Published in final edited form as:

Exp Clin Psychopharmacol. 2010 August ; 18(4): 350–358. doi:10.1037/a0020601.

Nicotine's Enhancing Effects on Responding Maintained by Conditioned Reinforcers are Reduced by Pretreatment with Mecamylamine, but not Hexamethonium, in Rats

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Abstract

Several studies have indicated that nicotine increases responding maintained by conditioned reinforcers. We assessed the effects of subcutaneous injections of 0.3 mg/kg nicotine and two nicotinic antagonists on responding maintained by conditioned and primary reinforcers and responding during extinction in 8 Long Evans rats. Mecamylamine, a central and peripheral nicotinic antagonist, and hexamethonium, a peripheral nicotinic antagonist, were administered prior to a subset of the experimental sessions. Nicotine selectively increased responding maintained by conditioned reinforcers and mecamylamine, but not hexamethonium, attenuated this effect. These results suggest that nicotine's enhancing effect on responding maintained by conditioned reinforcers is mediated in the central nervous system.

Keywords

Observing response procedure; rats; nicotine; hexamethonium; mecamylamine

Cigarette smoking provides numerous opportunities for establishing conditioned reinforcers – from the olfactory, gustatory, and tactile stimulation directly associated with nicotine administration to the environmental contexts and activities associated with smoking over time. Nicotine, the putative unconditioned stimulus, not only plays a role in establishing these stimuli as conditioned reinforcers, it may also enhance responding maintained by them (e.g., Chaudhri, Caggiula, Donny, Booth et al., 2006; Chaudhri, Caggiula, Donny, Palmatier et al., 2006; Donny et al., 2003). Indeed, enhanced responding for conditioned stimuli following nicotine administration may be partially responsible for the maintenance of cigarette smoking (Olausson, Jentsch, & Taylor, 2004a; Rose, 2006). Understanding why people smoke requires a systematic and thorough analysis of nicotine's effects on responding maintained by conditioned reinforcers (Clarke, 1991).

Experiments with humans suggest that conditioned reinforcement plays a role in the maintenance of smoking (Dallery, Houtsmuller, Pickworth & Stitzer, 2003; Donny, Houtsmuller & Stitzer, 2007; Perkins et al., 2001; Rose, 2006; Shahan, Bickel, Badger, &

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Giordano, 2001). One method to examine the role of conditioned reinforcers is by using de-nicotinized cigarettes, which provide similar sensory stimuli involved in smoking except for nicotine (Rose, 2006). Shahan, Bickel, Madden, and Badger (1999), for example, found similar break points for subjects responding for nicotinized and de-nicotinized cigarettes on a progressive-ratio schedule, and similar levels of consumption across both types of cigarettes at each unit price. Further, Donny et al. (2007) observed prolonged maintenance of denicotinized cigarette smoking during eleven days in a residential laboratory. De-nicotinized cigarettes also produced suppression in self-reports of craving throughout the study.

Several studies also suggest that conditioned reinforcers play a role in nicotine self-administration (SA) in laboratory animals (Caggiula et al., 2001; Chaudhri, Caggiula, Donny, Booth, et al., 2006; Palmatier et al., 2008). For instance, one study found that both contingent and noncontingent nicotine resulted in increased responding for a conditioned light-tone stimulus (Chaudhri, Caggiula, Donny, Booth, et al., 2006). Similarly, Palmatier et al. (2008) paired a neutral stimulus (i.e., 15 s light illumination) with nicotine infusions and subsequently made presentation of the paired stimulus contingent on a novel response. Rats that had received higher doses of nicotine emitted the novel response at higher rates than those that received lower doses.

