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Involvement of Nicotinic Receptor Subtypes in the Behavioral Effects of Nicotinic Drugs in Squirrel Monkeys

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ABSTRACT

Evidence suggests that the $\alpha4\beta2$, but not the $\alpha7$, subtype of the nicotinic acetylcholine receptor (nAChR) plays a key role in mediating the behavioral effects of nicotine and related drugs. However, the importance of other nAChR subtypes remains unclear. The present studies were conducted to examine the involvement of nAChR subtypes by determining the effects of selected nicotinic agonists and antagonists in squirrel monkeys either 1) responding for food reinforcement or 2) discriminating the nicotinic agonist (+)-epibatidine (0.001 mg/kg) from vehicle. In food-reinforcement studies, nicotine, (+)-epibatidine, varenicline and cytisine all produced dose-dependent decreases in rates of food-maintained responding. The rate-decreasing effects of nicotine were antagonized by mecamylamine (nonselective), not appreciably altered by dihydro-β-erythroidine ($\alpha4\beta2$ selective), and exacerbated by the nicotinic partial agonists, varenicline and cytisine. Results from discrimination studies show that non-nicotinic drugs did not substitute for (+)-epibatidine, and that except for lobeline, the nicotinic agonists produced either full [(+)-epibatidine, (−)-epibatidine, and nicotine] or partial (varenicline, cytisine, anabaseine, and isoarecolone) substitution for (+)-epibatidine. In interaction studies with antagonists differing in selectivity, (+)-epibatidine discrimination was substantively antagonized by mecamylamine, slightly attenuated by hexamethonium (peripherally restricted) or dihydro-β-erythroidine, and not altered by methyllycaconitine ($\alpha7$ selective). Varenicline and cytisine enhanced (+)-epibatidine's discriminative-stimulus effects. Correlational analysis revealed a close correspondence between relative behavioral potencies of nicotinic agonists in both studies and their published relative binding affinities at $\alpha4\beta2$ and $\alpha3\beta4$, but not $\alpha7$ nAChR, subtypes. Collectively, these results are consistent with the idea that the $\alpha4\beta2$ and $\alpha3\beta4$, but not $\alpha7$ nAChR subtypes play a role in the behavioral effects of nicotinic agonists.

Introduction

Several pharmacological agents that may reduce nicotine's addictive effects are currently available for smoking cessation in the United States [varenicline (Chantix, Pfizer, Groton, CT) and bupropion (Wellbutrin XL, Valeant Pharmaceuticals, Bridgewater, NJ)] and Central/Eastern Europe [cytisine (Tabex, Sopharma, Sofia, Bulgaria or Desmoxan, Aflofarm Farmacja, Pabianice, Poland)] (Etter et al., 2008; Benowitz, 2010; Walker et al., 2014). Administered alone or in combination with behavioral therapy, these medications produce short-term increases in quit rates but display low anti-relapse efficacy and may produce adverse effects—factors that limit their widespread clinical application (Hughes et al., 2003; Hays et al., 2008). These issues highlight the strong need for better understanding of the neurobiological and behavioral mechanisms that mediate the addiction-related effects of nicotine, and for novel medication strategies to combat them.

Several lines of evidence in rats and mice support a prominent role for $\alpha4\beta2$ nicotinic acetylcholine receptor (nAChR) mechanisms in the behavioral effects of nicotine. First, the $\alpha4\beta2$-selective nicotinic antagonist dihydro-β-erythroidine (DHβE) has been reported to block nicotine self-administration in rats (Watkins et al., 1999). Moreover, nicotine fails to maintain self-administration behavior in genetically modified mice lacking the $\beta2$ subunit (Picciotto et al., 1998; Epping-Jordan et al., 1999), whereas selective activation of $\alpha4$* subunits by nicotine suffices to produce rewarding effects (Tapper et al., 2004). Second, neurobiological data suggest that nicotine acts at $\alpha4\beta2$ nAChRs in the ventral tegmental area to concomitantly modulate excitatory (e.g., glutamate) and inhibitory (e.g., gamma-aminobutyric acid) neurotransmission (Picciotto et al., 1998; Quarta et al., 2007), and consequently elevate and diminish dopamine levels in the nucleus accumbens (D'Souza and Markou, 2013). Finally, the $\alpha4\beta2$ nAChR subtype has been implicated in the discriminative-stimulus effects of nicotine. For example, $\alpha4\beta2$ subtype–selective nicotinic agonists and antagonists mimic and attenuate nicotine's effects, respectively, whereas drugs acting exclusively at other subtypes of nAChRs (e.g., $\alpha7$ and $\alpha3\beta4$) or other pharmacological classes (e.g., muscarinic agents) fail to engender nicotine-like stimulus effects (for review, see Smith and Stolerman, 2009). Taken together, these converging lines of evidence suggest the $\alpha4\beta2$ subtype of nAChR plays a key role in nicotine's abuse-related actions.

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ABBREVIATIONS: DHβE, dihydro-β-erythroidine; FR, fixed ratio; MA, methamphetamine; nAChR, nicotinic acetylcholine receptor; TO, timeout.

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In addition to the $\alpha4\beta2$ nAChR, other subtypes of nAChRs also have been proposed to play a role in nicotine's behavioral effects (McGehee et al., 1995; Gotti et al., 1997). For example, selective antagonists at $\alpha7$ (Markou and Paterson, 2001; Brunzell and McIntosh, 2012), $\alpha6$ (Beckmann et al., 2015), and $\alpha3\beta4$ (e.g., Glick et al., 2002; Toll et al., 2012) nAChRs have been reported to attenuate nicotine self-administration or conditioned place preference. Moreover, based on results in knockout mice, a role has been proposed for $\alpha5$ and, in separate studies, $\alpha6$ nAChR subtypes in the abuse-related behavioral effects of nicotine, (Pons et al., 2008; Fowler et al., 2013; Faure et al., 2014; Sanjakdar et al., 2015). Finally, the $\alpha4\beta2$-selective nicotinic receptor antagonist DHβE antagonized the effects of nicotine but not the $\alpha4\beta2$ partial agonists varenicline and cytisine, in studies investigating their response-rate decreasing and discriminative-stimulus effects (Cunningham and McMahon, 2011; de Moura and McMahon, 2017).

The present studies were conducted to further explore the role of nAChR subtypes in the behavioral profile of nicotine by examining its effects and those of other nicotinic drugs in squirrel monkeys. First, we examined the rate-altering effects of nicotine and nicotine-related drugs on food-maintained operant performance, and in interaction studies we examined whether nicotine's effects on operant performance are altered by pretreatment with nAChR antagonists or partial agonists. Next, we employed drug discrimination methodology in a separate group of squirrel monkeys to determine whether agonists that varied in efficacy and selectivity at nAChR subtypes and non-nicotinic drugs [methamphetamine (MA; indirect monoaminergic agonist), citalopram (serotonin reuptake inhibitor), atropine (muscarinic antagonist), and arecoline (muscarinic agonist)] substituted for the nicotinic agonist (+)-epibatidine. We used (+)-epibatidine discrimination because previous research suggests that its behavioral actions, like those of nicotine, are mediated primarily through $\alpha4\beta2$ nAChR mechanisms, (e.g., Dukat and Glennon, 2003; Desai et al., 2016a). The nicotinic drugs studied included $\alpha4\beta2$ nAChR subtype–selective ligands previously characterized as either full agonists [nicotine, (−)-epibatidine, and (+)-epibatidine] or partial agonists (isoarecolone, varenicline, cytisine, and lobeline) (Anderson and Arneric, 1994; Badio and Daly, 1994; Hahn et al., 2003; Rollema et al., 2007). Substitution tests also were conducted with the $\alpha7$ nAChR subtype–selective agonist anabaseine (de Fiebre et al., 1995; Kem et al., 1997). Finally, we determined whether nAChR partial agonists (varenicline and cytisine) or antagonists varying in subtype selectivity attenuated (+)-epibatidine's discriminative-stimulus effects.

