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Naltrexone attenuation of conditioned but not primary reinforcement of nicotine in rats

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Abstract

Rationale—Opioid neurotransmission has been implicated in reinforcement-related processes for several drugs of abuse, including opiates, stimulants, and alcohol. However, less is known about its role in the motivational effects of nicotine and nicotine-associated environmental cues.

Objective—This study investigated whether pretreatment with naltrexone, an opioid receptor antagonist, alters conditioned incentive salience of nicotine cues under two conditions: cue-induced reinstatement of nicotine-seeking after extinction and cue-maintained responding during extinction. The effect of naltrexone on nicotine self-administration during the maintenance phase was also examined.

Materials and methods—Male Sprague–Dawley rats were trained in daily 1-h sessions to self-administer nicotine (0.03 mg/kg/infusion, i.v.) on a fixed-ratio 5 schedule and associate a conditioned stimulus (CS) with each nicotine delivery. Once responding was extinguished by saline substitution for nicotine and omission of the CS, the reinstatement tests were conducted following subcutaneous administration of naltrexone (0, 0.25, 1, 2 mg/kg). In separate groups of rats, naltrexone (0, 2 mg/kg) was chronically given before each extinction sessions, where responses on the active lever resulted in presentations of the CS without nicotine infusion (saline substitution). Self-administration/naltrexone tests were conducted in different groups of rats receiving similar nicotine self-administration training.

Results—Naltrexone significantly attenuated the CS-reinstated responding on the active, previously nicotine-reinforced lever in the reinstatement tests and the CS-maintained active lever responding during the extinction tests. In contrast, neither acute nor chronic naltrexone produced an effect on nicotine self-administration behavior.

Conclusions—These results indicate that activation of opioid receptors is implicated in mediation of the conditioned incentive properties of nicotine cues but not in the maintenance of nicotine self-administration. Therefore, these findings suggest that opioid receptor antagonists might have clinical potential for prevention of smoking relapse associated with exposure to environmental cues.

Keywords

Antagonist; Conditioned stimulus; Extinction; Naltrexone; Nicotine; Nicotine-seeking behavior; Opioid receptors; Reinstatement; Self-administration

Introduction

The environmental stimuli associated with administration and subjective effects of nicotine significantly contribute to the maintenance of and relapse to smoking behavior. For instance, clinical studies have demonstrated that smoking cues produce physiological responses (Abrams et al. 1988; Niaura et al. 1989, 1992; Saumet and Dittmar 1985), enhance desire to smoke (Drobes and Tiffany 1997; Droungas et al. 1995; Lazev et al. 1999; McDermut and Haaga 1998; Perkins et al. 1994), and increase the rate, intensity, and time of smoking (Mucha et al. 1998; Surawy et al. 1985). Denicotinized cigarettes (i.e., cue) produces subjective satisfaction, alleviates withdrawal symptoms, and sustains smoking behavior (Butschky et al. 1995; Donny et al. 2007; Gross et al. 1997; Rose et al. 2000). Recent animal studies have demonstrated that reintroduction of nicotine-associated cues after extinction resulted in increased responding on the previously nicotine-reinforced lever (Cohen et al. 2005; LeSage et al. 2004; Liu et al. 2006, 2007, 2008; Paterson et al. 2005), indicating conditioned incentive properties of the nicotine cues.

