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Reinforcement enhancing effect of nicotine and its attenuation by nicotinic antagonists in rats

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Abstract

Rationale—Recent studies have demonstrated that nicotine can enhance operant responding for other nonpharmacological reinforcing stimuli. However, the nature of the reinforcement-enhancing effect of nicotine remains largely unknown.

Objective—The present study determined the dose dependency of the ability of nicotine to increase lever-pressing responses maintained by a compound visual stimulus (VS) in rats and examined its sensitivity to pharmacological antagonism of nicotinic acetylcholine receptors (nAChRs).

Materials and methods—Male Sprague–Dawley rats were trained in daily 1-h sessions to lever press for delivery of a VS (1 s lever light on and 60 s house light off) on a fixed ratio 5 schedule. During these sessions, eight scheduled response-independent intravenous infusions of nicotine (total amount: 0, 0.06, 0.12, 0.24, 0.48 mg kg^{−1} h^{−1}) were delivered. In pharmacological tests, a nonselective nAChR antagonist mecamylamine, $\alpha 4\beta 2$ -selective antagonist dihydro- β -erythroidine (DH β E), and $\alpha 7$ -selective antagonist methyllycaconitine (MLA) were administered in different groups of rats 30 min before the session.

Results—The VS maintained a moderate level of lever-pressing responses and nicotine dose-dependently increased responses for the VS presentations. Pretreatment of mecamylamine and DH β E but not MLA significantly attenuated the nicotine-enhanced responding. However, mecamylamine had no effect on responding for the VS in rats that received scheduled saline infusions.

Conclusions—These results demonstrate dose dependency of the reinforcement-enhancing effect of nicotine and suggest that activation of the $\alpha 4\beta 2$ - but not $\alpha 7$ -containing nAChRs may mediate this effect.

Keywords

Nicotine; Nonpharmacological stimuli; Reinforcement-enhancing effect; Nicotinic acetylcholine receptors; Mecamylamine; Dihydro- β -erythroidine; Methyllycaconitine

Introduction

In the standard self-administration procedure, nicotine delivery is typically accompanied by contingent presentation of an environmental stimulus such as a cue light or a light plus tone, and response rates tend to be robust (Corrigall and Coen 1989; Donny et al. 1995; Chiamulera

et al. 1996; Shoaib et al. 1997; Watkins et al. 1999). However, when nicotine is self-administered without accompanying stimuli, response rates are much lower (Donny et al. 2003; Palmatier et al. 2006; Chaudhri et al. 2007). These findings suggest that the stimuli accompanying nicotine delivery play a critical role in maintaining reliable levels of nicotine self-administration. Goldberg et al. 1981 found a 50% decrease in intravenous (i.v.) nicotine self-administration in squirrel monkeys when a brief visual stimulus (VS) that had been associated with the drug was omitted. Recently, work from our laboratory has shown that nicotine synergizes with a compound VS to produce response rates substantially higher than those produced by either nicotine alone or the stimulus alone and that this synergism also results when nicotine is administered noncontingently (independent of the recipient rat's behavior) in a yoked design (Donny et al. 2003; Chaudhri et al. 2007). These findings are in agreement with those of Cohen et al. 2005, who reported that nicotine enhances responding for a tone/light stimulus and Olsson et al. 2004 who found that nicotine increased operant responding for the delivery of a stimulus that had become a conditioned reinforcer via prior Pavlovian conditioning. Taken together, these findings indicate that in addition to its primary reinforcing actions, nicotine can enhance the reinforcing effects of other non-nicotine stimuli (Caggiula et al. 2001; Chiamulera 2005; Chaudhri et al. 2006).