Materials and Methods

Subjects

Eight adult male squirrel monkeys (*Saimiri sciureus*; 750–950 g) were housed in stainless steel cages within a climate-controlled vivarium with a 12-hour light/dark cycle (lights on, 7:00 AM to 7:00 PM) in the McLean Hospital Animal Care Facility (licensed by the U.S. Department of Agriculture and compliant with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, Commission on Life Sciences, National Research Council, 2011). Except during experimental sessions, monkeys had unrestricted access to water and were fed a daily allotment of high-protein primate chow (Monkey Chow; Purina, St. Louis, MO) supplemented with fruit and multivitamins. Diets were adjusted as needed to maintain stable body weights. Behavioral experiments were conducted 5 days a week (Monday to Friday between 8:00 AM and 6:00 PM) under protocols approved by the Institutional Animal Care and Use Committee at McLean Hospital. All subjects had previously participated in behavioral studies involving drugs from differing pharmacological classes but had not participated in experiments for at least one month prior to this study.

Apparatus

During experimental sessions, subjects sat in customized Plexiglas chairs that were enclosed in ventilated, sound- and light-attenuating chambers. Subjects faced a panel with two sets of red stimulus lights and below each set, a response lever. Each lever press with a force >0.2 N produced an audible click, and was recorded as a response. In food-reinforced behavior studies, a Plexiglas receptacle for liquid delivery was positioned between the response levers. A syringe pump (Model PHM-100-10; Med Associates Inc., Georgia, VT) could be operated to deliver sweetened condensed milk via Tygon tubing into the Plexiglas receptacle (0.15 ml in 0.84 seconds). In drug discrimination studies, a shaved portion of the monkey's tail was secured under two brass electrodes by a small stock for the delivery of brief, low-intensity electrical stimulation (3 mA for 200 milliseconds). Electrode gel was applied to the shaved area of the tail before each session to ensure low-resistance electrical contact with the electrodes. These parameters are below values associated with painful stimuli in human subjects (Vierck et al., 2008). All experimental events and data collection were recorded and controlled using commercially available interfacing equipment and operating software (MED-PC, MedState Notation; Med Associates Inc., St. Albans, VT).

Food-Reinforced Behavior

All subjects were trained to respond under a 30-response fixed ratio (FR) schedule of food reinforcement (20% sweetened condensed milk in water). Under this schedule, the illumination of red stimulus lights on the front panel of the chair initiated the program. Completion of 30 consecutive responses on the active lever (left lever for two subjects and right lever for two subjects) turned off the stimulus lights and both triggered milk delivery and illuminated a white stimulus light above the active lever for 0.84 seconds. A 10-second timeout (TO) period during which all stimulus lights were off and responding had no scheduled consequences was initiated by each milk delivery. Thereafter, the red stimulus lights were reilluminated and the programmed schedule contingencies were again in effect. Responses on the inactive lever were recorded but had no programmed consequences. Daily sessions were comprised of four sequential components, each consisting of a 10-minute TO period followed by a 3-minute period during which the 30-response FR, 10-second TO schedule of food reinforcement was in effect.

Drug testing in each subject began after responding was stable, defined by <10% variability in overall response rates over 10 successive sessions. Subjects were tested twice weekly (Tuesdays and Fridays) and training sessions were conducted on the other days; no injections were given on training days. All schedule parameters and contingencies during test sessions were identical to those during training sessions. Cumulative dosing procedures were used to determine the effects of drugs; incremental doses of a drug were administered at the beginning of sequential 10-minute TO periods, permitting the evaluation of four cumulative doses during a single test session; if necessary a fifth component was initiated to allow testing of up to five cumulative doses in a single test session. The order of drug testing varied irregularly among individual subjects, and in each subject experiments with one drug were completed before the next drug was studied. For drug interaction studies, the effects of pretreatment with

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nicotinic antagonists and nicotinic partial agonists were studied by administering each pretreatment dose 10 minutes prior to the injection of the first test dose of a drug followed by incremental doses of the test drug as described previously.

(+)-Epibatidine Discrimination

The discrimination training procedure used to establish (+)-epibatidine discrimination has been described previously (Desai and Bergman, 2015; Desai et al., 2016a). Briefly, seated subjects were trained to turn off stimulus lights under a 10-response FR, 50-second TO schedule of stimulus termination. Under this schedule, the 10th lever press terminated visual stimuli associated with impending delivery of electric current to the tail (200 milliseconds; 3 mA). At the onset of the daily session, subjects received an intramuscular injection of (+)-epibatidine (0.001 mg/kg) or saline, initiating a 10-minute TO period during which stimulus lights were off. When stimulus lights were illuminated, current was delivered every 10 seconds (maximum of four deliveries) or until the 10-response FR response requirement was completed. Completion of the response requirement or the fourth current delivery initiated a 50-second TO. Each training session consisted of 10 presentations of the 10-response FR, 50-second TO schedule. The presession injection (drug or saline) sequence followed a double-alternation schedule (saline, saline, drug, drug, saline, saline, etc..). Testing began after subjects met criteria for stability, i.e., >90% of responses in the first FR and overall on the injection-associated lever during four of the last five training sessions. Thereafter, test sessions were conducted when subjects met the aforementioned criteria in two consecutive alternating [saline-drug or drug-saline] training sessions. Test sessions were identical to training sessions, with the following provisos: First, 10 successive responses on either lever turned off all stimulus lights; and second, the program of current delivery was switched off to prevent current-induced responding during testing. Test sessions occurred once or twice per week with training sessions on intervening days. Initially, tests were conducted to study the ability of single doses of nicotinic and non-nicotinic drugs to substitute for the training dose of 0.001 mg/kg (+)-epibatidine. Next, drug interaction studies were conducted to determine how pretreatment with nicotinic antagonists and nicotinic partial agonists modified (+)-epibatidine's discriminative-stimulus effects. In the latter studies, single doses of a test drug were given 10 minutes prior to the injection of single doses of (+)-epibatidine (0.0001–0.01 mg/kg), i.e., 20 minutes prior to the test session component.

