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The discriminative stimulus effects of mecamylamine in nicotine-treated and untreated rhesus monkeys

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Abstract

The extent to which chronic nicotine treatment can alter the effects of the nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine, and whether those effects can be attenuated by nicotine has not been clearly established in the literature. Here, the discriminative stimulus effects of mecamylamine were compared in one group of rhesus monkeys receiving continuous infusion of nicotine base (5.6 mg/kg/day s.c.) and another group of monkeys not receiving nicotine treatment. Both groups responded under a fixed ratio 5 schedule of stimulus-shock termination. Stimulus control was obtained at doses of 1.78 mg/kg of mecamylamine in monkeys receiving continuous nicotine and 5.6 mg/kg of mecamylamine in monkeys not receiving continuous nicotine treatment. Nicotine did not attenuate the discriminative stimulus effects of mecamylamine in either group. Discontinuation of continuous nicotine produced responding on the mecamylamine lever within 24 h in some but not all monkeys. This may indicate a qualitative difference in the discriminative stimulus effects of mecamylamine across groups, perhaps reflecting antagonism of nicotine and nicotine withdrawal in monkeys receiving continuous nicotine. The failure of nicotine to reverse the effects of mecamylamine is consistent with a non-competitive interaction at nAChRs and indicates that mecamylamine-induced withdrawal cannot be readily modified by nicotine.

Keywords

dependence; drug discrimination; mecamylamine; nicotine; rhesus; withdrawal

Introduction

Laboratory studies of drug dependence and withdrawal involve abrupt discontinuation of chronic drug treatment or administration of a pharmacologically related antagonist during chronic drug treatment. Withdrawal upon abrupt discontinuation of drug treatment has face validity for drug dependence that occurs outside the laboratory. However, the expression and magnitude of withdrawal emerging once drug treatment is discontinued can vary markedly over time between animals (e.g., diazepam; Lukas and Griffiths, 1982). In

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contrast, antagonist-induced withdrawal is typically less variable among animals due to a shorter onset and larger magnitude as compared with abrupt discontinuation of chronic drug treatment (e.g., morphine; Woods and Gmerek, 1985). Withdrawal can be measured by quantifying signs or elicited (i.e., unlearned) behaviors as well as disruption of normal physiology and/or learned behaviors. Drug discrimination assays have been used to assess dependence and withdrawal by training an antagonist as a discriminative stimulus in agonist-dependent animals (Li et al., 2011). Drug discrimination is particularly useful for assessing withdrawal that is not evidenced by robust changes in physiology or unlearned behavior (Stewart and McMahon, 2010).

In order for an antagonist to have utility for assessing dependence and withdrawal, it ideally should produce little or no effect in animals that are drug-naïve (i.e. not receiving chronic treatment) at the smallest dose of antagonist that reliably produces withdrawal in agonist-treated animals. The μ opioid receptor antagonist naloxone produces directly observable signs of withdrawal in morphine-treated animals at doses of naloxone much smaller than the doses producing signs in the absence of morphine treatment (Aceto et al., 1997). The benzodiazepine receptor antagonist flumazenil is also more potent in diazepam-treated monkeys than in monkeys not receiving diazepam treatment (Lukas and Griffiths, 1982). In addition to quantitative differences, agonist treatment often results in qualitative changes in the effects of an antagonist that can be detected in drug discrimination assays. In the absence of morphine treatment, pigeons could discriminate naltrexone; however, the training dose was 100-fold larger than the training dose in pigeons receiving 100 mg/kg/day of morphine (Valentino et al., 1983). Moreover, while various μ opioid receptor antagonists substituted for the effects of naltrexone in morphine-treated pigeons, substitution for naltrexone in morphine-naïve pigeons was not obtained for many of the same antagonists. Similar differences in discriminative stimulus effects, both quantitative and qualitative, were demonstrated for flumazenil in rhesus monkeys receiving 5.6 mg/kg/day of diazepam versus monkeys not receiving diazepam treatment (Gerak and France, 1999).