Little is known about the neurobiological mechanisms underlying the ability of nicotine to enhance the reinforcement maintained by a reinforcing VS. Therefore, the present study was designed (1) to characterize dose dependency of the reinforcement-enhancing effect of nicotine and (2) to examine whether this effect is sensitive to pharmacological blockade of nicotinic acetylcholine receptors (nAChRs). Specifically, rats were trained to press a lever for presentation of a moderately reinforcing VS while i.v. nicotine infusions of various doses were administered in a response-independent manner. The number and timing of nicotine infusions were scheduled throughout the sessions based on the pattern of nicotine infusions obtained in our previous studies in which rats self-administered nicotine without accompanying stimuli (Palmatier et al. 2006; Chaudhri et al. 2007). This i.v. nicotine regimen has been documented in our laboratory to produce reliable reinforcement-enhancing effect. Moreover, it would allow us to understand the reinforcement-enhancing effect of nicotine in a context of nicotine self-administration in rats and ultimately smoking behavior in humans. In pharmacological tests, rats were tested for the effect of pretreatment with a nonselective nAChR antagonist mecamylamine (Takayama et al. 1989) on the reinforcement-enhancing effect of nicotine in the rats that received scheduled nicotine infusions. Moreover, mecamylamine was also tested in the rats that received scheduled saline infusions to examine whether nonselective blockade of the nAChRs interferes with the intrinsic reinforcing properties of the VS. To further determine involvement of activation of subtypes of the nAChRs in mediating the reinforcement-enhancing effect of nicotine, dihydro- β -erythroidine (DH β E), an α 4 β 2-selective antagonist (Williams and Robinson 1984; Egan and North 1986; Rapier et al. 1990; Wang et al. 1991), and methyllycaconitine (MLA), an α 7-selective antagonist (Macallan et al. 1988; Alkondon et al. 1992), were tested in the rats that received scheduled nicotine infusions.

Materials and methods

Subjects

Male Sprague–Dawley rats (Harlan Farms) weighing 225–250 g upon arrival were used. Animals were individually housed in a humidity- and temperature-controlled (21–22°C) colony room on a reversed 12:12 h light/dark cycle (lights off at 7:00 A.M., on at 7:00 P.M.) with unlimited access to water. After 1 week of habituation, during which time food was made available ad libitum, the rats were placed on a food restriction regimen throughout all experiments as described below. All training and experimental sessions were conducted during the dark phase at the same time each day (9:00 A.M.–3:00 P.M.). All experimental procedures

were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Apparatus

All experimental sessions were conducted in experimental chambers located inside sound-attenuating, ventilated cubicles (Med Associates, St. Albans, VT). The chambers were equipped with two response levers on one side panel and with a 28-V white light above each lever as well as a house light on the top of the chambers. Between the two levers was a food receptacle. I.v. 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 controlled by an interfaced computer and software (Med Associates, MED-PC 2.0).

Food training

After 1 week of habituation to the colony room, animals were trained to respond for food reinforcement to facilitate learning of operant responses. Briefly, after overnight food deprivation, rats were allowed to consume 75 food pellets (45 mg) in a single magazine-training session. The following day, they were hand shaped to press the right (active) lever for 75 food pellets on a fixed ratio (FR) 1 schedule in a single session. Responses on the left (inactive) lever had no scheduled consequences. The criterion for completion of food training was to successfully earn 75 pellets. If rats failed to meet the criterion, they were again hand shaped on the following day. All rats completed food training in 2 days. There was no VS presented during food training.

Surgery

After food training, rats were anesthetized with halothane and implanted with jugular catheters as described previously (Caggiula et al. 2001). The rats were allowed at least 7 days to recover from surgery, and the cannulae were flushed twice a day with 0.1 ml of sterile saline containing heparin (30 U/ml), Timentin (66.67 mg/ml), and streptokinase (8,333 U/ml) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with the heparinized saline before and after sessions throughout the experiments.

Operant responses for presentations of the VS

Rats were trained in daily 1-h sessions to press the right lever for presentations of the VS. The sessions were initiated with extension of the two levers and illumination of a white house light. Once the rats reached the FR requirement at the active lever, the VS was presented that consisted of the onset for 1 s of a light above the active lever followed by the offset of the house light for 1 min, indicating a timeout period during which responses were recorded but not reinforced by the VS. An FR1 schedule was used for days 1–5, an FR2 for days 6–8, and an FR5 for the remainder of the experiment. Responses at the inactive lever were recorded but had no programmed consequence. Experimental sessions were conducted 5 days a week.