Drugs

(−)-Nicotine hydrogen tartrate [(−)-1-methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt], MA hydrochloride, (−)-lobeline hydrochloride, atropine sulfate, arecoline hydrobromide, hexamethonium hydrobromide, and mecamylamine hydrochloride (3-methylaminoisocamphane hydrochloride) were obtained from Sigma-Aldrich (St. Louis, MO). Citalopram hydrobromide was obtained from Lundbeck (Valby, Denmark). (+)-Epibatidine [(2*R*)-2-(6-chloro-3-pyridinyl)-7-azabicyclo[2.2.1]heptane monohydrochloride], (−)-epibatidine [(2*R*)-2-(6-chloro-3-pyridinyl)-7-azabicyclo[2.2.1]heptane monohydrochloride], isoarecolone hydrochloride (1-methyl-4-acetyl-1,2,3,6-tetrahydropyridine hydrochloride), methyllycaconitine citrate (MLA) [1α,4(*S*),6β,14α,16β]-20-Ethyl-1,6,14,16-tetramethoxy-4-[[[2-(3-methyl-2,5-dioxo-1-pyrrolidinyl)benzoyl]oxy]methyl]aconitane-7,8-diol citrate, and anabaseine dihydrochloride (3,4,5,6-tetrahydro-2,3′-bipyridine dihydrochloride) were obtained from the National Institute on Drug Abuse (Bethesda, MD). (-)-Cytisine [(1*R*,5*S*)-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one] and DHβE hydrobromide [(2*S*,13b*S*)-2,3,5,6,8,9,10,13-octahydro-2-methoxy-1*H*,12*H*-benzo[i]-pyrano[3,4-g]indolizin-12-one hydrobromide] were obtained from Tocris Bioscience (Minneapolis, MN). Varenicline [6,7,8,9-tetrahydro-6,10-methano-6*H*-pyrazino[2,3-hour][3]benzazepine] was generously donated by Dr. Hans Rollema at Pfizer Global Research and Development (Rollema Biomedical Consulting, Mystic, CT). All drugs were dissolved in sterile 0.9% saline solution or water and were administered by intramuscular injection (volume ≤0.3 ml). Drugs tested under the schedule of food-maintained behavior were administered using cumulative dosing procedures, whereas drugs tested in (+)-epibatidine discrimination studies were administered as single doses. The pH of nicotine was adjusted as needed to pH 7.0 with sodium hydroxide (0.1 N). Doses of each drug are expressed in terms of the free base.

Data Analysis

Food-Reinforced Behavior. Response rates were calculated for each component by dividing the total number of responses on the active lever by the duration of the component excluding the TO periods. Response rates were not significantly altered by vehicle injection, and therefore baseline values for each subject were determined by averaging data from all noninjection (training sessions) and vehicle injection control sessions throughout the study. The effect of each dose of a drug was calculated for individual subjects as a percentage of the mean control response rate. Grouped data were analyzed using repeated-measures one-way analysis of variance followed by Dunnett's *t* test (Prism version 5.0; GraphPad, La Jolla, CA).

(+)-Epibatidine Discrimination. The percentage of (+)-epibatidine-associated responding was calculated by dividing the number of responses on the drug lever by the total number of responses on both levers. Response rates were calculated for each session by dividing the total number of responses by the duration of the session excluding TO periods. If the mean response rate in a test session was <0.2 responses per second, data from that session were excluded from further analysis. Mean results for vehicle and each dose of a drug were calculated by averaging data for the four subjects. Complete, partial, and no substitution for (+)-epibatidine was defined as ≥90%, 31%–89%, and ≤30% of total responses on the (+)-epibatidine-associated lever, respectively.

ED_{50} Calculations. The dose of a drug needed to produce 50% reduction in response rate (food-reinforced behavior) and engender 50% (+)-epibatidine lever responding (ED_{50}) was calculated for individual subjects by interpolation of the linear portion of the dose-response curve. The mean ED_{50} value (± S.E.M.) for each drug was determined by averaging ED_{50} values for individual subjects. In pretreatment studies, modification of nicotine's effects on operant response rate or (+)-epibatidine discrimination was analyzed by comparing the ED_{50} values for nicotine and (+)-epibatidine (respectively) alone and in the presence of each dose of the test drug.

Pearson's Product-Moment Correlation Coefficient Calculation. Correspondence between the effects of drugs on behavior and competition receptor binding studies was examined by comparing the relative potency of nicotinic drugs in the present experiments (i.e., ED_{50} values divided by the ED_{50} value for nicotine alone) and their published relative affinities for binding $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta4$ nicotinic receptors in vitro (K_i values divided by the K_i value for nicotine alone). Relative affinity values for each drug were obtained from previously published radioligand binding experiments in rat brain. Affinity values from multiple studies in rat brain were averaged, and whenever possible were taken from studies using [^{3}H]nicotine binding for $\alpha4\beta2$ receptor subtype, [^{125}I]-α-bungarotoxin binding for the $\alpha7$ nicotinic receptor subtype, and [^{3}H]epibatidine binding for the $\alpha3\beta4$ nicotinic receptor subtype. The Pearson's product-moment correlation coefficient of these values was calculated to determine the relationship between relative behavioral potency and relative binding affinity at each nAChR subtype.

Results

Food-Reinforced Behavior

Control Performance. Rates of food-reinforced responding were stable over the course of the present experiments, with average response rates for individual subjects ranging from 4.16 to 6.0 responses per second (Table 1).

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TABLE 1
Mean rates of responding (responses per second) under the 30-response FR schedule of food-reinforced behavior for individual monkeys during noninjection and vehicle control sessions (n)

Monkey	n	Average Responses per Second ± S.E.M.
Ss 9	50	5.23 ± 0.12
Ss 80	34	5.61 ± 0.82
Ss 81	50	6.0 ± 0.15
Ss 106	49	4.16 ± 0.08

Effect of Nicotinic Agonists and Antagonists Alone. The nicotinic agonists nicotine (0.032–0.32 mg/kg) and (+)-epibatidine (0.000032-0.001 mg/kg) and the nicotinic partial agonists varenicline (0.0032–0.1 mg/kg) and cytisine (0.01–1.0 mg/kg) all produced dose-dependent and, at high doses, significant decreases in response rates under the FR schedule of food reinforcement ($P < 0.05$; Fig. 1). The rank order of potency based on the ED_{50} values was (+)-epibatidine > varenicline > nicotine ≈ cytisine (Table 2). In contrast, the nicotinic antagonists mecamylamine (0.032–1.0 mg/kg) and DHβE (0.032–0.56 mg/kg) did not significantly alter rates of responding (Fig. 1). The effects of higher doses of mecamylamine and DHβE alone were not determined to preclude potential adverse side effects.

