left in their home cages and handled three times a week.

Cue-Induced Cocaine-Seeking Behavioral Test

On day 1 or day 30 of forced abstinence, rats were again placed in the self-administration chambers, connected to the infusion line, and drug-seeking behavior was tested for 60 min. Upon active lever presses, only the contingent light cue appeared, without delivery of the drug.

Microdialysis Procedure

Twelve hours before the test, a microdialysis probe (2 mm length, 20 kDa cutoff value, CMA/10; Carnegie Medicine, Sweden) was inserted into the guide cannula. Artificial cerebrospinal fluid (aCSF; consisting of 126 mM NaCl, 2.4 mM $CaCl_2$, 1.2 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, and NaHCO; pH 7.4) was continuously pumped (l.0 μl/min) through the dialysis probe. Dialysates were collected at 20-min intervals, before the cue test (for baseline measurement, in home cages) and during the cue test (in the self-administration cages). Dialysates were immediately frozen on dry ice until assay of β-endorphin content.

Quantification of β-Endorphin Content

Microdialysate β-endorphin content was determined using an ELISA assay kit (Peninsula; CA, USA), according to the manufacturer's instructions. The linear portion of the standard curve was between 0.04 and 5 ng/ml of β-endorphin, and experimental levels of β-endorphin were within the linear portion. Intra-assay variation was 4%, and inter-assay variation was 13%.

Drugs and Intracranial Infusions

β-Endorphin-(1–17) (C157H254N42O44S, 100 ng, 28 nM, Sigma), anti-β-endorphin antibody (SC-18264, 4 μg, 28 nM, Santa Cruz Biotechnology, CA, USA), the selective δ receptor antagonist (naltrindole,1 μg; Sigma, MO, USA), and the selective μ receptor antagonist (CTAP, 3 μg and 30 ng, Sigma, St Louis, MO, USA) were dissolved in aCSF. Drugs were infused (5 min, 0.2 μl/min) into the NAc or dorsal striatum via the guide cannula, using an electronical syringe pump (CMA 400, CMA/Microdialysis). Control rats received similar infusions of aCSF only (5 min, 0.2 μl/min) into the same brain regions as treated rats from the respective groups. Infusions were performed immediately (<5 min) before testing of cue-induced cocaine-seeking behavior. The internal cannula remained in place for 5 min after infusion, to avoid reflux. Doses of β-endorphin, CTAP, naltrindole, and anti-β-endorphin were selected based on previous studies (Do Carmo *et al*, 2009; Roth-Deri *et al*, 2004).

Verification of Cannula Placement

On completion of the experiments, rats ($n = 7$–10 from each group) were anesthetized and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were removed and immersed in 4% paraformaldehyde for 24 h, then in phosphate buffer with 30% sucrose for 48 h. Brains were then frozen on dry ice and sliced (40-μm sections) with a cryostat. Sections were mounted on glass slides coated with 2% gelatin, and cannula placement was verified under a microscope. Average hit rate was 85%. Data were analyzed only after verifying that the guide cannula was accurately implanted (a total of eight rats were excluded from the experiment due to technical reasons, such as cannula placement, weakness of the animal, and so on. See Figure 1c and Supplementary Figure 1A of cannula placement in the NAc).

Statistical Analysis

One-way ANOVA was used to examine differences between the groups in active lever presses during cue tests, one-way ANOVA with repeated measures was used for analysis of β-endorphin levels during the cue test (day of abstinence