Scheduled nicotine infusions

During each 1-h session, eight i.v. infusions of nicotine were automatically administered independently of the animal's behavior. Nicotine infusions were dispensed by the drug delivery system in a volume of 0.1 ml in approximately 1 s. The number and timing of nicotine infusions were based on the pattern of nicotine infusions obtained in previous studies in which rats self-administered nicotine without accompanying stimuli (Palmatier et al. 2006; Chaudhri et al. 2007) and thereby scheduled across sessions at the following time points (in seconds): 30–78, 120–180, 250–450, 520–770, 830–1,060, 1,240–1,480, 1,750–1,940, and 2,100–2,300. If the scheduled infusion time happened to coincide with a VS delivery, this infusion was delayed at least 30 s so that there was no fortuitous association of nicotine infusion with VS presentation.

Dose–response study

Rats were randomly divided into five groups ($n=8-11$) for different nicotine doses of a total amount of 0 (saline control), 0.06, 0.12, 0.24, and 0.48 mg kg⁻¹ 3h⁻¹ calculated as free base. That is, the different groups received eight scheduled injections of nicotine with the unit doses of 0, 0.0075, 0.015, 0.03, and 0.06 mg/kg per infusion. All groups received i.v. infusions at the same schedule described above. Animal grouping and the events of the VS presentation and nicotine infusion described above were implanted from the first daily session after recovery from surgery. After completion of 20 daily sessions, nicotine dose was switched to 0.24 mg kg⁻¹ h⁻¹ or 0.03 mg/kg per infusion for seven daily sessions to determine whether or not operant behavior would change accordingly, another indication of dose dependency.

Effect of mecamylamine

In rats that received scheduled nicotine infusions—After completion of the dose–response study, a subset of animals ($n=8$) that had patent catheters remained on the daily sessions with the scheduled infusions of nicotine at 0.03 mg/kg per infusion. These rats were habituated to subcutaneous saline injection 30 min before the session for 3 days. Then, 30 min before sessions, rats received subcutaneous injections of mecamylamine (0, 0.5, 1, 2 mg/kg) using a within-subject design. The eight rats were randomly designated into four sets ($n=2$ for each). Each set of rats received one of the four orders of drug dose combination: 0–0.5–1–2, 0.5–1–2–0, 1–2–0–0.5, and 2–0–0.5–1. As such, both animals and the order of drug doses were counterbalanced in a pseudorandomized manner. Test sessions were conducted under conditions identical to that described above and arranged in such a manner that there were 2 days of nondrug sessions between testing to eliminate possible carryover effects of the drug; that is, rats received mecamylamine pretreatment on days 31, 34, 37, and 40.

In rats that received scheduled saline infusions—The saline rats ($n=10$) from the dose–response study that responded for the VS but without nicotine (saline infusions) were tested after pretreatment with mecamylamine at its highest dose (2 mg/kg) or saline vehicle. Pretreatments were given 30 min before each session in a counterbalanced order so that all rats received both saline and mecamylamine.

Effect of DH β E and MLA on nicotine-enhanced responding

A separate set of rats was trained to lever press for presentation of the VS with the eight scheduled response-independent infusions of nicotine at 0.03 mg/kg per infusion exactly the same as described above. After matched with the number of sessions received by the rats for mecamylamine tests, these rats were randomly divided into two groups for testing the effect of DH β E and MLA. In one group ($n=12$), DH β E (0, 1, 3, and 9 mg/kg) was subcutaneously administered 30 min before tests by using a within-subject design in a pseudorandomized order as described above for mecamylamine tests. The other group of rats ($n=11$) in a similar manner received intraperitoneal administration of MLA (0 and 10 mg/kg) 30 min before tests. The test sessions were arranged with 2 days of nondrug sessions in between.