To examine further the effects of nicotine on responding maintained by conditioned reinforcers, Raiff and Dallery (2006, 2008) employed an observing response procedure (Dinsmoor, 1985; Shahan, 2002a; Wyckoff, 1952). In an observing response procedure, a subject can respond on either of two alternatives. One alternative (e.g., a food-extinction lever) provides alternating access to a primary reinforcer or extinction. The other alternative (e.g., an observing lever) provides access to stimuli which indicate whether the primary reinforcer is available or whether extinction is in effect. The stimuli, displayed contingent on responses on the observing lever, have been explicitly paired with primary reinforcer availability (S+; e.g., solid house light) or extinction (S-; e.g., blinking house light). Raiff and Dallery (2006, 2008) found that nicotine increased responding maintained by conditioned visual stimulus reinforcers, but not responding maintained by primary sucrose pellet reinforcers or responding during periods of extinction in rats. Note that in the observing response procedure, a period of extinction means that food reinforcers are not available (it does not mean that the S+ undergoes extinction).

The current study expanded on the results of Raiff and Dallery (2008) to assess effects of mecamlamine and hexamethonium, two nAChR antagonists, on nicotine-induced increases in responding maintained by conditioned reinforcers. Mecamlamine is a centrally and peripherally active nicotinic antagonist whereas hexamethonium is only active peripherally (Goodman, Gilman, Brunton, Lazo, & Parker, 2006). Mecamlamine antagonizes many of nicotine's behavioral effects. For example, mecamlamine blocks reinstatement of responding for nicotine-paired stimuli following extinction (Liu et al., 2006), and pre-session injections of mecamlamine decreases nicotine SA in rats, whereas hexamethonium does not (DeNoble and Mele, 2006). Liu, Palmatier, et al. (2007) tested different nicotinic antagonists across groups of rats and found that nicotinic antagonists (i.e., mecamlamine and dihydro- β -erythroidine) result in decreased responding for a moderately primary-reinforcing visual stimulus. In humans, pre-cigarette administration of mecamlamine results in self-reported increases in craving and negative affect (McClernon & Rose, 2005) and reduced satisfaction of smoking and oral stimulation (Rose et al., 1994). In light of this previous research with animals and humans, if nicotine induced increases in responding maintained by conditioned reinforcers are centrally mediated, then mecamlamine, but not hexamethonium, should decrease responding maintained by conditioned reinforcers.

Method

Subjects

Subjects were eight experimentally naive male Long-Evans rats (Harlan; Indianapolis, IN). The rats were approximately 150 days old at the beginning of the experiment. They were individually housed in hanging polycarbonate cages with bedding, in a room which was temperature and humidity controlled. Subjects had free access to water and were maintained at approximately 85% of their 150 day old ad libitum weights, via post-session feeding (Lab Diet Rodent Diet, Formula 5001; PMI Nutrition International, LLC; Brentwood, MO). The colony room was on a 12:12 hr light dark cycle (lights on from 8am-8 pm).

Apparatus and Materials

Four Med Associates® extra tall operant-conditioning chambers (Model ENV 007; 30.48 cm L × 24.13 cm W × 29.21 cm H) were used to conduct experimental sessions. Chambers were contained in large sound attenuating boxes equipped with fans for ventilation and to mask experimental noise. Each intelligence panel contained a food receptacle (5 cm × 5 cm × 3 cm) which was equidistant between two levers (requiring approximately 0.31 N force), each of which measured 4.5 cm × 2 cm and were located 22 cm from the chamber ceiling. Seven cm above each lever were three light-emitting diodes (LED; red, yellow, green; 0.8 cm in diameter, 0.7 cm apart from each other). On the wall parallel to the intelligence panel was a house light (28 volt), centered left to right and 1.5 cm from the ceiling. Purified Rodent Tablets (45 mg sucrose food pellets; TestDiet®, Richmond, IN) were located outside of the chamber, but inside the sound attenuating box. Experimental events and data collection took place on a computer in the same room, using Med-PC software and hardware (MED Associates).