Mecamylamine (3-methylaminoisocamphane hydrochloride or Inversine®) is a secondary amine that penetrates the CNS and initially garnered interest because of its ability to inhibit transmission of impulses across autonomic ganglia (Stone et al., 1956). Mecamylamine is an antagonist of nAChRs (Garcha and Stolerman, 1993; Mariathasan and Stolerman, 1993; Webster et al., 1999). In binding studies with [3H] mecamylamine and nicotine, mecamylamine did not block nicotine binding but was able to block nicotine-induced seizures, indicating that mecamylamine binds to a different site than nicotine (Banerjee et al., 1990). Because the interaction between nicotine and mecamylamine is non-competitive, the effects of mecamylamine are not always reversed or surmounted by nicotine (Stolerman et al., 1983). Mecamylamine attenuates many of the behavioral effects of nicotine, including its positive reinforcing and aversive effects, its effects on rates of schedule-controlled behavior and locomotor activity (Clarke and Kumar, 1983; Fudala et al., 1985; Reavill and Stolerman, 1990), and the discriminative stimulus effects of nicotine in mice, rats, and non-human primates (Stolerman et al., 1999; Jutkiewicz et al., 2011; Cunningham et al., 2012). Mecamylamine, however, may have limited selectivity for nicotinic acetylcholine-gated ion channels. For instance, the N-methyl-D-aspartate (NMDA) antagonist phencyclidine and the calcium channel blocker verapamil both have high affinity for [3H]mecamylamine binding

sites, perhaps reflecting binding of mecamlamine at NMDA receptors and calcium channels (Banerjee et al., 1990). Furthermore, mecamlamine is reported to antagonize NMDA receptor-mediated norepinephrine release (O'Dell and Christensen, 1988) as well as attenuate NMDA-induced lethality (McDonough and Shih, 1995). Collectively, these data suggest that the effects of mecamlamine may not be solely mediated by nAChRs.

Mecamlamine has been used to assess nicotine dependence and withdrawal. Mecamlamine is reported to produce a larger number of directly observable and physiological signs in the presence of nicotine treatment as compared with the absence of treatment, consistent with antagonist-induced nicotine withdrawal (Watkins et al., 2000; Johnson et al., 2008; Weaver et al., 2012; Harris et al., 2013). Increased reward thresholds produced by mecamlamine in intracranial self-stimulation assays in nicotine-treated rodents are indicative of a deficit in brain reward function. Moreover, a dose of mecamlamine that increased reward thresholds in nicotine-dependent rats did not exert the same effect in saline-treated rats (Marcinkiewicz et al., 2009). Mecamlamine alone does have effects, however, as evidenced by mecamlamine being established as discriminative stimulus effects in the absence of nicotine treatment in rats (Garcha and Stolerman, 1993). Some, but not all, ganglion-blocking drugs substituted for the discriminative stimulus effects of mecamlamine in this study and the mecamlamine discriminative stimulus was not attenuated by nicotine (Garcha and Stolerman, 1993).

Here, drug discrimination was used to compare the effects of mecamlamine in one group of rhesus monkeys receiving nicotine treatment (5.6 mg/kg/day continuously) and in another group not receiving nicotine treatment. Two hypotheses were tested to examine the extent to which the discriminative stimulus effects of mecamlamine in nicotine-treated monkeys were related to withdrawal. One hypothesis was that mecamlamine would be discriminated in the presence of nicotine treatment at doses of mecamlamine smaller than doses that could be discriminated in the absence of nicotine treatment. A second hypothesis was that temporary discontinuation of nicotine treatment might result in a time-related increase in withdrawal and a corresponding switch in responding from the lever associated with vehicle, or the absence of withdrawal, to the lever associated with the antagonist or the presence of withdrawal. While both hypotheses were to some extent confirmed, other results consistent with a non-competitive interaction between mecamlamine and nicotine illustrate a limitation of the current approach for assessing the nicotine-withdrawal reversing effects of nAChR drugs.