Statistical analyses

The number of responses on the active and inactive levers in the dose–response study was separately analyzed by using two-way analysis of variance (ANOVA) with repeated measures (nicotine dose as the between-subject factor). The mean number of responses averaged across the last three sessions (sessions 18 to 20) of the FR5 phase was analyzed by one-way ANOVA. The data obtained from mecamylamine, DH β E, and MLA tests were separately analyzed by using one-way ANOVA with subsequent Newman–Keuls post-hoc tests to verify the significant difference among individual means.

Results

Dose dependence of the reinforcement-enhancing effect of nicotine

In saline (zero nicotine) rats, response-contingent presentation of the VS sustained a substantial level of lever-pressing responses (Fig. 1). Averaged across the last three sessions (sessions 18 to 20) of the FR5 schedule phase, rats emitted 74.5 ± 9.3 responses at the active lever and 3.7 ± 0.5 responses at the inactive lever (Fig. 2). Correspondingly, animals earned 13.4 ± 1.9 presentations of the VS during the daily 1-h sessions, indicating the moderately reinforcing property of the VS.

As shown in Fig. 1, top panel, response-independent i.v. infusions of nicotine dose-dependently increased lever pressing for the VS as compared to saline infusions. The mean of both the active and the inactive lever responses for each rat across the last three sessions of each FR schedule phases (sessions 3 to 5 on FR1, sessions 6 to 8 on FR2, and sessions 18 to 20 on FR5) was calculated, and a repeated-measures ANOVA on these means yielded significant main effect of dose ($F[4, 86]=17.63, p<0.0001$), lever ($F[1, 86]=524.41, p<0.0001$), and FR schedule phase ($F[2, 172]=289.74, p<0.0001$) as well as significant interactions of dose $\times$ phase ($F[8, 172]=6.13, p<0.0001$), lever $\times$ phase ($F[2, 172]=301.37, p<0.0001$), dose $\times$ lever ($F[4, 86]=14.57, p<0.0001$), and dose $\times$ lever $\times$ phase ($F[8, 172]=6.31, p<0.0001$). Further repeated-measure ANOVA analyses of the active response data across each FR schedule phase revealed significant main effect of dose ($F[4, 43]=6.71, p<0.001$ in FR1, $F[4, 43]=7.50, p<0.001$ in FR2, and $F[4, 43]=21.42, p<0.0001$ in FR5) and session ($F[4, 172]=19.68, p<0.0001$ in FR1, $F[2, 86]=10.45, p<0.0001$ in FR2, and $F(11, 473)=16.59, p<0.0001$ in FR5). There was a significant dose $\times$ session interaction in FR1 ($F[16, 172]=1.77, p<0.05$) but not FR2 or FR5. Similar analyses on the inactive lever responses produced neither main effect and nor interactions (details not shown).

When a single nicotine dose ($0.24 \text{ mg kg}^{-1} \text{ h}^{-1}$ or 0.03 mg/kg per infusion) replaced other doses, the number of active lever responses approached to similar levels within three sessions as shown in the FR5:0.24 phase of Fig. 1, indicating that the active lever-pressing responses closely followed the change of nicotine dose. A repeated-measures ANOVA on the active response data across this phase revealed neither significant difference among the four nicotine-injected groups nor group $\times$ session interaction. Similar analysis on the inactive lever responses showed no difference across nicotine-injected groups. This data from a different angle support the dose dependency of the reinforcement-enhancing effect of nicotine.

Figure 2 shows the number of lever responses that were averaged across the final three sessions (sessions 18 to 20) on the FR5 schedule when rats received nicotine injections at different doses. There was a systemic increase in the number of active lever responses with increasing dose of nicotine. One-way ANOVA analysis confirmed the significant group (dose) effect ($F[4, 43]=14.71, p<0.001$), and subsequent Newman–Keuls post-hoc tests verified the significant increase in the number of responses in nicotine-injected groups as compared to saline rats ($p<0.0001$ for $0.48 \text{ mg kg}^{-1} \text{ h}^{-1}$, $p<0.001$ for $0.24 \text{ mg kg}^{-1} \text{ h}^{-1}$ and $p<0.01$ for $0.12 \text{ mg kg}^{-1} \text{ h}^{-1}$) and systemic increase as nicotine dose increased (statistical details are shown in Fig. 2, top). However, there was no significant change in the number of inactive lever responses (Fig. 2, below).