Nicotine ([-]-Nicotine Hydrogen Tartrate Salt; Sigma, St. Louis, MO) was dissolved in potassium phosphate for all subcutaneous injections. Doses of 0.3 mg/kg were injected immediately prior to session (1mL/kg/injection; doses expressed as free base). A dose of 0.3 mg/kg nicotine was chosen because in previous studies, this dose increased response rates in the majority of subjects (Raiff & Dallery, 2006, 2008). Nicotine vehicle injections consisted of potassium phosphate buffer (0.1 molar) in isolation. Mecamylamine and hexamethonium were dissolved in saline. Doses of 1 and 5 mg/kg of mecamylamine hydrochloride and doses of 1 and 5 mg/kg of hexamethonium bromide (Sigma, St. Louis, MO) were injected subcutaneously 20 min before nicotine injections (Araki et al., 2004; Ise, Narita, Nagase, & Suzuki, 2000; Olausson, Jentsch, & Taylor, 2004b; Palmatier, Peterson, Wilkinson, & Bevins, 2004). Mecamylamine and hexamethonium vehicle injections consisted of saline in isolation (1 mL/kg).

Procedure

All sessions were conducted on separate days, seven days per week, at approximately the same time each day.

Lever Training—Research assistants used 45 mg sucrose pellets to reinforce successive approximations of the rats pressing the right and left levers. These initial lever training sessions ended after 30 min or after 20 responses on each lever. One response resulted in a food pellet, with the exception that after three consecutive responses on the same lever food could only be earned by pressing the other lever. Lever training continued for up to three additional sessions, as needed. Once lever pressing was acquired, subjects continued to earn food for pressing levers but were required to strictly alternate between the two levers such that the second consecutive response on one lever did not result in food. These alternation sessions lasted for a maximum of 30 min or until 30 responses had been made on each lever,

on seven separate days. The house light was continuously illuminated during all lever training sessions.

Discrimination Training—After subjects were trained to press both levers, the Discrimination Training condition began. The full discrimination training procedure has been described in detail previously (Raiff & Dallery, 2008). Components alternated between a continuously illuminated house light (S+) which signaled periods when food was available on a 20 s variable-interval (VI) schedule for pressing the left, food-extinction, lever (i.e., food components) and a blinking house light (S-; 0.2 s light-dark alternation) which signaled periods when food was not available for pressing the food-extinction lever (i.e., extinction components). Previous research in our lab (Raiff & Dallery, 2006) has shown that counterbalancing the levers across subjects resulted in the same outcomes for all subjects, thus lever position was held constant in the current study for all subjects. VI distributions (for food and visual-stimulus reinforcement) were based on the Fleshler-Hoffman distribution (Fleshler & Hoffman, 1962) and were composed of 15 interval values. Components lasted an average of 60 s (rectangular distribution ranging from 10 to 110 s); however, if the extinction component was scheduled to change to a food component, it would not change until 5 s elapsed without a response on either lever (i.e., differential reinforcement of other behavior [DRO] 5 sec). The DRO procedure was implemented to prevent adventitious pairings between responding during extinction components and subsequent transitions to food components. Aside from the DRO contingency, responses on the right, observing, lever did not have any additional programmed consequences during Discrimination Training. Discrimination Training lasted a minimum of 65 days.

Observing response procedure—The DRO contingency was discontinued when the observing response procedure began. At the beginning of the session, the computer randomly determined whether a VI 20 s food or extinction component would be in effect; however, the stimulus corresponding to the selected component was only shown contingent on a response to the right, observing lever. Initially, only one response on the observing lever was required to illuminate the schedule correlated stimulus for 10 s (i.e., fixed-ratio [FR] 1). If a component was scheduled to end during the 10 s stimulus presentation, the component continued until the stimulus turned off. Immediately after the stimulus turned off the schedule changed. For example, if after 5 s of S+ stimulus presentation the food component was scheduled to switch to extinction, the S+ and food schedule remained in effect for the final 5 s of the stimulus presentation and the component immediately changed to extinction when the stimulus turned off. For the first five sessions of the observing procedure, the first five observing responses resulted in the S+ stimulus being presented. If the extinction component was in effect when one of the first five responses occurred, the component switched to VI 20 s food (Shahan, 2002a). Otherwise, components alternated every 60 s on average as described for Discrimination Training. Observing responses during the stimulus presentation were recorded, but did not have programmed consequences.