Opioid neurotransmission is implicated in response and adaptation to emotionally salient stimuli in both animal models and human studies (Filliol et al. 2000; Kalin et al. 1988; Moles et al. 2004; Nelson and Panksepp 1998; Zubieta et al. 2003). It has been proposed that activation of opioid systems plays a role in mediating conditioned incentive effects of environmental stimuli associated with rewarding actions of drugs of abuse (Benedetti et al. 2005; Zubieta et al. 2005). In animal studies, opioid receptor antagonists have been shown to reverse cue-elicited reinstatement of heroin-seeking behavior (Shaham and Stewart 1996; Stewart 1983; Stewart and Wise 1992) and recently to reduce reinstatement of responding for oxycodone, the most abused prescription opiate (Leri and Burns 2005). Naltrexone, a long-lasting opioid receptor antagonist, has been approved by FDA as an anti-alcoholism drug. It effectively decreases alcohol craving associated with exposure to alcohol cues in abstinent alcoholics (Gerrits et al. 1999; Monti et al. 1999; Rohsenow et al. 2000) and reverses reinstatement of ethanol-seeking behavior induced by re-presentation of the ethanol cues in rats (Backstrom and Hyttia 2004; Bienkowski et al. 1999; Burattini et al. 2006; Ciccocioppo et al. 2002; Dayas et al. 2007; Katner et al. 1999; Liu and Weiss 2002). This agent also attenuates cue-induced reinstatement of methamphetamine- and cocaine-seeking behavior in rats (Anggadiredja et al. 2004; Burattini et al. 2008). In nicotine studies, mice that had lower level of expression of opioid receptors in the ventral tegmental area (Blendy et al. 2005) and were opioid-deficient by preproenkephalin knock-out (Berrendero et al. 2005) failed to develop nicotine-induced conditioned place preference (CPP). Walters et al. (2005) reported that opioid receptor antagonist naloxone blocked expression of nicotine-induced CPP. Based on these data, it is hypothesized that opioid neurotransmission may be implicated in the mediation of conditioned incentive properties of nicotine-associated cues.

Increasing evidence suggests that opioid neurotransmission, together with corticomesolimbic dopamine system, may be implicated in mediating rewarding actions and dependence of drugs of abuse including nicotine (Gianoulakis 2004; Koob and Le Moal 2001; Maldonado 2003; Pomerleau 1998; Watkins et al. 2000 for reviews). In animal studies, nicotine administration has been found to increase expression and release of opioid peptides in mesolimbic regions (Boyadjieva and Sarkar 1997; Houdi et al. 1991, 1998; Walters et al. 2005; Wewers et al. 1999). Opioid receptor antagonists decrease nicotine-induced dopamine release in the nucleus

accumbens (Tanda and Di Chiara 1998), reduce nicotine reward (Walters et al. 2005; Zarrindast et al. 2003), and precipitate withdrawal symptoms in rats treated chronically with nicotine (Malin et al. 1993). These data suggest the involvement of opioid neurotransmission in nicotine reinforcement and smoking behavior. However, in the self-administration paradigm, acute pretreatment with naltrexone or naloxone did not change operant responding for intravenous nicotine self-administration in rats (Corrigall and Coen 1991; DeNoble and Mele 2006). Clinical studies in the last three decades have produced inconsistency in the ability of opioid receptor antagonists to curb smoking (Brauer et al. 1999; Epstein and King 2004; King and Meyer 2000; Nemeth-Coslett and Griffiths 1986; Ray et al. 2006; Rohsenow et al. 2003; Sutherland et al. 1995; Wewers et al. 1998; Wong et al. 1999), and a recent meta-analysis on clinical trial data failed to find the effectiveness of naltrexone on smoking cessation (David et al. 2006). Therefore, it is not fully understood whether activation of opioid receptors critically contributes to nicotine reinforcement.

The present study was designed to address two issues. First, effect of naltrexone blockade of opioid neurotransmission on the conditioned incentive salience of nicotine cues was investigated under two conditions: cue-reinstated nicotine-seeking after extinction and cue-maintained nicotine-seeking during extinction. Second, the effect of not only acute but also chronic naltrexone on nicotine self-administration during the maintenance phase was also examined.

Materials and methods

Subjects

Male Sprague–Dawley rats (Charles River) weighing 225–250 g upon arrival were used. Animals were individually housed in a humidity- and temperature-controlled (21–22°C) vivarium on a reversed light/dark cycle (lights on 19:00 hours, off 07:00 hours) with unlimited access to water. After 1-week habituation to the vivarium, rats were placed on a food-restriction (20 g chow/day) regimen throughout the experiments. Training and experimental sessions were conducted during the dark phase at the same time each day (09:00–15:00 hours). All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Self-administration apparatus

Operant training, self-administration, and reinstatement tests were conducted in operant conditioning chambers located inside sound-attenuating, ventilated cubicles (Med Associates, St. Albans, VT, USA). The chambers were equipped with two retractable response levers on one side panel and with a 28-V white light above each lever as well as a red house light on the top of the chambers. Between the two levers was a food pellet trough. Intravenous nicotine injections were delivered by a drug delivery system with a syringe pump (Med Associates, model PHM100—10 rpm). Experimental events and data collection were automatically controlled by an interfaced computer and software (Med Associates, Med-PC 2.0).