Effect of mecamylamine on nicotine-enhanced responses

Pretreatment with mecamylamine significantly attenuated responses at the active lever as shown in Fig. 3, top. One-way ANOVA analysis yielded significant dose effect ($F(3, 21)=23.82, p<0.001$). Subsequent Newman–Keuls post-hoc tests verified the significant decreases in the number of responses in the mecamylamine-treated conditions as compared to the saline

control condition ($p < 0.001$ for all three doses). The number of responses under the highest dose (2 mg/kg) condition was significantly lower than that under 0.5 ($p < 0.01$) and 1 mg/kg ($p < 0.05$). There was no significant change in the number of inactive lever responses (Fig. 3, below).

Effect of mecamylamine in saline rats

In saline (zero nicotine) rats ($n = 10$), pretreatment with mecamylamine (at its highest dose, 2 mg/kg) did not change lever-pressing responses for presentation of the VS (Fig. 4). There was no difference in the number of responses between mecamylamine vs vehicle condition ($p > 0.05$).

Effect of DH β E and MLA on nicotine-enhanced responses

Pretreatment with DH β E substantially decreased the number of active lever responses (Fig. 5, top). One-way ANOVA analysis yielded a significant dose effect ($F(3, 44) = 8.24$, $p < 0.05$). Further Newman–Keuls post-hoc tests verified that the number of responses under all three doses of DH β E was significantly ($p < 0.01$) lower than saline control condition. However, as shown in Fig. 6, pretreatment with MLA did not change the nicotine-enhanced responding because there was no difference in the number of active lever responses between MLA vs the saline control condition ($p > 0.05$). Inactive lever responses in both drug groups remained at low levels indistinguishable across different dose conditions (Fig. 5, below, and Fig. 6).

Discussion

This study examined the dose–response function describing the ability of nicotine to enhance lever-pressing responses for the moderately reinforcing VS, the reinforcement-enhancing effect of nicotine. In pharmacological tests, nonselective blockade of the nAChRs by mecamylamine attenuated nicotine-enhanced responses but did not change the reinforcing value of the VS, and importantly, pretreatment with DH β E but not MLA decreased nicotine-enhanced responses. These data indicate that activation of the nAChRs containing $\alpha 4\beta 2$ - but not $\alpha 7$ -subunits is involved in mediation of the reinforcement-enhancing effect of nicotine.

In this study, rats responded at an active lever for presentations of a compound VS indicating the latter bears intrinsically reinforcing value. The VS may belong to the category of sensory stimuli that have long been evidenced to have reinforcing properties (Stewart 1960; Fowler 1971). Across the daily 1-h sessions, eight injections of i.v. nicotine were delivered in a response-independent manner, which were scheduled based on the pattern of nicotine infusions that happened in nicotine self-administration paradigms in our previous studies (Palmatier et al. 2006; Chaudhri et al. 2007). As previously shown, the VS (1 s turn on of lever light and 60 s turn off of house light) sustained substantial level of lever-pressing behavior, indicating its moderately reinforcing property (Caggiula et al. 2002; Donny et al. 2003; Palmatier et al. 2006; Chaudhri et al. 2007). Scheduled i.v. infusions of nicotine substantially enhanced the VS-maintained responding as compared to saline infusions. In light of the fact that nicotine has psychomotor stimulating effects, one may speculate that the response-enhancing effect might be due to its nonspecific stimulation of locomotor activity. However, the finding that responding at the inactive lever where no VS presented remained unchanged negates this possibility. Therefore, the noncontingently administered nicotine produced its enhancing effect selectively on operant responses that were reinforced by the moderately reinforcing VS. This finding confirms previous observations from our laboratory (Caggiula et al. 2002; Donny et al. 2003; Palmatier et al. 2006; Chaudhri et al. 2007). In another line of studies using brain self-stimulation reward procedures, nicotine has been found to decrease the threshold of electrical self-stimulation of the medial forebrain bundle, lateral hypothalamus, and ventral tegmental area and therefore potentiate the rewarding effects of electrical stimulation in these