Effects of Pretreatment with Nicotinic Antagonists. Mecamylamine (0.1–1.0 mg/kg) antagonized the effects of cumulatively administered nicotine on response rate, as indicated by dose-dependent rightward shifts of the nicotine dose-effect function and corresponding significant changes in ED_{50} values (Fig. 2A, top left panel; Table 3). To assess the specificity with which mecamylamine antagonized nicotine's effects on response rate, we examined its ability to modify the rate-decreasing effects of the indirect monoaminergic agonist MA. Like nicotine, MA alone produced dose-dependent and significant decreases in the rate of responding for food reinforcement (Fig. 2A, top right panel). Overall, these effects of MA were not attenuated by any pretreatment dose of mecamylamine; in fact, the combination of 1.0 mg/kg mecamylamine and the intermediate dose of 0.1 mg/kg MA doses that were alone without effect significantly decreased food-maintained responding, resulting in a corresponding decrease in ED_{50} value compared with MA alone (Fig. 2A, top right panel; Table 3). In contrast to mecamylamine, administration of DHβE (0.1–0.56 mg/kg) did not substantively modify nicotine's effects on response rate (Fig. 2A, bottom left panel), and the ED_{50} values for nicotine's effects on rates of responding after pretreatment with DHβE were not significantly different from the ED_{50} value of nicotine alone (Table 3). In additional studies, the highest dose of DHβE (0.56 mg/kg) also failed to significantly alter the rate-decreasing effect of (+)-epibatidine, reflected both in overlapping dose-effect curves and comparable ED_{50} values (Fig. 2A, bottom right panel; Table 3).

Effects of Pretreatment with Varenicline and Cytisine. Doses of varenicline and cytisine that did not decrease response rates alone (Fig. 1) did serve to alter the effects of nicotine. Thus, pretreatment with 0.032 mg/kg of varenicline produced a leftward shift of the nicotine dose-effect curve,

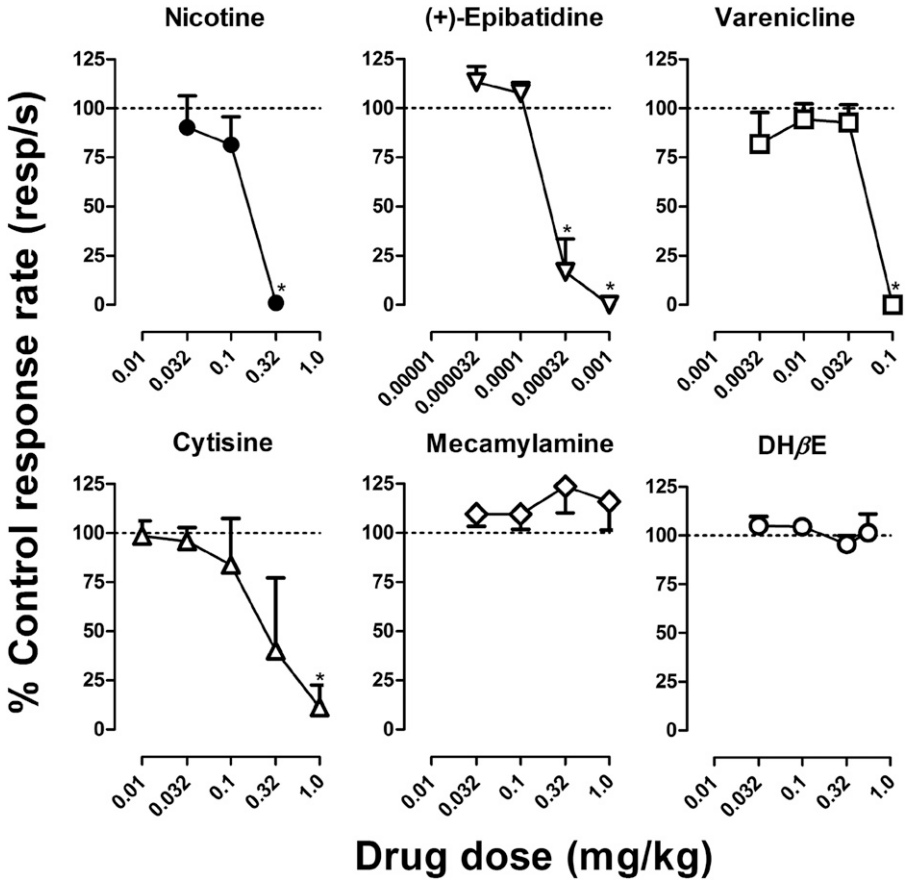

Fig. 1. Effects of nicotinic agonists [(+)-epibatidine: (+)-Epi; nicotine: Nic; varenicline: Var; and cytisine: Cyt] and antagonists (mecamylamine: Mec; and DHβE) on schedule-controlled behavior in squirrel monkeys. Each data point represents the average effect determined in four monkeys. Dashed line represents effects of vehicle; * indicates significant difference from vehicle levels; Dunnett's t test, $P < 0.05$, on mean values derived from the main effect of dose. [(+)-Epi, Nic, Var, Cyt: $Fs_{3\text{-}18} \geq 3.82$; ps < 0.05]; Mec, DH$\beta$E: ($Fs_{4\text{-}15} \leq 0.80$; ps > 0.05). Abscissa: cumulative drug dose (milligrams per kilogram). Ordinates: response rates expressed as a percentage of the average rate obtained during vehicle and noninjection test sessions.

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TABLE 2

ED_{50} values (±S.E.M.) and relative potencies with which nicotinic agonists produce increases in (+)-epibatidine-associated lever responding and decreases in food-reinforced behavior in squirrel monkeys (nicotine = 1) and their relative affinity at $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta4$ nAChR (nicotine = 1)

The in vitro values for inhibiting [^{3}H]nicotine binding at $\alpha4\beta2$ nAChR, [^{125}I]a-bungarotoxin binding at $\alpha7$ nAChR, and [^{3}H]epibatidine binding at $\alpha3\beta4$ are the average and range of values taken from the references cited. Data for nornicotine, anabasine, myosmine, and anatabine are from a previous study in squirrel monkeys trained under similar experimental conditions (Desai et al., 2016a) and data for (−)-epibatidine's effects on schedule-controlled behavior are from unpublished experiments. Data for these drugs are included in this study to permit determination of any relationship between relative potencies of nicotinic drugs in both drug discrimination and food-reinforced responding, and their relative affinities at $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta4$ nAChRs in radioligand binding studies. The $ symbol indicates that value is for (±)-epibatidine; # indicates binding data from rat forebrain and K_i determinations were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract No. HHSN-271-2013-00017-C, which is directed by Bryan L. Roth M.D., Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at the National Institute of Mental Health, Bethesda, MD. The protocol was adapted from Xiao et al. (1998).