Methods

Subjects

Adult rhesus monkeys (*Macaca mulatta*) had no prior drug or experimental history at the beginning of the experiment. Four males and one female received continuous nicotine infusion; two males and two females did not receive continuous nicotine treatment. The monkeys were housed individually in stainless steel cages on a 14-h light/10-h dark schedule (lights on at 06.00 h). They were maintained at 95% free-feeding weight (range 6-10.5 kg) with a diet consisting of primate chow (Harlan Teklad, High Protein Monkey Diet; Madison, WI), fresh fruit, and peanuts; water was continuously available in the home cage. Monkeys

were maintained, and experiments were conducted in accordance with, the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio and the *National Institutes of Health's Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 2011).

Apparatus

Monkeys were seated individually in a chair (Model R001, Primate Products; Miami, FL) in front of a stainless steel wall, two levers, and two lights (i.e., one above each lever) within ventilated, sound-attenuating chambers. Feet were maintained in contact with brass electrodes to which a brief electric stimulus (3 mA, 250 ms) could be delivered from an a/c generator (Coulbourn Instruments; Whitehall, PA). An interface (MedAssociates) connected the chambers to a computer, which controlled and recorded lever responses with Med-PC software.

Osmotic minipump and continuous nicotine treatment

Nicotine was administered continuously through an osmotic minipump (Alzet 2ML4, Durect Corp; Cupertino, CA) implanted in the mid-scapular region or the flank. The pH of the nicotine solution was adjusted to 7 with buffer and the concentration was adjusted per individual monkey according to body weight at the time of minipump implantation for a dose of 5.6 mg/kg/day. Every 28 days monkeys were given ketamine (10 mg/kg) for immobilization and transportation to the experimental surgery department where anesthesia was maintained with inhaled isoflurane. At this time depleted pumps were removed and new, sterile pumps were implanted.

Discrimination training

Responding was maintained under an FR5 schedule of stimulus-shock termination. Illumination of the lights signaled that an electric stimulus was scheduled for delivery in 10 s; however, five consecutive responses on a lever extinguished the lights, prevented delivery of the electric stimulus, and postponed the schedule for 30 s. The schedule of stimulus-shock termination ended after 10 min or the delivery of four electric stimuli, whichever occurred first.

Experimental sessions began with a 30-min timeout followed by a 10-min schedule of stimulus shock termination. The lever designated correct during training was assigned based on whether the monkey received saline or the training dose of mecamlamine. Mecamlamine lever assignments were balanced across the groups for left and right levers and remained the same per monkey for the duration of the study. Five consecutive responses on the correct lever postponed the shock schedule; incorrect responses reset the response requirement. The first test was conducted when for five consecutive or six out of seven training sessions at least 80% of the total responses occurred on the correct lever and fewer than five responses occurred on the incorrect lever prior to completion of the first fixed ratio on the correct lever.

Discrimination testing

Test sessions were identical to training sessions except that five consecutive responses on either lever postponed the schedule of stimulus-shock termination and animals received saline or dose(s) of test compound. Further tests were conducted when performance for consecutive training sessions, including both saline and mecamylamine training sessions, satisfied the test criteria.

To establish a dose-response function, a dose of mecamylamine or hexamethonium was administered at the beginning of a session (i.e., timeout) or 30 min before the schedule of stimulus-shock termination. For tests with nicotine alone, the pretreatment interval was decreased to 10 min before the schedule of stimulus shock termination, i.e., 20 min after administration of saline. For some tests, immediately after the 10-min schedule of stimulus shock termination, up to five cycles were added; the subsequent cycles consisted of a 10-min pretreatment followed immediately by 10 min of responding under the schedule of stimulus-shock termination. The combined effects of mecamylamine and nicotine were assessed by administering the training dose of mecamylamine 30 min beforehand, followed by saline or a dose of nicotine 10 min before a subsequent 10-min response period. To examine the duration of action of mecamylamine, saline or 5.6 mg/kg of mecamylamine was administered on separate days either at the beginning of or in 100-min intervals before a test session consisting of 6 cycles. To examine the effects of abrupt discontinuation of nicotine treatment, monkeys were tested on consecutive days with no intervening training and with an injection of saline given 30 min before a 10-min response period, one day before and each of the next 4 days after the removal of the osmotic minipumps containing nicotine.