After 11 sessions the VI 20 s food schedule was increased to a VI 30 s schedule. After 10 more sessions the schedule on the observing lever was increased to VI 5 s. Thus, the terminal parameters of the observing response procedure consisted of a VI 30 s food schedule alternating with extinction approximately every 60 s, and observing stimuli were presented for 10 s on a VI 5 s schedule. All sessions from this point forward were arranged according to these terminal parameters and were 30 min in duration.

Drug Administration

Establishing increases with nicotine—Drug administration began after a minimum of 65 sessions on the observing response procedure. During this phase, antagonist vehicle (i.e.,

saline) was administered 20 minutes pre-session and 0.3 mg/kg nicotine was administered immediately pre-session. The purpose of this phase was to assess whether nicotine reliably increased observing response rates before evaluating effects of the nicotinic antagonists. Injections were administered every fourth day for the first six injections; thereafter they were administered every other day for the remainder of this phase and the remainder of the experiment. Sessions were always conducted during the intervening days; however, nicotine vehicle was not administered during this phase of the experiment. Thus, sessions were conducted, but no injections were given in between drug days.

Antagonist administration—Drug administration continued every other day. As stated above, injections of the antagonist occurred 20 minutes prior to drug administration sessions and injections of nicotine occurred immediately prior to the start of the session. Subjects were randomly divided into two groups and received a counterbalanced order of antagonist drug. Group 1 received mecamylamine first and Group 2 received hexamethonium first. The order of doses is shown in Table 1. The antagonists were given in descending order of dose (i.e., 5.0 then 1.0 mg/kg). The complete series of probe injections was administered two times.

Data Analyses

Repeated measures analyses of variance (ANOVAs), with Huynh-Feldt correction, were performed to assess effects of nicotine and antagonist administration. Bonferroni post-hoc analyses were performed when significant main effects were observed. Percent of vehicle responding was used for all analyses. Visual inspection was employed to assess whether individual subject data were representative of the group effects (data not shown). Alpha was set to 0.05 for all analyses.

Results

Discrimination Training

Performance during the Discrimination Training phase was evaluated using the discrimination index (DI) equal to the rate of responding in the S+ divided by the combined rate of responding in the S+ and S-. Average Discrimination Indices (DI) for each subject from the last three days of the Discrimination Training phase of the experiment are presented in Table 2. All but two subjects displayed a DI of 0.75 or higher. Two subjects had an average DI below 0.75 (Subject 287 = 0.68, Subject 288 = 0.72) for the last three days of Discrimination Training.

Observing Response Procedure

Once the observing response phase began, extinction and food responses were recorded in both the presence and the absence of the S+ and S-. The S+ and S- were only displayed following an observing response if one was scheduled. Thus, if the observing lever was not pressed then a mixed schedule was in effect, meaning there were no schedule-correlated stimuli displayed. At the end of the *Establishing Increases with Nicotine* phase, a repeated measures ANOVA revealed a main effect of type of responding ($F[2, 92] = 15.686, p < .001$) and condition ($F[1, 46] = 9.976, p < .005$) and a significant interaction between response type and condition ($F[2, 92] = 13.985, p < .001$). Average increases in responding under 0.3 mg/kg nicotine + antagonist vehicle versus no-injection control were 81.9%, 5.8%, and 22.2% for observing, food, and extinction responses, respectively. Post-hoc analyses revealed that only the increases in observing responses were statistically significant. For these results and those presented below, individual subject data was representative of the observed group effects. Observing responses during the mixed schedule, food responses in the presence of the S+, and extinction responses in the presence of the S- are presented as

responses per minute in Table 3, for each dose combination in the *Antagonist Administration* phase.

Mecamylamine—A repeated measures ANOVA revealed a main effect of type of responding ($F[2,168] = 14.43, p < .001$) and condition ($F[5,84] = 4.03, p < .005$), but not for order of antagonist administration. Figure 1 (left panels) shows the aggregated effects of both doses of mecamylamine on observing, food, and extinction responses when administered with vehicle and nicotine.