Food training

Rats received food training sessions in order to facilitate learning of operant responding for nicotine self-administration (see below). In these sessions, responding on the active lever was rewarded with delivery of a food pellet (45 mg). Sessions lasted 1 h and were repeated until all animals earned 75 food pellets on a fixed-ratio (FR) 1 schedule in a single session. The reinforcement schedule was increased to FR5 and training continued until the same criterion was achieved. Successful food training was achieved within two to five sessions. During the food training sessions, the visual/auditory stimulus that later was used as a nicotine conditioned stimulus (CS, see below) was not presented.

Surgery

After food training, the rats were anesthetized with isoflurane and implanted with jugular catheters as described previously (Donny et al. 1998). The rats were allowed at least 7 days to recover from surgery. For the first 2 weeks after surgery, the catheters were flushed twice a day with 0.1 ml of sterile saline containing heparin (20 units/ml), ticarcillin (14 mg/ml), and streptokinase (5 mg/ml) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with the heparinized saline prior to and after the experimental sessions throughout the experiments.

Nicotine self-administration/conditioning

After recovery from surgery, rats were trained to intravenously self-administer nicotine (0.03 mg/kg/infusion, free base) and associate a CS with nicotine delivery. In the training sessions, animals were placed in the operant conditioning chambers and connected to a drug delivery system. The daily 1-h sessions were initiated by introduction of the two levers with illumination of the red house light. Once the FR requirement on the active lever was met, an infusion of nicotine was dispensed by the drug delivery system in a volume of 0.1 ml in approximately 1 s. Each nicotine infusion was paired with a presentation of the CS consisting of a 5-s tone and illumination of the lever light for 20 s. The latter signaled a 20-s timeout period during which time responses were recorded (included in the total number of responses) but not reinforced. Responses on the inactive lever had no consequence. An FR1 schedule was used for days 1–5, an FR2 for days 6–8, and an FR5 for remainder of the experiments. All rats received 30 daily self-administration/conditioning sessions.

Extinction

After completion of the self-administration/conditioning phase, rats were subjected to daily extinction sessions. During the extinction sessions, lever responding was extinguished by withholding nicotine and the CS. Specifically, the daily 1-h extinction sessions began with introduction of the levers and illumination of the red house light. Responses on the active lever resulted in the delivery of saline rather than nicotine, and the CS presentation was omitted. The FR5 schedule and 20-s timeout period was still in effect for saline infusions. The criterion for extinction was three consecutive sessions, in which the number of responses/session was less than 20% of the average over the last three self-administration/conditioning sessions.

Reinstatement test and effect of naltrexone

One day after the final extinction session, reinstatement tests were conducted under conditions identical to nicotine self-administration/conditioning sessions, with the exception of saline substitution for nicotine. As such, during the test sessions, responses on the active lever resulted in representation of the CS and saline infusion (no availability of nicotine) on the FR5 schedule with a 20-s timeout period. Thirty minutes before the test sessions, naltrexone (0, 0.25, 1, 2 mg/kg) was subcutaneously administered to separate groups of rats ($n=12$ for each group). Every rat received only one reinstatement/naltrexone test.

Effect of naltrexone on nicotine cue-maintained responding

Two groups of rats ($n=12$ for each group) were used for these tests. Animals also received the 30 daily 1-h self-administration/conditioning training sessions. During the following six daily extinction test sessions, however, responses on the active lever still resulted in presentation of the CS on the FR5 schedule with saline substitution of nicotine. Thirty minutes before each test sessions, naltrexone (2 mg/kg) was subcutaneously administered to one group of rats, with the other group receiving saline administration.