Drug	Drug Discrimination		Schedule-Controlled Behavior		$\alpha4\beta2$		$\alpha7$		$\alpha3\beta4$	
	ED_{50} ± S.E.M.	Relative Behavioral Potency	ED_{50} ± S.E.M.	Relative Behavioral Potency	In Vitro Affinity	Relative Affinity	In Vitro Affinity	Relative Affinity	In Vitro Affinity	Relative Affinity
	mg/kg		*mg/kg*		*nM*		*nM*		*nM*	
Nicotine	0.12 ± 0.02	1	0.15 ± 0.018	1	3.6 (0.6–10[a, b, c, d, e, f, g])	1	4895[c]	1	183[c]	1
(+)-Epibatidine	0.00040 ± 0.000053	0.0033	0.00022 ± 0.000044	0.0015	0.05 (0.045–0.055[a,b])	0.01	255[h]	0.05	0.15[c],[$]	0.0008
(−)-Epibatidine	0.00042 ± 0.000094	0.0035	0.00054 ± 0.000047	0.0036	0.57 (0.055–0.058[a,b])	0.02	109[h]	0.02	0.30[i]	0.0002
Cytisine	1.06 ± 0.23	8.83	0.18 ± 0.21	1.2	0.99[j]	0.28	4515 (260–15,000 [d,k,l,m])	0.92	56[j]	0.31
Varenicline	N.D.	N.D	0.053 ± 0.0030	0.35	0.29 (0.17–0.4[n,o])	0.08	620[n]	0.13	460[p]	2.51
Isoarecolone	1.63 ± 0.79	13.58	N.D.	N.D.	611[c]	169.72	>1000,000[c]	20.43	10,384[c]	56.74
Lobeline	N.D.	N.D	N.D.	N.D.	7.3 (1.5–16[e,q,r])	2.03	12,350 (11,600–13,100[m,r])	2.52	480[j]	2.62
Anabaseine	0.48 ± 0.53	4.0	N.D.	N.D.	32[s]	8.88	58[t]	0.01	107[i]	0.58
Nornicotine	1.19 ± 0.29	9.92	1.58 ± 0.086	10.53	21.05 (13.1–29[e,u])	5.85	730[u]	0.15	N.A.	—
Anabasine	0.58 ± 0.15	4.83	0.59 ± 0.13	3.93	168 (76–260[j,t])	46.67	58[t]	0.01	2100[j]	11.48
Myosmine	29.29 ± 3.77	244.08	30.40 ± 11.25	202.67	3300[t]	916.67	N.A.	—	N.A.	—
Anatabine	2.09 ± 0.23	17.42	1.02 ± 0.34	6.8	279.9[#]	77.75	N.A.	—	2874[#]	15.70

N.A., not available; N.D., not determined.
[a]Badio and Daly (1994).
[b]Damaj et al. (1994).
[c]Hahn et al. (2003).
[d]Jensen et al. (2003).
[e]Anderson and Arneric (1994).
[f]Xiao and Kellar (2004).
[g]Decker et al. (1995).
[h]Sullivan et al. (1994).
[i]Matera et al. (2016).
[j]Parker et al. (1998).
[k]Mukhin et al. (2000).
[l]Marks et al. (1986).
[m]de Fiebre et al. (1995).
[n]Rollema et al. (2007).
[o]Rollema et al. (2010).
[p]Yenugonda et al. (2013).
[q]Damaj et al. (1997).
[r]Miller et al. (2003)
[s]Kem et al. (1997).
[t]Ferretti et al. (2003).
[u]Schmitt et al. (1999).

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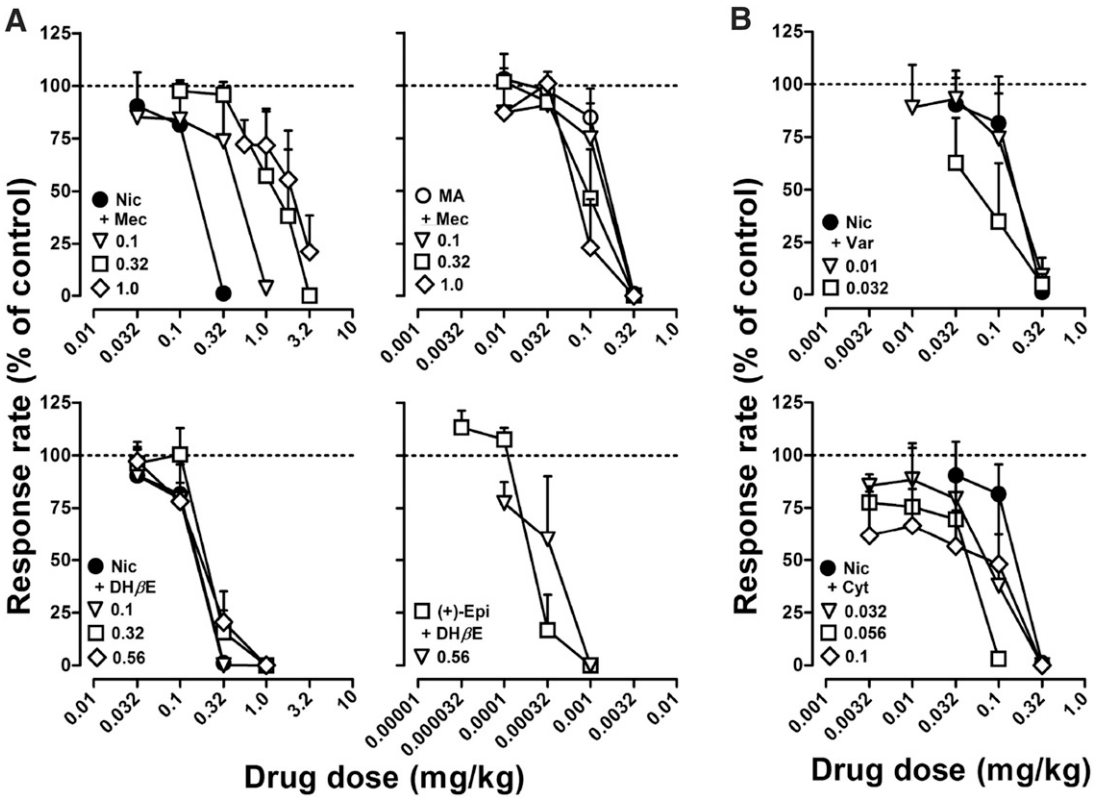

Fig. 2. (A) Dose-effect functions of nicotine alone or after pretreatment with the nicotinic antagonists mecamylamine [(Mec) top left panel] and DHβE (bottom left panel), and dose-effect functions of MA alone or after pretreatment with the nicotinic antagonist mecamylamine (top right panel) and (+)-epibatidine [(+)-Epi] alone or after pretreatment with DHβE (bottom right panel) on schedule-controlled behavior in squirrel monkeys. (B) Dose-effect functions of nicotine alone or after pretreatment with varenicline [(Var) top panel] and cytisine [(Cyt) bottom panel]. Results are presented as mean percent control (± S.E.M.). Abscissa: cumulative drug dose (milligrams per kilogram). Ordinates: response rates expressed as a percentage of the average rate obtained during vehicle and noninjection test sessions.

resulting in an approximately 2-fold decrease in nicotine's ED_{50} value (Fig. 2B, top panel; Table 3). Similarly, cytisine (0.032–0.1 mg/kg), enhanced the rate-decreasing effects of nicotine. This was evident in a leftward shift in the nicotine dose-effect curve following 0.032 and 0.056 mg/kg cytisine and a downward shift in the nicotine dose-effect curve following 0.1 mg/kg cytisine (Fig. 2B, bottom panel), resulting from the effects of 0.1 mg/kg cytisine alone (Fig. 1). Correspondingly, pretreatment with 0.032–0.1 mg/kg cytisine resulted in a 3- to 6-fold decrease in the ED_{50} value for the rate-decreasing effects of nicotine (Table 3).