Post-hoc analyses also revealed that observing responses, represented as percentage of vehicle, under antagonist vehicle + 0.3 mg/kg nicotine were significantly greater than under antagonist vehicle + nicotine vehicle, 5.0 mg/kg mecamylamine + 0.3 mg/kg nicotine, and 1.0 mg/kg mecamylamine + 0.3 mg/kg nicotine. Additionally, 5.0 mg/kg mecamylamine + nicotine vehicle resulted in decreased observing responses when compared to antagonist vehicle + nicotine vehicle.

Hexamethonium—Figure 1 (right panels) shows the effects of both doses of hexamethonium on observing, food, and extinction responses when administered with vehicle and nicotine. As before, repeated measures ANOVA revealed a significant main effect for type of responding ($F[2,168] = 17.18, p < .001$) and condition ($F[5,84] = 5.93, p < .001$), but not for order of antagonist administration. Additionally, post-hoc analyses revealed that observing responses, represented as percentage of vehicle, were significantly greater under 1.0mg/kg hexamethonium + 0.3 mg/kg nicotine than under antagonist vehicle + nicotine vehicle.

Discussion

The results of the current study systematically replicated and extended the growing body of data suggesting that nicotine has a selective, enhancing effect on responding maintained by conditioned visual stimulus reinforcers (e.g., Raiff & Dallery, 2006, 2008). Nicotine did not reliably increase responding maintained by primary reinforcers or responding during periods of extinction. Mecamylamine, but not hexamethonium, antagonized the nicotine-induced increase in responding maintained by conditioned reinforcers. Because mecamylamine crosses the blood-brain barrier and hexamethonium does not, these results suggest that nicotine's effect on responding maintained by conditioned reinforcers is mediated via nAChRs in the central nervous system.

Nicotinic antagonists, such as mecamylamine, result in a number of effects on nicotine SA and other responses influenced by nicotine. Mecamylamine attenuates nicotine SA (DeNoble & Mele, 2006; Donny et al., 1999) and increases preference for higher doses of nicotine in rats (Glick, Visker, & Maisonneuve, 1996). Mecamylamine also blocks reinstatement of nicotine SA induced by nicotine-paired stimuli (Liu et al., 2006; Liu, Caggiula et al., 2007). Nicotine is active at multiple sites throughout the nervous system, and it is likely that these and other behavioral effects are mediated through particular nAChR subtypes. The $\beta 2$ subunit of nAChRs appears to play a role in the reinforcing properties of nicotine (Wannacott, Sidhpura, & Balfour, 2005). Because mecamylamine is known to be active on $\beta 2$ nAChR subtypes (Papke, Sanberg, & Shytle, 2001), some of its behavioral effects are likely mediated through $\beta 2$ receptor subtypes. Nicotine also affects behavior in ways which may be indirectly related to SA - it has been shown to have a reinforcer-enhancing effect in rats (Chaudhri, Caggiula, Donny, Palmatier et al., 2006; Olausson et al., 2004a; Olausson et al., 2004b; Raiff & Dallery, 2008). Olausson and colleagues demonstrated that nicotine selectively increases responding maintained by conditioned reinforcers and that mecamylamine offsets this effect (Olausson et al., 2004b).

Olausson et al. also found that nicotine did not increase responding for other non-reinforcing sensory stimuli, suggesting that the increase in responding depended on the conditioned reinforcing aspects of the stimulus and was not due to general, undifferentiated increases in responding. Additionally, the selective increase in responding maintained by conditioned reinforcement has been shown to be present whether nicotine delivery is response-dependent or response-independent (Chaudhri, Caggiula, Donny, Booth et al., 2006).