Effect of naltrexone on nicotine self-administration

Acute test—Eleven rats were used to test the effect of acute naltrexone treatment on nicotine self-administration. These rats also received the 30 daily self-administration training sessions. Then, the naltrexone test sessions began. Naltrexone (0, 0.25, 1, 2 mg/kg) was subcutaneously administered 30 min before the session in a within-subject design. Every rat received each dose of naltrexone once in a counterbalanced order. Test sessions were performed every other day, with a no-drug pretreatment session in between to eliminate possible carry-over effect of the drug.

Chronic test—Two groups of rats ($n=8$ for each group) were used. The chronic naltrexone treatment tests were conducted after completion of the 30 daily self-administration training sessions. Thirty minutes before self-administration test sessions, one group of rats received subcutaneous administration of naltrexone (2 mg/kg), and the other group had saline injection. This self-administration/naltrexone test lasted for seven consecutive days.

Statistical analyses

Data are presented as the mean ($\pm$ SEM) number of lever responses and nicotine infusions. The data obtained during self-administration and from the reinstatement/naltrexone, extinction/naltrexone, and the self-administration/acute and chronic naltrexone tests were separately analyzed by using either two- or one-way ANOVA, with repeated measures wherever appropriate. Subsequently, the Fisher's PLSD post hoc tests were used to verify differences among individual means.

Results

Nicotine self-administration/conditioning and extinction

After 30 daily 1-h self-administration/conditioning training sessions, rats developed stable levels of operant responding for nicotine delivery administered intravenously. Table 1 shows the profiles of lever responding emitted by the rats that were used for the reinstatement/naltrexone tests. Averaged across the final three sessions (session 28 to 30), rats made a mean $\pm$ SEM number of responses of 83.3 ± 3.2 on the active lever and 10.8 ± 1.4 on the inactive lever. Correspondingly, rats earned 15.5 ± 0.6 infusions of nicotine, with a total intake of 1.6 ± 0.1 mg/kg/h. Since rats were divided into four groups for subsequent reinstatement/naltrexone tests in a counterbalanced manner, there was no difference among groups in lever responses. In the first extinction session, animals emitted a mean $\pm$ SEM number of responses of 80.8 ± 4.6 on the active lever and 7.8 ± 1.3 on the inactive lever. During subsequent extinction sessions, lever responses gradually decreased. All rats reached extinction criterion within ten sessions.

Effect of naltrexone on cue-induced reinstatement of nicotine-seeking after extinction

A repeated measure ANOVA on the number of active lever responses revealed a significant main effect of session [reinstatement vs. extinction (averaged across the final three sessions); $F(1,44)=46.50$, $p<0.0001$] and a session $\times$ group interaction [$F(3,44)=3.00$, $p<0.05$]. Further post hoc analysis showed that in vehicle-treated rats, the number of active lever responses in the reinstatement test was significantly higher than that of extinction ($p<0.0001$), indicating re-presentation of the CS effectively reinstated the extinguished nicotine-seeking behavior. One-way ANOVA on the active lever responses during reinstatement tests yielded significant group (naltrexone dose) effect [$F(3,44)=2.89$, $p<0.05$], and subsequent Fisher's PLSD post hoc test verified significant difference of 2 ($p<0.01$) and 1 mg/kg ($p<0.05$) vs. vehicle, indicating that naltrexone dose dependently decreased the cue-induced reinstatement (Fig. 1, top). However, responses on the inactive lever remained at low levels indistinguishable from extinction responses (Fig. 1, bottom).

Effect of naltrexone on cue-maintained responding during extinction sessions

During the six daily extinction test sessions where active lever responses resulted in presentations of the CS without nicotine (saline substitution), pretreatment of naltrexone significantly suppressed the cue-maintained lever responding. A repeated measure ANOVA on the number of active lever responses revealed a significant main effect of drug [naltrexone ($n=12$) vs. saline ($n=12$); $F(1,22)=26.73$, $p<0.0001$] and session [$F(5,110)=25.26$, $p<0.0001$]. Further, one-way ANOVA showed a significant effect of session in both naltrexone [$F(5,66)=13.22$, $p<0.0001$] and saline control [$F(5,66)=10.66$, $p<0.0001$] groups. In each session, the number of active lever responses in naltrexone-treated rats was significantly lower than that of saline control animals (Fig. 2).