brain regions (Olds and Domino 1969; Pradhan and Bowling 1971; Newman 1972; Huston-Lyons and Kornetsky 1992; Ivanova and Greenshaw 1997; Wise et al. 1998; Harrison et al. 2002; Kenny and Markou 2006). Taken together, these data demonstrate that nicotine produces reinforcement-enhancing effect. More interestingly, this effect has been found to depend on the reinforcing value of the non-nicotine stimuli. Recent studies have shown that although nicotine does not produce enhancement effect on the low level of operant responses maintained by relatively neutral stimuli, after these stimuli were endowed with reinforcing value by being conditioned to sucrose pellets or water, nicotine significantly increased responses reinforced by these conditioned stimuli (Olausson et al. 2004; Liu et al. 2005). However, it should be noted that the reinforcement-enhancing effect may not be a unique feature of nicotine only. There has been evidence showing that other drugs also produce similar response enhancement. For instance, other psychostimulants (e.g., amphetamine, cocaine) and drugs of different pharmacological categories (e.g., opiates) as well have been found to increase operant responses supported by reinforcing stimuli including conditioned reinforcers (Hill 1970; Robbins 1976; Robbins and Koob 1978; Phillips and Fibiger 1990; Cunningham and Kelley 1992; Harmer and Phillips 1998; Taylor and Horger 1999; Taylor and Jentsch 2001; Chaudhri et al. 2006). Comparing the reinforcement-enhancing effect across these psychoactive substances would warrant future studies.

The first significant finding of this study was the dose dependency of the reinforcement-enhancing effect of nicotine. Nicotine at doses from 0.06 to 0.48 mg kg⁻¹ h⁻¹ systemically increased responses for the VS, although at the lowest dose (0.06 mg kg⁻¹ h⁻¹), this effect did not reach statistical significance. Moreover, the dose dependency was also reflected by the convergence of response rates of different groups when they were all switched to a single dose of nicotine (0.24 mg kg⁻¹ h⁻¹). Rats in groups with previously lower nicotine doses (0.06 and 0.12 mg kg⁻¹ h⁻¹) substantially increased lever-pressing responses after nicotine dose was switched and reached level similar to that of the 0.24 mg kg⁻¹ h⁻¹ group by the second session. Rats in the highest dose group (0.48 mg/kg h⁻¹) decreased the rate of responding to the level of that of the 0.24 mg kg⁻¹ h⁻¹ group in three sessions. It is noted that the linear dose–response curve describing the reinforcement-enhancing effect of nicotine in the present study differs from the inverted “U”-shaped curve typically reported for i.v. self-administration of nicotine, which presents the maximum effect produced by 0.03 mg/kg per infusion (Shoaib et al. 1997; Donny et al. 1999; Watkins et al. 1999). This could be attributable to two facts. First, if both the nicotine doses expressed as 0.0075–0.06 mg/kg per infusion and the limited, predetermined eight infusions were taken into account, nicotine doses used in this study did not go beyond the ascending limb of a typical dose–response curve obtained from nicotine self-administration paradigms, where there was no limitation on the number of self-administration of nicotine (Shoaib et al. 1997; Donny et al. 1999; Watkins et al. 1999). In this regard, even compared to one of our previous study (Chaudhri et al. 2007) where the noncontingent nicotine injections were yoked to other self-administering rats rather than predetermined, the present study not only supports the finding in that study that the reinforcement-enhancing effect of nicotine can be observed within a wide range of nicotine doses but also for the first time describes the linear dose–response function of the reinforcement-enhancing effect of nicotine. Second, nicotine probably has aversive effects at higher doses, which would reduce responding when both nicotine and the stimulus are contingent on the same operant response, thus leading to the descending limb of the dose–response curve in the standard self-administration paradigm. The argument has gained support from our recent studies. When responding for the VS is dissociated with responses for the nicotine infusion, rats respond at one lever for nicotine injections at a much lower level than that obtained from standard self-administration paradigms, while responses at another lever for the VS presentations reaches levels similar to that of standard self-administration paradigm (Palmatier et al. 2006).