One interpretation of the present findings is that the visual stimuli were serving as primary reinforcers. Indeed, several studies suggest that visual stimuli that have not been paired with primary reinforcers can maintain responding (Palmatier et al., 2007; Raiff & Dallery, 2009; Stewart, 1960), and that nicotine can increase such responding (Palmatier et al., 2007; Raiff & Dallery, 2009). Raiff & Dallery (2008) found that response rates for visual stimuli increased following explicit pairing with food, and the rates of observing for the visual stimuli in the present study exceeded response rates maintained by unpaired (primary) visual stimuli by a factor of two (see Raiff & Dallery, 2009). The findings within (e.g., Raiff & Dallery, 2008) and across (present study, Raiff & Dallery, 2008, 2009) studies indicate that pairings with food increase response rates for visual stimuli, suggesting that the value of the stimuli are increased via respondent conditioning. Determining the function of the visual stimuli, however, is obviously more complicated than comparing response rates. Indeed, even a comparison of responding on the observing lever when food is available or not (i.e., S+ versus S- responding), is complicated by the presence of food during S+. Food maintained responding has been shown to compete with observing responses during food components (Shahan, 2002b). Additional support for the inference that the visual stimuli were serving as conditioned reinforcers in the present study is derived from a number of studies showing that the conditioned reinforcing properties of the S+ are responsible for maintaining observing responses (Case & Fantino, 1981; Dinsmoor, 1985; Dinsmoor et al., 1982; Dinsmoor, Browne, & Lawrence, 1972; Fantino, 1977; Gaynor & Shull, 2002; Jwaideh & Mulvaney, 1976; Shahan & Podlesnik, 2005; see Shahan, 2010 for recent review of the role of conditioned reinforcers).

The effects of nicotine on responding across the different responses in the present experiment could be characterized as rate-dependent. Rate dependence occurs when baseline response rates predict changes in response rate following drug administration (e.g., low baseline rates are increased and high baseline rates are decreased following drug administration). In the present study, observing responses occurred at a relatively low rate and food maintained responses occurred at a relatively high rate. To test for rate dependence, we plotted the ratio of baseline to drug response rates as a function of baseline response rates in log-log coordinates. Because we were interested in whether the differences across response types (i.e., observing versus food maintained responses) could be characterized as rate-dependent, and not differences within a response type, all of the response types were included in the analysis (i.e., observing, food-extinction S+ and S-; based on the method used in Raiff & Dallery, 2008). The correlation was -0.72, which indicates a significant rate-dependent relationship. Rate dependence is, however, a description of the relationship between vehicle and drug responding and does not account for why the relationship exists (Branch, 1984; Lancaster & Dallery, 1999). It is possible that the rate-dependent relationship in the current study is due to the selective effects of nicotine on conditioned reinforcers. To assess further whether nicotine selectively increases responding maintained by conditioned reinforcers compared to primary reinforcers, independent of their rates, it would be desirable to equate response rates prior to drug administration. For example, the schedules of reinforcement for food and light stimuli could be adjusted in order to maintain equal rates of responding (cf. Lucki & DeLong, 1983). Thus, although the present results may be characterized as rate-dependent, rate-dependence

does not explain the differences in increases across response types, or for the selective effect of mecamylamine on responding maintained by conditioned reinforcers.

A few limitations of the current study should be noted. First, each subject received the same dose of nicotine (0.3 mg/kg). Although this dose has been the most behaviorally active in previous experiments (e.g., Raiff & Dallery, 2006, 2009), testing for the dose of nicotine that resulted in the greatest increases in responding maintained by conditioned reinforcers might have resulted in clearer effects of the two antagonists. For example, although the individual data were generally representative of the group data, differences in the magnitudes of nicotine-induced increases might be due to different sensitivities to the 0.3 mg/kg nicotine dose (see Raiff & Dallery, 2006 for dose-response curves using the observing response procedure). Additionally, a greater range of doses for the antagonists might help to clarify whether their effects were due to general behavioral decreases or to a selective antagonism of nicotine-induced increases in responding maintained by conditioned reinforcers. As shown in Figure 1 (top, left panel), 5.0 mg/kg mecamylamine decreased observing responses relative to both vehicle and 0.3 mg/kg nicotine. Thus, the 5.0 mg/kg dose of mecamylamine might produce general decrements in responding maintained by conditioned reinforcers (i.e., it may not be specific to nicotine-induced increases in responding). It should be noted, however, that mecamylamine's rate-reducing effect was only for observing responses, and not for food or extinction responses, indicating that mecamylamine resulted in a selective, rather than general, rate reduction.