Effect of acute and chronic naltrexone on nicotine self-administration

One-way ANOVA on the number of active lever responses rats ($n=11$) emitted during the acute naltrexone test sessions produced no significant dose effect. A repeated measure ANOVA on the data from chronic tests also failed to show significant drug [naltrexone ($n=8$) vs. saline ($n=8$)] effect. Therefore, neither acute nor chronic naltrexone pretreatment changed nicotine self-administration behavior (Fig. 3).

Discussion

This study for the first time demonstrates that naltrexone blockade of opioid neurotransmission attenuates nicotine cue-maintained responding during extinction and cue-induced reinstatement of nicotine-seeking behavior after extinction. This finding indicates that activation of opioid receptors may play a role in mediation of the conditioned incentive properties of nicotine-associated cues and suggests that opioid receptor antagonists, especially naltrexone, would be of clinical potential for prevention of smoking relapse triggered by exposure to the environmental smoking cues. However, neither acute nor chronic naltrexone pretreatment changed nicotine self-administration, suggesting that activation of opioid receptors may not be critically involved in the primary reinforcing effects of nicotine.

In the reinstatement tests, response-contingent re-presentation of the CS significantly reinstated extinguished responding on the previously nicotine-reinforced active lever in rats that received saline vehicle pretreatment, indicating conditioned motivational effect of the nicotine-associated cue. It is a replication of previous observation showing that nicotine-associated cues effectively reinstated nicotine-seeking behavior in rats (Cohen et al. 2005; LeSage et al. 2004; Liu et al. 2006, 2008; Liu et al. 2007; Paterson et al. 2005). Importantly, pretreatment with naltrexone dose dependently attenuated the cue-induced reinstatement of nicotine-seeking responding in the reinstatement tests conducted after lever responding was extinguished. Besides, in the extinction tests performed in separate groups of rats, naltrexone significantly suppressed the CS-maintained responding, because responses on the active lever resulted in only the CS presentation but not nicotine infusion (saline substitution). These effects could not be readily attributable to nonspecific impairment of general arousal and/or locomotor activity by this agent because of the facts that responses on the inactive lever during the reinstatement tests and responses on the active lever for nicotine reinforcement during the self-administration tests remained unchanged and that in a recent study, naltrexone at a dose even higher than that of this study did not change cue-induced sucrose-seeking behavior (Burattini et al. 2008). Therefore, naltrexone produced a selective suppressant effect on motivational actions of the nicotine cue. This finding indicates that activation of opioid receptors may play a role in mediation of conditioned incentive properties of nicotine cues, which underlies the cue-induced reinstatement of nicotine-seeking in animals and relapse of smoking behavior in humans. This argument gains support from an observation showing that naloxone, another opioid receptor antagonist, blocked expression of nicotine-induced CPP (Walters et al. 2005). It is also in line

with clinical studies showing that naltrexone decreased smoking cue-induced urge to smoke (Hutchison et al. 1999; King and Meyer 2000; Lee et al. 2005).

Increasing animal studies have shown that blockade of opioid neurotransmission attenuates drug cue-induced reinstatement of operant responding for previously self-administered drugs of abuse, including opiates (Leri and Burns 2005; Shaham and Stewart 1996; Stewart 1983; Stewart and Wise 1992), alcohol (Backstrom and Hyytia 2004; Bienkowski et al. 1999; Burattini et al. 2006; Ciccocioppo et al. 2002; Dayas et al. 2007; Katner et al. 1999; Liu and Weiss 2002), methamphetamine (Anggadiredja et al. 2004), and cocaine (Burattini et al. 2008). These data, together with the present finding, suggest that activation of the opioid receptors may to some extent play a general role in expression of the conditioned incentive properties of environmental stimuli previously associated with drug taking and subjective effects of the drugs. However, it should be noted that naltrexone does not alter drug priming-elicited reinstatement of cocaine and methamphetamine seeking (Anggadiredja et al. 2004; Gerrits et al. 2005), operant responding and its associated neuronal activation under a negative cue condition predictive of the unavailability of ethanol (Dayas et al. 2007), and cue-induced reinstatement of natural reward sucrose-seeking behavior in rats (Burattini et al. 2008). These negative results further suggest that opioid neurotransmission may be implicated differentially in the associative learning process involved in addictive drugs vs. natural rewards and the motivational effects of drug cues vs. drug priming. Therefore, it is proposed that naltrexone might have a broad clinical potential for prevention of relapse to drug use, including cigarette smoking, which is associated with exposure to environmental drug cues.