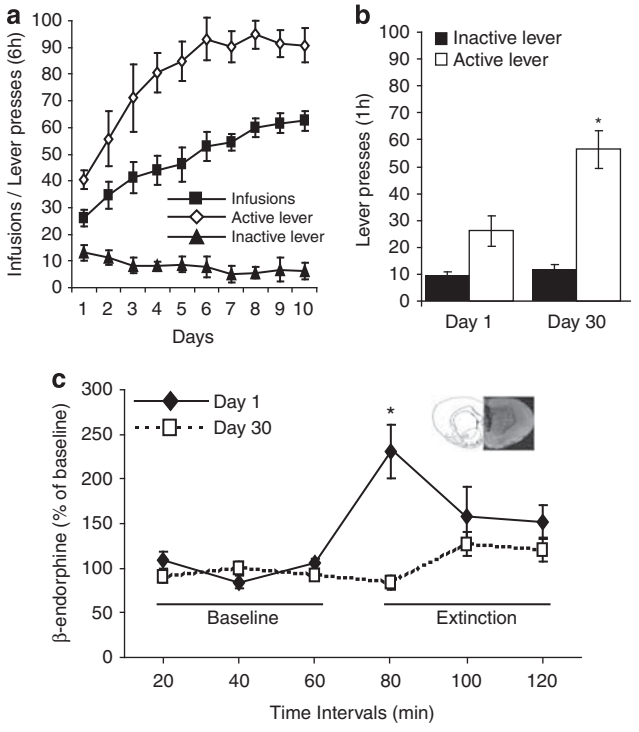

Figure 1 Incubation of cocaine craving and β-endorphin extracellular levels. (a) Self-administration training: Number of active and inactive lever presses and cocaine infusions over the 10-day, 6 h/day self-administration sessions. Cocaine (0.75 mg/kg per infusion) induced intensive pressing on the active lever, whereas the amount of presses on the inactive lever was minimal. (b) Cue-induced drug-seeking behavior. The number of active lever presses in cocaine-trained rats tested on day 30 of abstinence was significantly higher than on day 1. Values are expressed as mean ± SEM; $n = 9$ rats per group. *$p < 0.001$ *vs* day 1. (c) Cue-evoked β-endorphin levels in the NAc were significantly higher after 1 day of abstinence from cocaine than after 30 days. Values are expressed as mean ± SEM; $n = 9$ rats per group.*$p < 0.001$ *vs* baseline and day 30. Top right corner: representative image for cannula placement in the NAc.

as the between-subjects factor and time intervals as the within-subjects factor). Student–Newman–Keuls was used as *post-hoc* analysis. $p<0.05$ was considered significant. Results are presented as mean ± SEM.

RESULTS

For all the trials, rats were implanted with a guide cannula into the NAc or dorsal striatum (according to the specific experiment). After recovery, experiments consisted of three phases: cocaine self-administration training (10 days, 6 h/day), a period of forced abstinence (1 or 30 days), and then testing for cue-induced cocaine seeking (without cocaine reinforcement).

Extracellular β-Endorphin Levels During Cue-Induced Cocaine Seeking After Abstinence

Rats demonstrated reliable cocaine self-administration behavior during the 10-day, 6-h/day training sessions (Figure 1a). Similar results were also obtained during training sessions for subsequent experiments.

After training, rats were subjected either to a 1-day or a 30-day period of forced abstinence. Cue-induced cocaine seeking was then tested, and NAc β-endorphin levels were concurrently measured using the microdialysis technique.

For the cue test, one-way ANOVA showed a main effect of group ($F(1,32)=28$, $p<0.0001$). *Post-hoc* comparisons showed a significantly higher number of cue-elicited active lever presses on day 30 of abstinence, as compared with day 1 (Student–Newman–Keuls, $p<0.001$). The number of inactive lever presses was similarly low on both days ($p>0.05$; Figure 1b; $n=9$ per group).

Repeated measures ANOVA for NAc β-endorphin levels during cocaine seeking revealed a main effect of group ($F(1,80)=42.06$; $p<0.0001$), main effect of fractions ($F(5,80)=23.85$; $p<0.0001$), and an interaction between group and fractions ($F(5,80)=30.43$; $p<0.0001$). NAc β-endorphin levels were inversely related to active lever responding, demonstrating a significant twofold increase during cocaine seeking on day 1, relative to day 30 (Student–Newman–Keuls *post hoc*; $p<0.0001$; Figure 1c). Exposure to the cue transiently increased extracellular levels of β-endorphin, which returned to baseline within 20 min. There was no significant differences between baseline and all extinction time points (A one-way repeated measures ANOVA, followed by Dunnet's *post-hoc* test, ($F(3,7)=3.51$; $p>0.05$). There was no statistical difference between basal levels of β-endorphin on day 1 and day 30 of abstinence (0.483 ± 0.041 mg/μl *vs* 0.466 ± 0.1 mg/μl, respectively, $p>0.05$, Student's *t*-test).