Drugs

Nicotine hydrogen tartrate salt, hexamethonium bromide (Sigma-Aldrich; St. Louis, MO), and mecamylamine hydrochloride (Waterstone Technology; Carmel, IN) were dissolved in physiological saline and administered s.c. in the midscapular region of the back in volumes less than 3 ml. Nicotine dose was expressed as the weight of the base only. For both mecamylamine and hexamethonium, doses in mg/kg were expressed as the combined weight of the base and salt. Ketamine was obtained in a commercial solution of 100mg/ml (Butler Animal Health Supply, Dublin, OH).

Data analyses

Data were expressed per individual monkey or as the mean of individual monkeys. Discrimination data were calculated as a percentage of responses on the mecamylamine lever out of total responses on the saline and mecamylamine levers. Rate of responding on both levers (i.e., mecamylamine and saline) was calculated as responses per second excluding responses during timeouts. Rate of responding during a test was expressed as the percentage of the control response rate for individual animals. The control was defined as the average response rate for all cycles during the five previous saline training sessions excluding sessions during which the test criteria were not satisfied. Discrimination and rate data were averaged among subjects and plotted as a function of dose or time.

Potency of a drug to produce mecamylamine-appropriate responding was calculated by simultaneously fitting straight lines to individual dose-effect data by means of GraphPad Prism version 5.0 for Windows (San Diego, CA) with linear regression. Straight lines were fitted to the linear portion of dose-effect curves, defined by doses producing 20-80% mecamylamine-lever responding, including not more than one dose producing less than 20% mecamylamine-lever responding and not more than one dose producing greater than 80% mecamylamine-lever responding. Other doses were excluded from the analyses. Doses corresponding to the 50% level of the effect (ED_{50}) and 95% confidence limits were calculated by parallel line analyses of data from individual subjects (Tallarida, 2000).

The time course of mecamylamine was constructed by combining 6-cycle test data obtained on different days and with different pretreatment times. The pretreatment intervals between tests were selected so that the sixth cycle of a test would coincide with the first cycle of a second test; data from these cycles were averaged in individual monkeys for further analysis. Significant changes in discrimination or response rate over time were analyzed by repeated measures one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test ($p < 0.05$). When mean response rate was not decreased to less than 50% at any dose of a drug, significant changes in response rate were examined with linear regression and an F-ratio test comparing the slope to 0. If the slope was not significantly different from 0, then there was no significant effect of the drug on response rate as a function of dose.

RESULTS

The effects of mecamylamine in nicotine-treated monkeys

Monkeys receiving 5.6 mg/kg/day of nicotine were initially trained with 1 mg/kg of mecamylamine. After 24 sessions consisting of both mecamylamine and saline training sessions, the training dose was increased to 1.78 mg/kg of mecamylamine. The total number of training sessions (including training at both 1 and 1.78 mg/kg mecamylamine) required for each monkey to pass the test criteria was 121, 125, 128, 160, and 204.

In monkeys receiving continuous nicotine treatment, mecamylamine dose-dependently increased the percentage of responses on the mecamylamine lever to 95% at the training dose (1.78 mg/kg s.c.; Figure 1, top left), whereas saline produced 1% of responses on the mecamylamine lever (Figure 1, top left, square). The ED_{50} value (95% confidence limits) of mecamylamine to produce discriminative stimulus effects was 0.82 (0.59-1.12) mg/kg. The control rate of responding for individual monkeys was 1.31, 1.50, 1.74, 2.03, and 2.50 responses per s. Mecamylamine did not significantly modify response rate as a function of dose (Figure 1, bottom left).

The effects of mecamylamine in untreated monkeys

After 120 sessions (including both mecamylamine and saline training sessions) of training at the initial training dose (1 mg/kg) of mecamylamine, the training dose was increased to 3.2 mg/kg. After 105 additional training sessions at 3.2 mg/kg, the training dose was increased further to 5.6 mg/kg. Monkeys satisfied the criteria for testing after 5-74 additional training

sessions at 5.6 mg/kg. The total number of training sessions required for each monkey to pass the test criteria was 230, 234, 250, and 299.