Animal studies have provided important information on neuroanatomical substrates for mediation of the conditioned incentive of the CS and its attenuation by naltrexone. For example, Dayas et al. (2007), by concomitantly examining suppressant effect of naltrexone on cue-induced ethanol seeking and expression of immediately early gene *c-fos*, implicates hippocampus, amygdale, and hypothalamus as potential brain regions in mediation of naltrexone attenuation of conditioned ethanol seeking. Gerrits et al. (1999), by using autoradiographic technique, found activation of opioid receptors in these brain regions of rats under conditioned motivational effects of cocaine cues. Studies with human smokers and rats receiving association of nicotine injection with specific context demonstrated implication of these brain regions in response to nicotine-related cue exposure (Due et al. 2002; Schiltz et al. 2005; Scott et al. 2007). Opioid receptors and its related phosphorylation of the gene transcription factor cAMP response element-binding proteins are required for the expression of nicotine-induced CPP (Walters et al. 2005). Based on these data, it is conceivable to propose that these brain regions may also be implicated in opioid mediation of the motivational effects of the nicotine cues. The role of opioid-dependent signal transduction in these neuroanatomical substrates in mediation of conditioned incentive by nicotine cues warrants future investigation.

The second goal of this study was to determine whether naltrexone alters nicotine intake after animal acquired stable level of nicotine self-administration. Parallel to the rats used for the cue tests described above, animals for the self-administration tests also received 30 daily nicotine self-administration sessions so that they had similar history of lever experience and nicotine exposure. The lack of effect of acute naltrexone pretreatment on lever responding and nicotine infusions is consistent with previous observations showing that naltrexone and naloxone did not change nicotine self-administration (Corrigall and Coen 1991; DeNoble and Mele 2006). Additionally, taking into consideration of the long-term use of this drug in humans, this study also examined whether chronic treatment with naltrexone would interfere with nicotine intake. Seven daily pre-session administration of naltrexone at its highest dose (2 mg/kg) did not change the number of nicotine infusions earned as compared to vehicle control rats. Although this agent may produce differential effects in nicotine-dependent vs. nicotine-nondependent subjects and the rats used in this daily 1-h self-administration study might not become nicotine

dependent (Paterson and Markou 2004), these negative results from animal studies mesh with ambiguous clinical observations: Some studies indicated that naltrexone attenuates smoking pleasure and cigarette consumption (Epstein and King 2004; King and Meyer 2000; Sutherland et al. 1995; Wewers et al. 1998), whereas others failed to show an effect of naltrexone on smoking reinforcement (Brauer et al. 1999; Nemeth-Coslett and Griffiths 1986; Ray et al. 2006; Rohsenow et al. 2003; Sutherland et al. 1995; Wong et al. 1999). A recent meta-analysis of clinical data found no significant difference in quit rates between naltrexone and placebo (David et al. 2006), and the authors recommended that more large-scale clinical studies are needed to determine the effectiveness of naltrexone for smoking cessation. To interpret the inconsistent clinical results, it has been proposed that opioid response may be only one of the reinforcement mechanisms for nicotine dependence/smoking and probably that mediation of nicotine reinforcement by endogenous opioid activity may not be significant in normal smokers under ordinary conditions (Pomerleau 1998; Sutherland et al. 1995). In fact, opioid activation may be implicated in nicotine reinforcement mainly under conditions of stress (Pomerleau 1998). In addition, most clinical studies allowed smokers to smoke ad libitum without eliminating the conditioned rewarding aspects of smoking (Robinson et al. 2000; Rose et al. 2000; Rusted et al. 1996) so that naltrexone possibly interfered with conditioned reward rather than primary reinforcement of nicotine. Therefore, the results of this study, together with conflicting clinical observations on the ability of naltrexone to reduce cigarette consumption, suggest that clinical trials should focus on the potential of naltrexone for prevention of smoking relapse triggered by cue exposure rather than the influence of naltrexone on reinforcement of nicotine and cigarette consumption.