Intra-NAc β-Endorphin After Long-Term Abstinence Attenuates Cue-Induced Cocaine Seeking

In this experiment, we infused a synthetic β-endorphin peptide into the NAc or dorsal striatum on day 30 of abstinence and examined response to cocaine cues ($n=9$/group). A two-way ANOVA revealed a main effect of treatment ($F(1,31)=9.19$, $p<0.005$), an interaction between brain region and treatment ($F(1,31)=4.71$, $p<0.05$), but no main effect of brain region ($F(1,31)=0.36$, $p>0.05$).

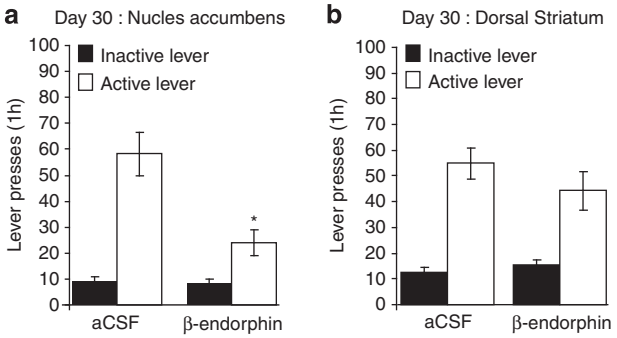

Figure 2 Effect of intra-NAc infusion of β-endorphin on cue-induced cocaine-seeking behavior on day 30. β-Endorphin microinjections into the NAc (a) but not into the dorsal striatum (b) significantly decreased cocaine-seeking behavior after 30 days of abstinence. Values are expressed as mean ± SEM; $n=10$ rats per group. *$p<0.002$ *vs* aCSF-infused rats.

Cue-elicited cocaine-seeking was significantly decreased in intra-NAc β-endorphin-infused rats, as compared with aCSF-infused control rats (Student–Newman–Keuls *post-hoc* test, $p<0.01$; Figure 2a). For rats which received dorsal striatum infusions, there were no differences in active lever pressing between β-endorphin-treated rats and controls (Student–Newman–Keuls *post-hoc* test, $p>0.05$; Figure 2b). There were no group differences in inactive lever pressing for either intra-NAc- or dorsal striatum-infused rats ($p>0.05$).

Intra-NAc Anti-β-Endorphin Antibody After Short-Term Abstinence Increases Cue-Induced Cocaine Seeking

Here, we infused an anti-β-endorphin antibody into the NAc or dorsal striatum on day 1 and then examined cocaine-seeking behavior ($n=7$–8/group). A two-way ANOVA revealed a main effect of brain region ($F(1,27)=18.93$; $p<0.0005$), an interaction between brain region and treatment ($F(1,27)=4.61$; $p<0.05$), but no main effect of treatment ($F(1,27)=3.09$; $p>0.05$). Responding on the active lever was significantly higher for rats that received intra-NAc anti-β-endorphin infusion, as compared with aCSF-infused controls (Student–Newman–Keuls *post-hoc* test, $p<0.01$; Figure 3a). For rats which received a dorsal striatum infusion, there were no differences in active lever presses between the anti-β-endorphin-treated and aCSF-treated groups (Student–Newman–Keuls *post-hoc*, $p>0.05$; Figure 3b). There were no group differences in inactive lever pressing for either intra-NAc- or dorsal striatum-infused rats ($p>0.05$).

The Effect of Intra-NAc μ or δ Receptor Antagonists on Cue-Induced Cocaine Seeking After Abstinence

Here, we infused either naltrindole (a δ receptor antagonist) or CTAP (a μ receptor antagonist) into the NAc on day 1. On day 30, rats received the same intra-NAc antagonist treatments, preceded by infusion of a synthetic β-endorphin peptide. Controls received intra-NAc aCSF infusion. Additionally, treatment groups on day 1 were compared with results obtained for intra-NAc anti-β-endorphin, and