(+)-Epibatidine Discrimination

Consistent with our previous observations (Desai and Bergman, 2015; Desai et al., 2016a), reliable discriminative-stimulus control by 0.001 mg/kg (+)-epibatidine was maintained in all four subjects throughout the present study (Fig. 3, top panel). No significant changes in response rates were observed following administration of 0.001 mg/kg (+)-epibatidine compared with vehicle values (Fig. 3, bottom panel). There was no significant change in (+)-epibatidine's (0.0001–0.001 mg/kg) discriminative-stimulus effects or response rates over the length of the study (>24 months). The ED_{50} values (± S.E.M.) for (+)-epibatidine's discriminative-stimulus effects were 0.00031 ± 0.00011 versus 0.00055 ± 0.000024 at the beginning and end of the study, respectively. Thus, discrimination and response rate data for (+)-epibatidine at the beginning and end of these experiments were averaged to provide control values for all analyses and graphic presentations.

Effects of Nicotinic Agonists. The (+)- and (−)-enantiomers of epibatidine (0.0001–0.001 mg/kg) produced dose-dependent increases in responding on the (+)-epibatidine-associated lever, with full substitution occurring at the 0.001 mg/kg dose of (+)- and (−)-epibatidine in all subjects (Fig. 3, top panel). Nicotine (0.01–0.32 mg/kg) also produced dose-dependent substitution for 0.001 mg/kg (+)-epibatidine, with full substitution following intramuscular administration of 0.32 mg/kg dose of nicotine (Fig. 3, top panel). In contrast, the nicotinic partial agonists varenicline (0.01–0.18 mg/kg), cytisine (0.0032–3.2 mg/kg), anabaseine (0.032–1.0 mg/kg), and isoarecolone (0.32–3.2 mg/kg) produced partial substitution (Fig. 3, top panel). Maximum responding on the (+)-epibatidine lever was observed following administration of 0.032 mg/kg varenicline (46% ± 26%), 1.8 mg/kg cytisine (60% ± 23%), 3.2 mg/kg isoarecolone (51% ± 21%), and 1.0 mg/kg anabaseine (72% ± 16%). These data typically reflect effects that somewhat varied among individual monkeys, i.e., two subjects produced greater than 80% responding on the (+)-epibatidine lever, whereas the remaining two monkeys did not generalize for (+)-epibatidine discrimination. The nAChR partial agonist, lobeline, did not generalize for (+)-epibatidine (8% ± 5%). The effects of higher doses of each of these nicotinic partial agonists were not

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TABLE 3

Doses of nicotine alone and after pretreatment with mecamylamine, DHβE, varenicline, and cytisine which were required to produce 50% decreases in food-reinforced behavior. Doses of (+)-epibatidine and MA alone after treatment with DHβE and mecamylamine which were required to produce 50% decreases in food-reinforced behavior, respectively

Treatment	ED_{50} value ± S.E.M. mg/kg
Nicotine Alone	0.15 ± 0.018
+ 0.1 mg/kg Mecamylamine	0.40 ± 0.11*
+ 0.32 mg/kg Mecamylamine	0.94 ± 0.26*
+ 1.0 mg/kg Mecamylamine	1.67 ± 1.20*
+ 0.1 mg/kg DHβE	0.11 ± 0.033
+ 0.32 mg/kg DHβE	0.19 ± 0.054
+ 0.56 mg/kg DHβE	0.17 ± 0.0067
+ 0.01 mg/kg Varenicline	0.13 ± 0.046
+ 0.032 mg/kg Varenicline	0.093 ± 0.035*
+ 0.032 mg/kg Cytisine	0.048 ± 0.037*
+ 0.056 mg/kg Cytisine	0.027 ± 0.0047*
+ 0.1 mg/kg Cytisine	0.057 ± 0.058*
(+)-Epibatidine Alone	0.00022 ± 0.000044
+ 0.56 mg/kg DHβE	0.00032 ± 0.00014
MA Alone	0.14 ± 0.032
+ 0.1 mg/kg Mecamylamine	0.12 ± 0.025
+ 0.32 mg/kg Mecamylamine	0.086 ± 0.022
+ 1.0 mg/kg Mecamylamine	0.072 ± 0.022*

determined because in the present or previous studies 0.25 or 0.5 log-unit higher doses either markedly disrupted responding or produced untoward effects in squirrel monkeys (Desai and Bergman, 2014; Desai et al., 2016a). Based on the mean ED_{50} values, the rank order of potency of drugs that produced epibatidine-like effects was (+)-epibatidine ≈ (−)-epibatidine > nicotine > cytisine > isoarecolone > anabaseine (Table 1). Response rates were not significantly altered by any dose of (+)-epibatidine, (−)-epibatidine, varenicline, cytisine, anabaseine, isoarecolone, or lobeline, although nicotine did produce a significant increase in rates of responding at 0.1 mg/kg (Fig. 3, bottom panel).

Effects of Non-Nicotinic Drugs. At the doses examined, MA (0.032–0.56 mg/kg; monoaminergic psychomotor stimulant), atropine (0.01–0.1 mg/kg; muscarinic antagonist), arecoline (0.01–0.1 mg/kg; partial muscarinic agonist), and citalopram (0.32–10 mg/kg; serotonin-selective reuptake inhibitor) did not substitute for (+)-epibatidine (Fig. 3, top panel). Except for a small decrease in responding produced by the highest doses of MA (0.56 mg/kg) and arecoline (0.1 mg/kg), none of the drugs produced significant changes in rates of responding (Fig. 3, bottom panel).

Effects of Pretreatment with Nicotinic Antagonists. Administration of nAChR antagonists prior to saline produced limited responding on the (+)-epibatidine-associated lever and did not significantly alter response rates compared with control values (Fig. 4). In drug interaction studies, pretreatment with the nonselective nicotinic antagonist mecamylamine (0.032–0.32 mg/kg) dose dependently antagonized the discriminative-effects of (+)-epibatidine (Fig. 4, top panel; Table 4). Thus, a 3-fold higher dose of (+)-epibatidine was required to produce full substitution after pretreatment with 0.1 mg/kg mecamylamine, whereas doses of (+)-epibatidine up to 0.0056 mg/kg failed to produce any (+)-epibatidine-like effects after pretreatment with 0.32 mg/kg mecamylamine (Fig. 4, top panel; Table 4). In contrast, the nAChR subtype–selective antagonists were relatively ineffective in attenuating (+)-epibatidine's discriminative-stimulus effects. The two highest doses of the α4β2-selective nicotinic antagonist DHβE (0.32 and 0.56 mg/kg) produced only an approximately 2-fold rightward shift in the (+)-epibatidine dose-response curve and a corresponding decrease in potency, whereas pretreatment with a 10-fold range of doses of the α7-selective nicotinic antagonist MLA (0.01–0.1 mg/kg) was without effect (Fig. 4, top panel; Table 4). Finally, pretreatment with the highest dose of hexamethonium (5.6 mg/kg), a nonselective peripherally acting nicotinic antagonist, produced a slight rightward shift in the (+)-epibatidine-dose response curve resulting in a small but significant increase in the ED_{50} value of (+)-epibatidine (Fig. 4, top panel; Table 4). There were no decreases in response rates following (+)-epibatidine in combination with any of the antagonists tested (Fig. 4, bottom panel).