Second, extinction responses increased under certain dose conditions (e.g., antagonist vehicle + 0.3 mg/kg nicotine). The rate-increasing effects of the different antagonist doses across extinction responses were not reliable, however. Notably, similar increases in extinction responses were observed by Raiff and Dallery (2008) following acute administration of 0.3 mg/kg nicotine, but these effects were attenuated following chronic administration. Furthermore, these increases in extinction responses were not consistent across a range of doses (Raiff & Dallery, 2006), whereas increases in observing responses were consistent across a range of nicotine doses.

Third, although there were large relative increases in observing responses as a function of nicotine exposure, the absolute changes were not large (see Table 3). The magnitude of the increases in observing responses shown in the current study is consistent with those shown in previous studies demonstrating the reinforcer-enhancing effects of nicotine (note that the current study reported responses per minute, whereas other researchers have reported total responses per 60 minute session; e.g., Donny et al., 2003; Liu, Palmatier, Caggiula, Donny, & Sved, 2007). For example, Donny et al. (2003, Figure 4) demonstrated an increase of approximately 1.1 rsp/min for a visual stimulus following noncontingent nicotine versus noncontingent saline administration. Liu, Palmatier, et al. (2007, Figure 2) demonstrated a similar increase of 1.00 rsp/min with a dose of 0.12 mg/kg/h. These results are consistent with the average of 1.09 rsp/min increases in observing responses in the present study.

Finally, Clarke (1987) suggested that hexamethonium is not as potent as mecamylamine in rats. Higher doses of hexamethonium have produced antihypertensive effects (10, 20, & 30 mg/kg; Santajuliana, Hornfeldt, & Osborn, 1996) and attenuation of cardiovascular reactivity following cocaine administration (10 & 30 mg/kg; Tella, Schindler, & Goldberg, 1992). These studies using larger doses measured physiological reactions to various doses of drug, whereas the current study, as well as others using doses similar to ours (e.g., Besheer, Palmatier, Metschke, & Bevins, 2004; DeNoble and Mele, 2006), measured behavioral responses. It is unknown whether higher doses of hexamethonium might have produced similar dampening in behavioral responses. Therefore, future studies should include higher

doses of hexamethonium, or use more potent peripheral nAChRs such as chlorisondamine (Clarke, 1987).

The results of the current study implicate central, but not peripheral, nAChRs in the mediation of nicotine's reinforcer-enhancing effects on responding maintained by conditioned reinforcers. Given the potential importance of the role of conditioned reinforcers in maintaining smoking (e.g., Dallery, Houtsmuller, Pickworth & Stitzer, 2003; Donny, Houtsmuller & Stitzer, 2007; Perkins et al., 2001; Rose, 2006; Shahan, Bickel, Badger, & Giordano, 2001), and relapse to smoking (e.g., Juliano, Donny, Houtsmuller, & Stitzer, 2006; Liu et al., 2006), further research into the utility of central nAChRs in reducing increases in responding maintained by conditioned reinforcers could lead to more targeted and comprehensive pharmacological interventions for smoking.

Acknowledgments

This research was supported by US Public Health grant R03DA019467. We would like to thank Matthew Capriotti, Rachel Cassidy, Carla Lagorio, Gabe Mazur, and Steve Meredith for their assistance in conducting this research. This research was conducted in partial fulfillment of the requirements of the Master of Science degree at the University of Florida for the first author, Jeb Jones.

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Figure 1.

Antagonist Administration: Mean percent of vehicle responding for mecamylamine (left panels) and hexamethonium (right panels) on observing (top panels), food (middle panels), and extinction responses (bottom panels) as a percent of vehicle responding. Bars represent group means and closed circles are percent of vehicle responding from individual sessions. Note: The bars for the two saline conditions are redundant across graphs to aid comparison of the data and the scaling of the y-axis on the lower left panel is different. Bars sharing the same letter were significantly different from one another.