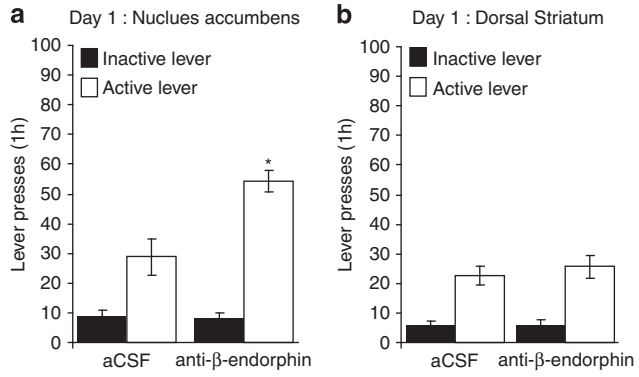

Figure 3 Effect of intra-NAc infusion of anti-β-endorphin antibody on cue-induced cocaine-seeking behavior on day 1. β-Endorphin antibody infusion into the NAc (a) but not dorsal striatum (b) significantly increased cocaine-seeking behavior after 1 day of abstinence. Values are expressed as mean ± SEM, $n = 7$-8 rats per group. *$p < 0.01$ *vs* aCSF-infused rats.

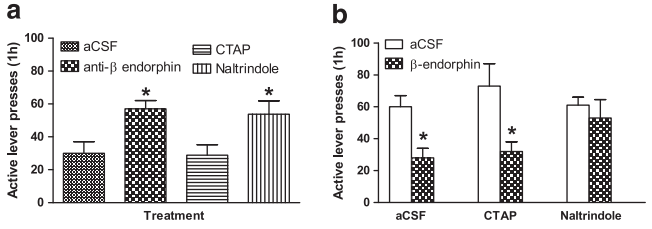

Figure 4 Effect of intra-NAc infusion of δ or μ antagonist cue-induced cocaine-seeking behavior after 1 day or 30 days of forced abstinence. (a) Day 1: Number of active lever presses after infusion of naltrindole (δ antagonist) is significantly higher than after infusion of CTAP (μ antagonist; 3 μg) and showed the same effect as anti-β-endorphin antibody infusion (anti β-endorphin). *$p < 0.05$ *vs* aCSF-infused rats. (b) Day 30: Antagonism of the δ receptor reversed the effect of exogenous β-endorphin on cue-induced cocaine-seeking behavior. The number of active lever presses after infusion of naltrindole together with β-endorphin is significantly higher than after infusion of CTAP (3 μg) with β-endorphin or infusion of a β-endorphin alone. *$p < 0.01$ *vs* aCSF-infused rats. Values are expressed as mean ± SEM; $n = 9$ rats per group.

treatment groups on day 30 were compared with results obtained for intra-NAc synthetic β-endorphin injection.

For day 1, one-way ANOVA revealed a significant difference between treatments ($F(3,32) = 4.799$, $p < 0.01$). Treatment with naltrindole, similar to anti-β-endorphin, resulted in significantly increased cocaine-seeking behavior, as compared with CTAP (3 μg) or aCSF (Student–Newman–Keuls *post-hoc*, $p < 0.05$; $n = 9$ per group; Figure 4a). Injection of CTAP at a lower dose (30 ng) showed results similar to that of 3 μg CTAP (active lever presses: 21 ± 5 *vs* 28 ± 6, respectively; $p > 0.05$).

For day 30, a two-way ANOVA revealed a main effect of group ($F(1, 38) = 12.97$; $p < 0.001$) but no main effect of treatment ($F(2,38) = 0.33$; $p > 0.05$) and no interaction ($F(2,38) = 1.65$; $p > 0.05$). Subsequent *post-hoc* tests showed that CTAP (3 μg) co-injected with β-endorphin, similar to injection of β-endorphin alone, significantly decreased active lever responding compared with aCSF (Student–Newman–Keuls, $p < 0.01$; Figure 4b). Co-injection of CTAP at a lower dose (30 ng) showed results similar to those of co-injected 3 μg CTAP (active lever presses: 24 ± 6 *vs* 34 ± 7, respectively; $p > 0.05$). However, infusion of naltrindole together with β-endorphin reversed the effect of β-endorphin alone and of co-injected CTAP, by significantly elevating the amount of active lever presses to control levels (Student–Newman–Keuls, $p < 0.05$; $n = 9$ per group; Figure 4b).

Infusion of either CTAP (3 μg) or naltrindole (1 μg) without co-injection of β-endorphin showed no significant effect on cocaine-seeking behavior, as compared with aCSF (one-way ANOVA; $F(3,29) = 6.456$, followed by Student–Newman–Keuls *post-hoc* test, $p > 0.0.5$).