In monkeys not receiving nicotine treatment, mecamylamine dose-dependently increased the percentage of responses on the mecamylamine lever up to 98% at the training dose (5.6 mg/kg s.c.; Figure 1, top right), whereas saline produced no responses on the mecamylamine lever (Figure 1, top right, square). The ED₅₀ value (95% confidence limits) of mecamylamine to produce discriminative stimulus effects was 2.95 (1.27-6.83) mg/kg. When the training dose (5.6 mg/kg) of mecamylamine was studied over time, mecamylamine-lever responding varied significantly as a function of time ($F_{14,28}=8.00$, $p<0.001$). From 30-90 min, drug-lever responding was at least 98% (Figure 2, top). Drug-lever responding was least 73% up to 230 min after mecamylamine and then decreased to 28-33% by 270-310 min. The control rate of responding for individual monkeys was 1.54, 1.90, 2.25, and 2.51 responses per s. Response rate did not vary significantly as a function of mecamylamine dose (1-5.6 mg/kg) or as a function of time after the training dose ($p>0.05$).

The effects of nicotine alone and in combination with mecamylamine

When administered alone, nicotine base (1-3.2 mg/kg) produced a maximum of 4% mecamylamine-lever responding and did not significantly alter response rate (data not shown). Nicotine base, up to 5.6 mg/kg in nicotine-treated and 10 mg/kg in untreated monkeys, did not alter the discriminative stimulus effects of mecamylamine in either discrimination (Figure 3, top left and right, respectively). When tested in combination with mecamylamine (5.6 mg/kg) in untreated monkeys, nicotine base dose-dependently decreased response rate to 33% of control at a dose of 10 mg/kg of nicotine (Figure 3, bottom right). The ED₅₀ value (95% confidence limits) for nicotine to decrease rate of responding was 8.37 (1.77-39.5) mg/kg.

The effects of hexamethonium in nicotine-treated and untreated monkeys

Hexamethonium dose-dependently increased responding on the mecamylamine lever in nicotine-treated and untreated monkeys (Figure 4, top left and right, respectively). A dose of 10 mg/kg hexamethonium produced 94% mecamylamine-lever responding in nicotine-treated monkeys. In untreated monkeys, three of four monkeys responded greater than 95% on the mecamylamine-lever at 17.8 and 32 mg/kg of hexamethonium and one of four monkeys responded a maximum of 5% on the mecamylamine lever, resulting in a group average maximum of 76% at 32 mg/kg of hexamethonium (Figure 4, top right). The ED₅₀ values (95% confidence limits) of hexamethonium to produce mecamylamine-lever responding were 5.89 (3.50-9.94) mg/kg in nicotine-treated monkeys and 9.36 (2.50-35.0) mg/kg in control monkeys. Hexamethonium did not significantly modify response rate at the doses tested (Figure 4, bottom).

Discontinuation of nicotine treatment

At the end of the study monkeys who were receiving continuous nicotine-treatment were tested on consecutive days immediately before and after nicotine-containing osmotic minipumps were removed. All five monkeys responded primarily on the saline-associated lever one day prior to pump removal (Figure 5). However, one day after the removal of the

osmotic minipumps three of the five monkeys responded, at least partially, on the mecamylamine-lever (46%, 65%, and 87%). The relationship between days of nicotine deprivation and amount of mecamylamine lever responding differed for these three monkeys; mecamylamine-lever responding lasted only one day in a monkey (Figure 5, triangles), two days in another monkey (Figure 5, diamonds), and persisted for the duration of the experiment (4 days) in the final monkey (Figure 5, inverted triangles). Two of the five monkeys responded predominantly on the saline-lever before and after pump removal. Discontinuation of continuous nicotine administration did not modify response rate over time (data not shown) as compared to the saline control determined during continuous nicotine treatment. Moreover, systematic changes in directly observable behavior that might be indicative of a nicotine withdrawal syndrome, either after mecamylamine during nicotine treatment or after abrupt discontinuation of nicotine treatment, were not consistently observed in all monkeys during any of the experiments reported here. Mecamylamine produced ptosis and paleness in the face; however, these effects did not appear to differ in nicotine-treated versus untreated monkeys.