Table 1

Dose order by group

Group 1		Group 2	
20 min presession	Immediately presession	20 min presession	Immediately presession
Saline	0.3 Nicotine	Saline	0.3 Nicotine
Saline	KPO ₄	Saline	KPO ₄
Mecamylamine 5.0	0.3 Nicotine	Hexamethonium 5.0	0.3 Nicotine
Mecamylamine 1.0	0.3 Nicotine	Hexamethonium 1.0	0.3 Nicotine
Hexamethonium 5.0	0.3 Nicotine	Mecamylamine 5.0	0.3 Nicotine
Hexamethonium 1.0	0.3 Nicotine	Mecamylamine 1.0	0.3 Nicotine
Mecamylamine 5.0	KPO ₄	Hexamethonium 5.0	KPO ₄
Mecamylamine 1.0	KPO ₄	Hexamethonium 1.0	KPO ₄
Hexamethonium 5.0	KPO ₄	Mecamylamine 5.0	KPO ₄
Hexamethonium 1.0	KPO ₄	Mecamylamine 1.0	KPO ₄

Order of probe doses presented to both groups. Probes were administered every other day. This order of doses was presented twice.

Table 2

Discrimination Indices

Subject	Average Discrimination Index ($\pm$ Standard Deviation)	Days in Discrimination Training
281	0.86 ($\pm$ 0.02)	69
282	0.88 ($\pm$ 0.04)	69
283	0.83 ($\pm$ 0.08)	69
284	0.80 ($\pm$ 0.03)	65
285	0.89 ($\pm$ 0.01)	69
286	0.76 ($\pm$ 0.07)	69
287	0.68 ($\pm$ 0.05)	75
288	0.72 ($\pm$ 0.04)	75

Average Discrimination Indices for the last three days of Discrimination Training ($\pm$ Standard deviation) and total days in Discrimination Training. Discrimination Index is equal to the rate of responding in the presence of the S+ divided by the combined rate of responding in the presence of the S+ and S-.

Table 3

Responses per minute ($\pm$ standard deviation) for Each Dose Combination

Nicotinic Antagonist – 20 min pre-session						
	Veh (Sal)	Mec 5.0	Mec 1.0	Hex 5.0	Hex 1.0	
Observing Responses (Mixed Schedule)						
Veh (KPO ₄)	2.17 $\pm$ 1.21	1.23 $\pm$ 0.93	1.90 $\pm$ 1.18	1.88 $\pm$ 1.12	1.95 $\pm$ 1.09	
0.3 Nic	3.26 $\pm$ 1.47	1.09 $\pm$ 0.70	1.98 $\pm$ 1.17	3.13 $\pm$ 1.99	3.33 $\pm$ 1.49	
Food Responses (S+)						
Nicotine - Immediately pre-session						
Veh (KPO ₄)	39.28 $\pm$ 23.32	29.30 $\pm$ 14.60	37.24 $\pm$ 17.81	35.65 $\pm$ 18.01	36.32 $\pm$ 17.70	
0.3 Nic	30.84 $\pm$ 13.46	25.71 $\pm$ 15.06	38.08 $\pm$ 18.05	36.52 $\pm$ 17.11	31.07 $\pm$ 12.29	
Extinction Responses (S-)						
Veh (KPO ₄)	7.83 $\pm$ 4.04	8.51 $\pm$ 3.83	9.57 $\pm$ 5.37	7.56 $\pm$ 3.48	7.33 $\pm$ 3.19	
0.3 Nic	8.60 $\pm$ 2.76	7.49 $\pm$ 3.72	9.39 $\pm$ 4.36	9.67 $\pm$ 4.03	8.24 $\pm$ 2.63	

Note. Responses per minute for observing responses under the mixed schedule, food responses in the presence of S+, and extinction responses in the presence of the S-; Veh = Vehicle; Sal = Saline; Mec = Mecamylamine; Hex = Hexamethonium; Nic = Nicotine.