DISCUSSION

β-Endorphin levels in the NAc are associated with cocaine self-administration and craving (Roth-Deri *et al*, 2003, 2008). In the present study, we demonstrate novel evidence supporting the involvement of this opioid peptide in the incubation of cocaine craving via its interaction with the δ receptor.

In addition to its involvement in cocaine self-administration (Roth-Deri *et al*, 2003, 2008), β-endorphin also possesses rewarding and reinforcing properties of its own (Amalric *et al*, 1987). Moreover, it can moderate behavioral responses to stressful stimuli (Merenlender-Wagner *et al*, 2009; Young *et al*, 1990). Thus, we suggest that the increase in extracellular β-endorphin levels on day 1 may facilitate coping both with the stress caused by the sudden lack of cocaine supply and with the resultant heightened drug craving. This lowering of craving can account for the reduction in cue-induced cocaine seeking on day 1, demonstrated herein and in previous reports (Grimm *et al*, 2001; Lu *et al*, 2004b).

We additionally showed that intra-NAc infusion of anti-β-endorphin antibody on day 1 enhanced cue-induced cocaine seeking, whereas infusion of exogenous β-endorphin on day 30 significantly attenuated cocaine seeking. This further suggests that release of β-endorphin in the NAc may function as a mechanism for lowering of cue-induced craving. However, this mechanism appears to be short-lived, as during cue exposure on day 30 extracellular β-endorphin levels remained unchanged, when cocaine craving was high. Interestingly, basal levels of β-endorphin did not change after cocaine abstinence, indicating that tonic release remained unchanged yet phasic release was altered following the exposure to the cue on day 30. Incubation is usually interpreted as an increase in the expression of an existing effect or mechanism. Herein we postulate that during abstinence, an endogenous braking mechanism, ie, the endogenous release of NAc β-endorphin in response to the cue, is apparently lost.

Contrary to our findings, a recent study demonstrated that intra-NAc infusion of β-endorphin reinstates previously extinguished cocaine seeking (Simmons and Self, 2009). These different effects of β-endorphin may be due to the differences in methodological factors, such as daily dosage of cocaine and intensity of exposure and the between-session analysis applied. Yet most significant, in our opinion, is the different paradigms applied: the

frequently used extinction training by daily exposure to a cue, employed in the study by Simmons and Self (2009), as opposed to a prolonged forced abstinence period followed by a single exposure to the cue, employed herein.

Several recent findings suggest involvement of β-endorphin and the δ receptor, in cocaine seeking (reviewed in (Yadid *et al*, 2012)). It was shown that cocaine-induced reward is reduced in β-endorphin-deficient but not μ receptor knockout mice. This implies that β-endorphin is essential for the rewarding action of cocaine while the μ receptor may not mediate this regulatory action (Nguyen *et al*, 2012).

Our findings, showing reduced β-endorphin release concurrently with heightened drug craving after long-term abstinence, are consistent with previous studies demonstrating attenuated β-endorphin response during ethanol or nicotine relapse. One study examined the extent to which β-endorphin response to stress is associated with early smoking relapse. The authors found that smokers who relapsed exhibited an attenuated β-endorphin response to stressors, compared with those who maintained abstinence over the same period (Shaw and al'Absi, 2008). Moreover, smokers who underwent weekly exercise sessions had higher β-endorphin plasma levels and demonstrated a reduced smoking rate (Leelarungrayub *et al*, 2010). In another study, a 10-day withdrawal from ethanol consumption led to decreased β-endorphin plasma levels. Chronic treatment with acamprosate, which increases β-endorphin plasma concentrations, caused a significant reduction in ethanol intake. The authors concluded that restoring alcohol-induced deficits in β-endorphin levels may be an important factor in preventing craving and maintaining abstinence (Zalewska-Kaszubska *et al*, 2008).

Altogether, our results reveal a novel role for β-endorphin and the δ opioid receptor in the incubation of cocaine craving (Roth-Deri *et al*, 2008). To our knowledge, this study provides the first evidence for modulation of cue-induced incubation of cocaine craving by intra-NAc application of exogenous β-endorphin. Future research can focus on β-endorphin-releasing agents and δ opioid agonists for alternative treatment of enhanced cocaine craving that incubates during extended abstinence periods.

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The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)