Discussion

The non-competitive nAChR antagonist mecamylamine was established as a discriminative stimulus in one group of monkeys not receiving continuous nicotine and in another group of monkeys receiving 5.6 mg/kg/day of nicotine base. Monkeys receiving continuous nicotine could discriminate a smaller dose of mecamylamine than monkeys not receiving nicotine (1.78 and 5.6 mg/kg, respectively). The peripheral nAChR antagonist hexamethonium substituted for mecamylamine in both groups. Nicotine was unable to attenuate the effects of mecamylamine in either group. There were individual differences in test performance when continuous nicotine treatment was abruptly discontinued. Three out of five monkeys switched their response choice from the saline- to the mecamylamine-associated lever after one day of discontinuation, whereas two other monkeys continued to respond on the saline lever throughout four days of nicotine deprivation. While the performance of some monkeys is consistent with antagonism of nicotine and nicotine withdrawal, the inability of nicotine to attenuate the mecamylamine discriminative stimulus demonstrates a limitation of the assay for assessing the withdrawal-modifying effects of nAChR agonists.

Drug discrimination has utility for examining antagonist-induced withdrawal from opioids, benzodiazepines, and cannabinoids (Li et al., 2011). Here, the nAChR antagonist mecamylamine was established as a discriminative stimulus in rhesus monkeys receiving continuous subcutaneous administration of 5.6 mg/kg/day of nicotine. Two outcomes suggested that the discrimination in nicotine-treated monkeys was related to withdrawal. First, nicotine treated monkeys were more sensitive to the discriminative stimulus effects of mecamylamine inasmuch as a larger dose of mecamylamine was needed to maintain stimulus control over behavior in monkeys not receiving nicotine treatment. Second, abrupt discontinuation of nicotine treatment substituted for the mecamylamine discriminative stimulus in a subset of monkeys. These results notwithstanding, nicotine was unable to attenuate mecamylamine-induced nicotine withdrawal. In contrast, a variety of drugs including the dependence-inducing drug modify the discriminative stimulus effects of their respective antagonists in monkeys receiving chronic treatment with opioids,

benzodiazepines, or cannabinoids (Li et al., 2011). The failure of nicotine to modify the effects of mecamylamine limits the utility of the current assay as compared with other withdrawal-related discrimination assays.

The daily dose (5.6 mg/kg) of nicotine and method of drug delivery (continuous administration via osmotic minipump) was selected based not only on previous studies but also on the limits of dosing with a single osmotic minipump. Robust nicotine withdrawal has been observed in rats treated with 3.2 mg/kg/day of nicotine, a dose approximately 10-fold larger than a training dose for nicotine discrimination in rats (i.e. 0.4 mg/kg; Stolerman et al., 1984). In nonhuman primates, doses of 1-2 mg/kg have been studied (Howell, 1995; Grove et al., 2001), which are similar to doses of nicotine that have been used as a training dose (1.78 mg/kg) for drug discrimination in rhesus monkeys (Cunningham et al., 2012). The dose chosen for daily treatment in rhesus monkeys (5.6 mg/kg/day nicotine base) was larger than the training dose of nicotine previously used in rhesus monkeys, but was limited by the maximum concentration and volume allowable in most commercially-available osmotic minipumps.

In nicotine-treated monkeys, the failure of additional nicotine to attenuate, or surmount, the discriminative stimulus effects of mecamylamine is most likely due to a non-competitive interaction. While enhanced sensitivity to mecamylamine as a function of chronic nicotine treatment has been reported here and elsewhere (Hildebrand et al., 1997; Vann et al., 2006), the extent to which additional, acute administration of nicotine prevents or reverses mecamylamine-induced nicotine withdrawal is currently unclear. The results of several drug discrimination studies suggest that nicotine is unable to surmount the antagonism by mecamylamine (Jutkiewicz et al., 2011; Cunningham et al., 2012). Among alternative nAChR antagonists suitable for behavioral studies, dihydro- β -erythroidine is a competitive nAChR antagonist (Williams and Robinson, 1984). However, dihydro- β -erythroidine produced limited (i.e., 2-fold) antagonism of a nicotine discriminative stimulus in rhesus monkeys up to doses of dihydro- β -erythroidine that could be safely studied (Cunningham et al., 2012). This contrasts with the marked antagonism produced by mecamylamine in the same nicotine discrimination assay. Sensitivity to the effects of dihydro- β -erythroidine might have been enhanced in nicotine-treated animals, not only because of precipitated withdrawal, but also because chronic nicotine treatment up-regulates nAChR (Barik and Wonnacott, 2009). However, dihydro- β -erythroidine was not studied here because of concerns over toxicity.