Effects of Pretreatment with Varenicline and Cytisine. The effects of pretreatment with nicotinic partial agonist varenicline (0.001–0.1 mg/kg) or cytisine (0.032–1.0 mg/kg) on (+)-epibatidine discrimination are shown in Fig. 5. Low-to-intermediate doses of varenicline (0.001–0.032 mg/kg) had no significant effect on (+)-epibatidine discrimination (Fig. 5, top left panel; Table 4). Administration of the highest dose of 0.1 mg/kg varenicline produced an apparent leftward and upward shift in the (+)-epibatidine dose-response function (Fig. 5, top left panel). Thus, pretreatment with this dose of varenicline prior to injections of the lowest two doses of (+)-epibatidine (0.00001 or 0.000032 mg/kg) resulted in intermediate-to-high levels of responding on the (+)-epibatidine-associated lever, precluding determination of potency values.

Cytisine produced dose-dependent leftward shifts in (+)-epibatidine's dose-response curves (Fig. 5, top right panel; Table 4). Thus, the lowest pretreatment dose of 0.032 mg/kg cytisine did not modify (+)-epibatidine's discriminative-stimulus effects, whereas pretreatment with higher doses of cytisine (0.1 or 1.0 mg/kg) produced a leftward shift in (+)-epibatidine's dose-response curve and a corresponding decrease in ED_{50} values (Fig. 5, top right panel; Table 4). As with varenicline, the highest pretreatment dose of cytisine (1.0 mg/kg) prior to injections of all doses of (+)-epibatidine produced >50% responding on the (+)-epibatidine-associated lever, precluding determination of ED_{50} values (Fig. 5; Table 4). There were no decreases in response rates following (+)-epibatidine in combination with varenicline or cytisine (Fig. 5, bottom panel).

Discussion

The present studies were conducted to elucidate the role of nAChR subtypes in the behavioral effects of nicotinic ligands in squirrel monkeys. Results show that nicotine and the enantiomers of epibatidine engendered dose-dependent and full substitution for (+)-epibatidine's stimulus effects and produced dose-dependent decreases in rates of food-maintained behavior. Unlike the well-known stereoselective actions of nicotine (e.g., Goldberg et al., 1989), the two enantiomers of epibatidine produced the same behavioral effects with equal potency. These results agree with previous findings (Damaj et al., 1994; Smith and Stolerman, 2009; Desai and Bergman, 2014) and are consistent with the nonstereoselective binding

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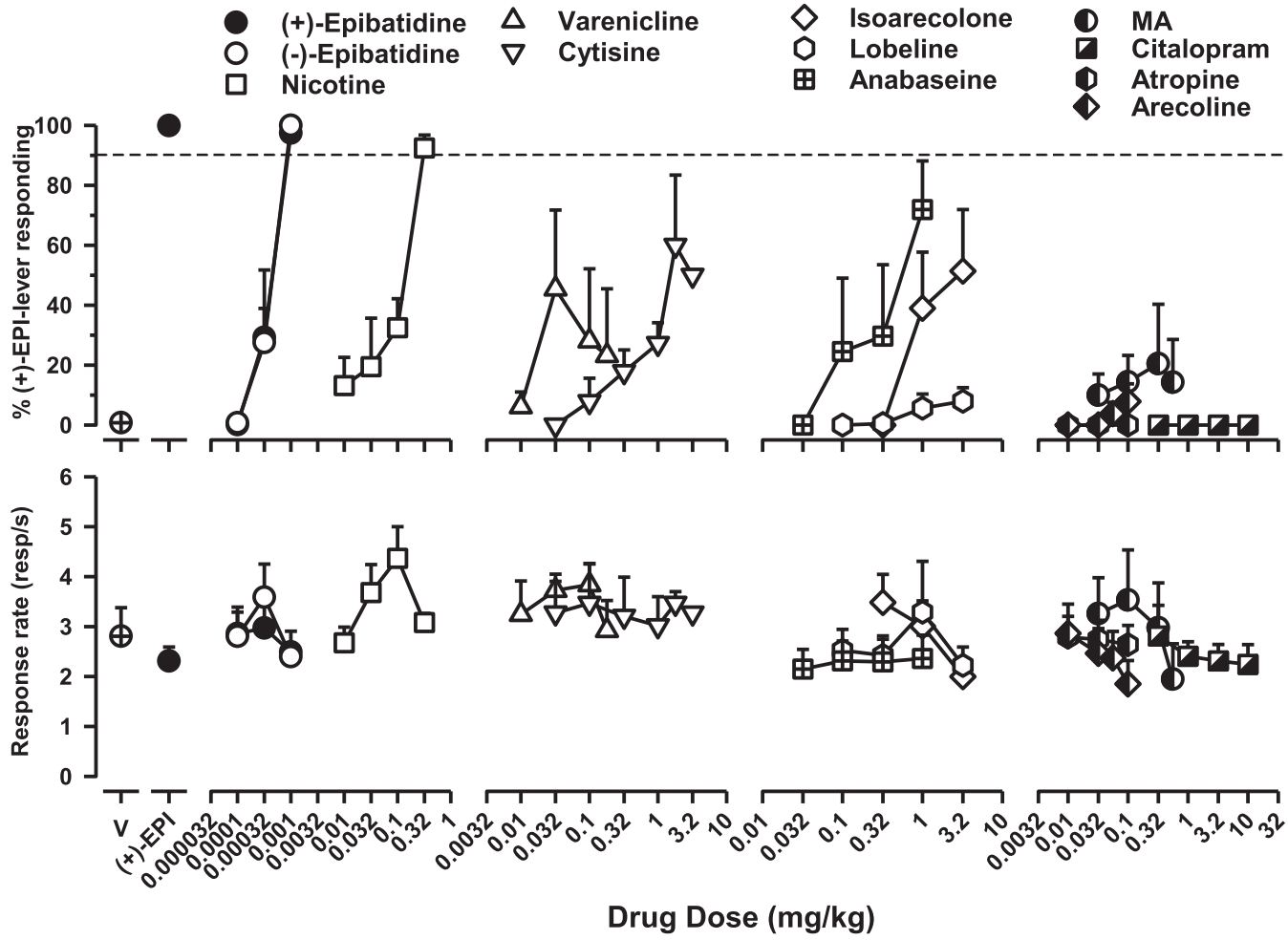

Fig. 3. Effects of the nicotinic ligands [(+)-epibatidine, (−)-epibatidine, nicotine, varenicline, cytisine, isoarecolone, lobeline, and anabaseine] and non-nicotinic drugs [MA, citalopram, atropine, and arecoline] on percent (+)-epibatidine-lever responding (top panel) and response rates (bottom panel) in squirrel monkeys trained to discriminate 0.001 mg/kg (+)-epibatidine from saline. Each data point represents the average (± S.E.M.) of effects in four subjects tested at each dose. Abscissa: drug dose (milligrams per kilogram). Ordinates: top panel: percentage (+)-epibatidine-lever responding; bottom panel: response rate.

profile displayed by the two optical isomers of epibatidine at $\alpha4\beta2$ nAChR subtypes (e.g., Dukat and Glennon, 2003). The nAChR ligands varenicline, cytisine, isoarecolone, and anabaseine also engendered dose-related (+)-epibatidine-like discriminative-stimulus effects but the maximum effect was less than that of nicotine or (+)-epibatidine. These results along with the dose-dependent decreases in food-maintained responding produced by varenicline and cytisine are consistent with those from several studies in which, depending on the experimental conditions, one or more of these drugs either partially or fully reproduced the behavioral effects of nicotine (e.g., LeSage et al., 2009; Smith and Stolerman, 2009; Jutkiewicz et al., 2011; Cunningham et al., 2012; Desai and Bergman, 2015; Desai et al., 2016a; de Moura and McMahon, 2017). The effects of these compounds are consistent with their in vitro classification as nicotinic partial agonists at the $\alpha4\beta2$ nAChR subtype (Kem et al., 1997; Hahn et al., 2003; Shoaib, 2006; Rollema et al., 2010). However, varenicline, cytisine, and anabaseine also have activity at other nAChR subtypes, which may have interfered with the ability of higher doses to fully express $\alpha4\beta2$-mediated agonist-like behavioral effects.