The second purpose of this study was to determine whether the reinforcement-enhancing effect of nicotine is sensitive to pharmacological antagonism of nicotinic neurotransmission. Pretreatment with a nonselective nicotinic antagonist, mecamylamine (0.5, 1, 2 mg/kg) significantly attenuated lever-pressing for the VS, when compared to vehicle, in rats that received scheduled nicotine infusions. One may speculate that this agent would produce nonspecific impairment of general locomotor activity because systemic administered mecamylamine might also produce peripheral effects such as ganglionic blockade and hypotension besides its antagonizing action at nicotinic receptors located in brain (Varanda et al. 1985; Loiacono et al. 1993; Eissenberg et al. 1996). However, this possibility can be readily ruled out based on the facts that this agent did not change responses in rats receiving scheduled saline infusions and attenuated responses at only the active but not the inactive lever in nicotine rats and that it produced no depression on operant responses reinforced by other reinforcers such as food (Liu et al. 2007), water (Glick et al. 2002), or stimulus conditioned to food reinforcement (Liu et al. 2007). Another speculation is that the inhibitory effect of mecamylamine might be a result of its attenuating the reinforcing value of the VS per se. However, two facts should be noted: First, mecamylamine only abolished the enhancement of lever pressing produced by nicotine; even at the highest dose of mecamylamine, response rates for the VS were comparable to those of animals received saline infusions (without the reinforcement-enhancing effect of nicotine), and second, in rats that received infusions of saline rather than nicotine, mecamylamine pretreatment had no effect on responding for the VS. Therefore, the inhibitory effect of mecamylamine in nicotine rats was not due to interference with the reinforcing value of the VS and instead resulted from its attenuation of the reinforcement-enhancing effect of nicotine. Our results are in agreement with other findings that mecamylamine reversed nicotine-enhanced lever pressing for a conditioned reinforcer (Olausson et al. 2004) and that in brain self-stimulation studies this agent effectively blocked nicotine-induced decrease in electrical self-stimulation threshold (Huston-Lyons and Kornetsky 1992; Ivanova and Greenshaw 1997). Taken together, these data extend the role of nicotinic neurotransmission in mediating the direct positive reinforcing actions of nicotine (Watkins et al. 1999; Mathieu-Kia et al. 2002; Wonnacott et al. 2005) to the involvement in the reinforcement-enhancing effect of nicotine.

Significantly, the present study further determined involvement of subtypes of the nAChRs. Antagonists selective for the two most characterized subtypes of the nAChRs, $\alpha 4\beta 2$ and $\alpha 7$ (Lena and Changeux 1997; Lukas et al. 1999; Gotti et al. 2006), were tested. DH β E, an $\alpha 4\beta 2$ -selective antagonist, effectively decreased the nicotine-enhanced lever responses. This finding is in line with previous studies showing that knockout of $\beta 2$ -subunit of the nAChRs cancelled the ability of nicotine to enhance responding for a food-conditioned reinforcement (Brunzell et al. 2006) and that DH β E reversed nicotine-induced potentiation of the electrical stimulation reward (Harrison et al. 2002; Kenny and Markou 2006). However, the $\alpha 7$ -selective antagonist MLA did not change magnitude of nicotine-enhanced responding. Because the dose of MLA used in this study was the highest one or higher than the effective dose ever reported in literature (Gommans et al. 2000; Grottick et al. 2000; Markou and Paterson 2001), this single dose should not give rise to doubt on the lack of effect of this agent. Together, these data suggest that the nAChRs containing $\alpha 4\beta 2$ - but not $\alpha 7$ -subunits may play an important role in mediating the reinforcement-enhancing effect of nicotine. In addition, the report that MLA also has antagonistic actions on the $\alpha 3/\alpha 6$ -containing nAChRs (Mogg et al. 2002) suggests exclusion of these nAChR subtypes in the mediation of the reinforcement-enhancing effect of nicotine.