Nicotine treatment appears to have increased the potency of mecamylamine to produce discriminative stimulus effects. Monkeys receiving continuous nicotine treatment were able to discriminate a dose (1.78 mg/kg) of mecamylamine 0.5 log unit smaller than the dose (5.6 mg/kg) discriminated by monkeys not receiving continuous nicotine. A similar difference in potency has been reported in rhesus monkeys discriminating the benzodiazepine antagonist flumazenil in the presence versus the absence of daily diazepam treatment (Gerak and France, 1999). In contrast, the potency of naltrexone as a discriminative stimulus is markedly (e.g., 100-fold) different in morphine-treated versus untreated pigeons (Valentino et al., 1983). Moreover, monkeys not receiving continuous nicotine treatment required more time to acquire the mecamylamine discrimination than nicotine-treated monkeys. Increased

sensitivity to the effects of an antagonist resulting from continuous treatment with a pharmacologically related agonist is due to the displacement of agonist binding and, to the extent that the discrimination is related to withdrawal, the dose of agonist and magnitude of dependence. In contrast, the effects of an antagonist in drug-naïve animals are mediated by changes in endogenous tone at the primary site of action or by actions of the antagonist at secondary site(s), especially at relatively large doses. While the pharmacology of the mecamlamine discriminative stimulus is not further described here, a mecamlamine discrimination established elsewhere in rats had a pharmacologic profile consistent with actions at both nAChR and N-methyl-D-aspartate receptors (Garcha and Stolerman, 1993). The training dose of mecamlamine in rats (3.5 mg/kg) was much larger than a dose (0.1 mg/kg mecamlamine) of mecamlamine that antagonizes the discriminative stimulus effects of nicotine (Stolerman et al., 1983).

The relative potency of mecamlamine for producing multiple effects in rats and monkeys facilitates interpretation of the current data. The training dose of mecamlamine in rats not receiving nicotine treatment was 3.5 mg/kg and the dose of mecamlamine required to produce full substitution was 5.6 mg/kg (Garcha and Stolerman, 1993). Smaller doses (0.1 mg/kg) of mecamlamine antagonized the effects of nicotine in rats (Stolerman et al., 1983). In rhesus monkeys, a dose of 0.32 mg/kg of mecamlamine significantly antagonized a nicotine discriminative stimulus (Cunningham et al., 2012), demonstrating that the relative potency of mecamlamine to antagonize nicotine and to maintain stimulus control over behavior in the absence of nicotine treatment did not differ between rhesus monkeys and rats. Overall, these data strengthen the notion that 5.6 mg/kg of mecamlamine is near the smallest dose that can be discriminated without nicotine treatment, and provide further evidence that discrimination of mecamlamine at the smaller dose (1.78 mg/kg) in nicotine-treated monkeys is related to antagonism of nicotine.

The non-competitive nAChR antagonist hexamethonium substituted for the mecamlamine discriminative stimulus in both nicotine-treated and untreated monkeys, suggesting that nAChR receptors mediated both discriminations. Hexamethonium is a quaternary compound that does not readily cross the blood-brain-barrier and is used clinically as an anti-hypertensive at a dose of 1 mg/kg. Therapeutic effects result from antagonism of ganglionic nAChR. In rhesus monkeys, doses (7-10 mg/kg) of hexamethonium larger than those used clinically and similar to those used here were reported to decrease mean arterial blood pressure (Dews and Herd, 1974; Gaide et al., 1980). If the pharmacological mechanism(s) underlying the discriminative stimulus and cardiovascular effects of hexamethonium in rhesus monkeys are the same, then nAChR located outside the brain mediate the discriminative stimulus effects of mecamlamine. However, that doses of hexamethonium larger than 10 mg/kg were needed to fully substitute for mecamlamine in the absence of nicotine treatment might reflect penetration into and actions at receptors in the brain.