Another issue that needs to be discussed is the differential effects of naltrexone on conditioned incentive of nicotine cues vs. nicotine self-administration. Increasing data have demonstrated that the conditioned incentive of drug cues and the primary reinforcing actions of the drug may recruit distinct neurocircuitries and thereby show different pharmacological profiles. Notably, basolateral amygdala has been demonstrated to mediate the conditioned but not primary effects of cocaine (Everitt et al. 1999; Grimm and See 2000; Meil and See 1997), and most nucleus accumbens neurons have been found to exhibit excitation in response to conditioned stimuli but inhibition to primary reinforcer (Wilson and Bowman 2004). Liu and Weiss (2004) found that nitric oxide synthesis inhibition attenuated conditioned reinstatement of ethanol seeking, but not the primary reinforcing actions of ethanol. Similarly, Martin-Fardon et al. (2007) demonstrated that antagonism of an orphan sigma (1) receptor reversed cue-induced cocaine-seeking but did not change cocaine self-administration. Even in some cases where one drug produced an effect on both the conditioned and primary reinforcement, sensitivity is different. For example, responding motivated by stimuli conditioned to cocaine is more sensitive to glutamate antagonists than behavior maintained by cocaine itself (Baptista et al. 2004; Newman and Beardsley 2006). Therefore, it is justifiable to argue that conditioned incentive properties of nicotine cues and the primary reinforcing actions of nicotine would be mediated to some extent by different neurobiological substrates.

In summary, naltrexone effectively attenuated motivational effect of nicotine cues in animal models of drug-seeking but did not change nicotine self-administration. Considering the fact that although there have been pharmacotherapies for smoking cessation such as nicotine replacement therapies, bupropion, and recently varenicline, majority of smokers who try to quit relapse (Aubin et al. 2008; Bala et al. 2008; Gonzales et al. 2006; Jorenby et al. 2006; Oncken et al. 2006); this finding lends support for continued clinical assessment of the effectiveness of naltrexone as an adjunctive pharmacotherapy for smoking cessation but suggests that focus should shift from decrease of nicotine intake/cigarette consumption to prevention of smoking relapse associated with exposure to the environmental smoking cues. These data also suggest that nicotine reinforcement and conditioned incentive of nicotine cues

may be mediated by distinct neurocircuitries at least in terms of implication of opioid neurotransmission.

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Fig. 1.
Effect of naltrexone on lever responses in the reinstatement tests conducted after extinction.
For comparison, extinction responses averaged across the final three sessions were shown.
* $p < 0.05$, ** $p < 0.01$ different from control group

Fig. 2. Effect of chronic naltrexone on nicotine cue-maintained responses. Lever responses made during the last five sessions of the self-administration/conditioning training phase were shown for reference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ different from vehicle control group

Fig. 3. Nicotine infusions earned after acute (*top*) and chronic (*below*) naltrexone treatment in rats

Table 1

Lever responses made and nicotine infusions earned during the self-administration/conditioning phase (averaged across the final three sessions) and in the first extinction session as well as body weight measured after completion of nicotine self-administration/conditioning training in the rats ($n=12$ for each group) used for reinstatement/naltrexone tests

	Group (naltrexone, mg/kg)			
	0	0.25	1	2
Self-administration/conditioning				
Active lever responses	81.5±8.2	82.1±8.8	82.5±3.4	86.8±4.3
Nicotine infusions	15.3±1.3	14.6±1.6	16.4±1.1	15.9±0.8
Inactive lever responses	8.4±2.8	9.7±2.8	12.6±2.9	12.4±2.8
Extinction (first session)				
Active lever responses	79.3±12.1	77.4±10.1	81.0±5.4	85.6±9.3
Inactive lever responses	7.4±3.9	7.8±3.4	9.1±2.6	9.3±2.2
BW (g) after self-administration	313±11	312±9	307±10	310±8