Mesolimbic dopamine circuitry has been implicated in mediating reinforcing properties of pharmacological reinforcers and natural rewards as well (Koob 1987; Robinson and Berridge 1993; Di Chiara 2000; Salamone et al. 2003). There is evidence showing that nicotine modulates dopaminergic neurotransmission. For instance, nicotine increases burst firing of dopamine neurons in the ventral tegmental area and dopamine release in the nucleus accumbens

(Nisell et al. 1994; Nisell et al. 1996; Marshall et al. 1997). Recently, Rice and Cragg 2004 found that nicotine selectively enhances dopamine release during phasic but not tonic neuronal firing activity. Because dopamine neurons respond to salient stimuli such as primary or conditioned rewards by switching from tonic to phasic firing (Schultz et al. 1997), it has been suggested that amplification of mesolimbic dopaminergic neurotransmission in response to reinforcing stimuli might be a mechanism for nicotine to enhance reinforcement. This argument is supported by the finding that nicotine-induced potentiation of brain electrical stimulation reward was blocked by dopamine antagonists (Huston-Lyons et al. 1993; Ivanova and Greenshaw 1997; Harrison et al. 2002). In light of the fact that the $\beta 2$ -containing nAChRs are mostly expressed on dopaminergic neurons in the midbrain (Klink et al. 2001) and activation of these receptors increases dopaminergic firing rates and dopamine release in this brain region (Picciotto et al. 1998; Klink et al. 2001; Marubio et al. 2003; Schilström et al. 2003), it is argued that a neural pathway of signal transduction via DH β E-sensitive, $\beta 2$ -containing nAChR–dopamine cascade may underlie the reinforcement-enhancing effect of nicotine.

In summary, this study characterized the dose dependency of the reinforcement-enhancing effect of nicotine. The finding that pretreatment with mecamylamine attenuated the nicotine-enhanced operant responses but did not change lever responding in saline rats indicates that the blockade of nicotinic neurotransmission produces no influence on the reinforcing properties of the VS but selectively interferes with the reinforcement-enhancing effect of nicotine. The finding that DH β E but not MLA effectively decreased the nicotine-enhanced responses suggests that activation of the $\alpha 4\beta 2$ - but not $\alpha 7$ -containing nAChRs is implicated in mediating the reinforcement-enhancing effect of nicotine.

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Fig. 1. Profiles of responses on the active (*top*) and inactive (*below*) levers. Note that the doses of nicotine in the figure legend are the total amount of nicotine rats received during the 1-h sessions. *FR5:0.24* means that nicotine dose was switched to 0.24 mg kg⁻¹ h⁻¹ (0.03 mg/kg per infusion) in all nicotine-infused rats. See text for statistical details

Fig. 2. Dose-dependent effect of nicotine administered in a response-independent manner on responses on the active (*top*) and inactive (*below*) levers ($n=8-11$). The numbers of responses were averaged across the last three sessions (sessions 18 to 20) of the FR5 phase. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ different from saline group. ^ $p < 0.05$, ^^ $p < 0.01$, and ^^^ $p < 0.0001$ different from the 0.06 mg kg⁻¹ h⁻¹ group. + $p < 0.05$ different from the 0.12 mg kg⁻¹ h⁻¹ group

Fig. 3. Effect of mecamylamine on lever responses in rats ($n=8$) that received scheduled nicotine infusions. *** $p<0.001$ different from vehicle. ^^ $p<0.01$ different from 0.5 mg/kg. + $p<0.05$ different from 1 mg/kg

Fig. 4.
Effect of mecamylamine on lever responses in rats ($n=10$) receiving no nicotine infusions

Fig. 5.
Effect of DHβE on lever responses in rats ($n=12$) that received scheduled nicotine infusions.
** $p<0.01$ different from vehicle

Fig. 6.
Effect of MLA on lever responses in rats ($n=11$) that received scheduled nicotine infusions