In agonist-dependent animals discriminating an antagonist, abrupt discontinuation of agonist treatment can lead to a time-related increase in withdrawal and a corresponding switch in responding from the lever associated with vehicle, or the absence of withdrawal, to the lever associated with the antagonist or the presence of withdrawal (Becker et al., 2008; Stewart and McMahon, 2010). Discontinuation of nicotine treatment (5.6 mg/kg/day) resulted in a

switch from the saline lever to the mecamylamine lever in three of five rhesus monkeys. In two monkeys, responding on the mecamylamine lever decreased over time. The effects of nicotine deprivation in the other monkey might also have been time-limited; however, the deprivation period did not extend beyond four consecutive days. Discontinuation of nicotine treatment did not mimic the effects of mecamylamine during nicotine treatment in all monkeys. At a minimum, there are differences in the magnitude of discriminative stimulus effects between the two conditions, and perhaps also a difference in quality such that discriminative stimulus effects are unrelated to nicotine withdrawal for one of the conditions.

In summary, the discriminative stimulus effects of mecamylamine appeared to change both quantitatively and qualitatively as a function of nicotine treatment. Furthermore, discontinuation of nicotine treatment produced mecamylamine lever responding in some, but not all monkeys. While the overall profile suggests that the mecamylamine discrimination in nicotine-treated monkeys is related to nicotine withdrawal, nicotine was unable to attenuate the discriminative stimulus effects of mecamylamine. The non-competitive nature of the interaction between mecamylamine and nicotine at nAChRs appears to limit the combined use of mecamylamine and drugs that bind to the same site as nicotine as a strategy for assessing the neuropharmacology of nicotine dependence.

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Figure 1. Discriminative stimulus effects of mecamylamine in nicotine-treated (left) and untreated (right) rhesus monkeys

The training dose of mecamylamine was 1.78 mg/kg in monkeys receiving 5.6 mg/kg/day of continuous nicotine base. The training dose of mecamylamine in untreated monkeys was 5.6 mg/kg. Horizontal axes: Saline or dose in mg/kg body weight administered s.c. Vertical axes: mean ($\pm$ S.E.M.) percentage of responding on the mecamylamine lever (upper pane;) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (lower panel).

Figure 2. Time course of discriminative stimulus effects after mecamylamine (5.6 mg/kg) in rhesus monkeys with no continuous nicotine treatment
Horizontal axes: Time in minutes. Vertical axes: mean ($\pm$ S.E.M.) percentage of responding on the mecamylamine lever (upper panel) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (lower panel).

Figure 3. Discriminative stimulus effects of the training dose of mecamylamine in combination with nicotine in nicotine-treated (left) and untreated (right) rhesus monkeys

The training dose of mecamylamine was 1.78 mg/kg in monkeys receiving 5.6 mg/kg/day of continuous nicotine base. The training dose of mecamylamine in untreated monkeys was 5.6 mg/kg. Mecamylamine was administered 30 min before, and nicotine base 10 min before, experimental sessions. Horizontal axes: Saline or dose in mg/kg body weight administered s.c. Vertical axes: mean ($\pm$ S.E.M.) percentage of responding on the mecamylamine lever (upper panel) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (lower panel).

Figure 4. Discriminative stimulus effects of hexamethonium in nicotine-treated (left) and untreated (right) rhesus monkeys discriminating mecamylamine (1.78 and 5.6 mg/kg, respectively)

Horizontal axes: Saline or dose in mg/kg body weight administered s.c. Vertical axes: mean ($\pm$ S.E.M.) percentage of responding on the mecamylamine lever (upper panel) and mean ($\pm$ S.E.M.) rate of responding expressed as a percentage of control (lower panel).

Figure 5. Time course of mecamlamine-like discriminative stimulus effects after discontinuation of continuous nicotine-treatment in individual monkeys

Horizontal axis: Time in days. Vertical axis: percentage of responding on the mecamlamine lever.