All nAChR agonists and partial agonists tested in both behavioral studies decreased food-reinforced responding and produced (+)-epibatidine-like stimulus effects over a similar range of doses, and with the exception of cytisine at similar ED_{50} values (Table 2). On the other hand, nicotinic full or partial agonists that produced significant decreases in rates of responding under the schedule of food reinforcement had little or no effect on rates of responding under the stimulus-termination schedule. This agrees with previous studies showing that the potency of nicotine (Spealman et al., 1981) and other drug classes—i.e., benzodiazepines (Barrett, 1976) and opioids (Bergman and Warren, 1989)—to decrease responding may differ when behaviors are maintained by dissimilar consequences. For example, although nicotine demonstrated similar behavioral effects under schedules of both food presentation and stimulus-shock termination, the potency of nicotine to decrease rates of responding was higher under schedules of food presentation than under schedules of stimulus-shock termination (Spealman et al., 1981).

We found no overlap in the discriminative-stimulus effects of (+)-epibatidine and the partial nAChR agonist lobeline,

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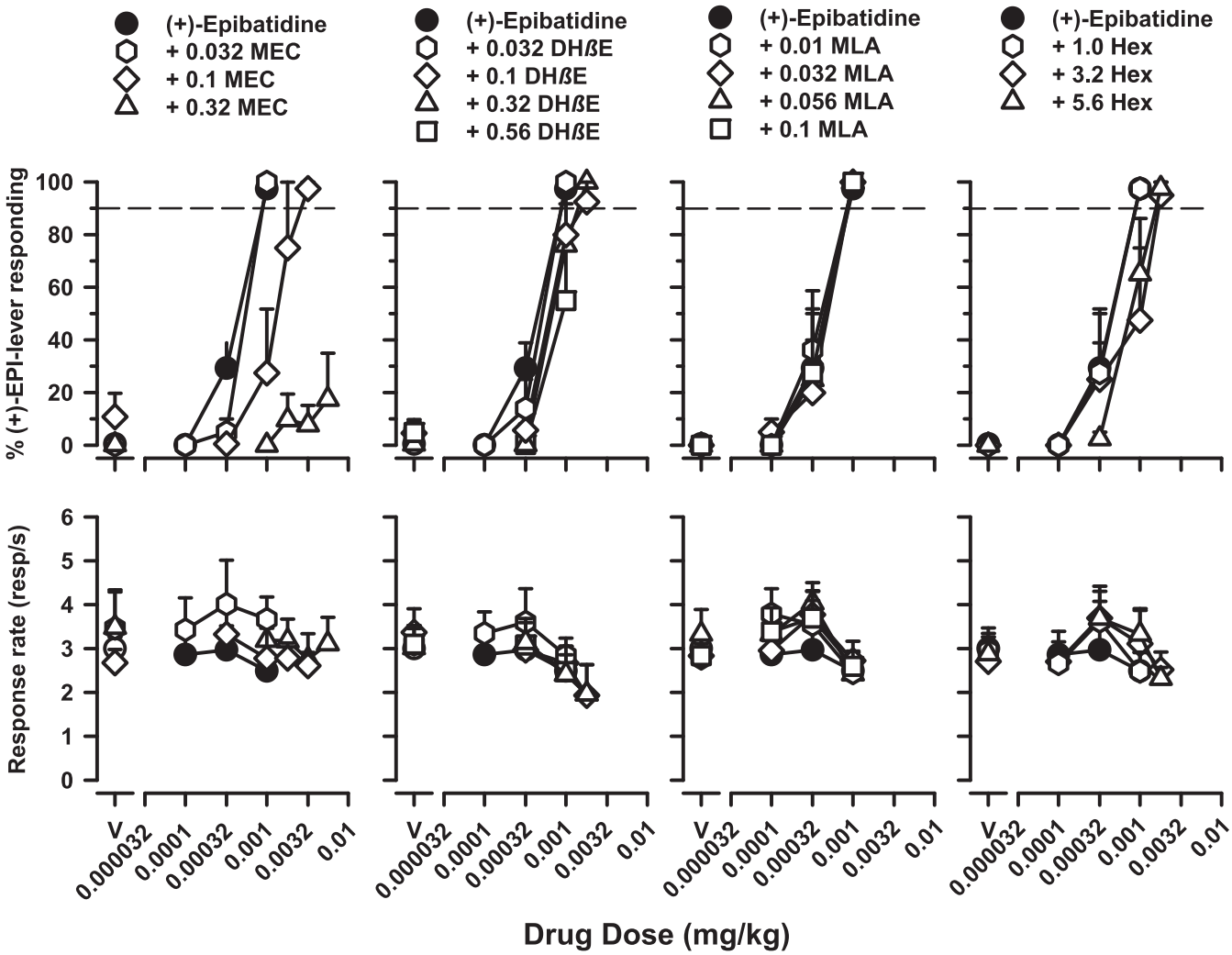

Fig. 4. Dose-effect functions of (+)-epibatidine in squirrel monkeys trained to discriminate 0.001 mg/kg (+)-epibatidine from saline either alone (filled circles) or after several pretreatment doses (10 minutes) of the nicotinic antagonists mecamylamine (MEC), DHβE, MLA, and hexamethonium (HEX). Effects on percent (+)-epibatidine-lever responding (top panels) and effects on response rates (bottom panel) are shown. Abscissa: drug dose (milligrams per kilogram). Ordinates: top panel: percentage (+)-epibatidine-lever responding; bottom panel: response rate.

consistent with the absence of nicotine-like discriminative effects in prior studies. Although the reasons for these findings are unclear, non-nicotinic pharmacological actions of lobeline (e.g., monoaminergic activity) might obscure the expression of its nicotinic activity (e.g., Stolerman et al., 1995; Dwoskin and Crooks, 2002; Harrod et al., 2003; Desai and Bergman, 2014). Similarly, our data with arecoline, atropine, and citalopram agree with previous results showing that muscarinic and serotoninergic mechanisms do not contribute appreciably to the behavioral effects of nicotinic agonists (Smith and Stolerman, 2009). With regard to MA, although the monoaminergic stimulant did not engender (+)-epibatidine-like discriminative-stimulus effects in the present experiments, we previously found that both (+)-epibatidine and nicotine fully substitute for MA in MA-trained subjects (Desai and Bergman, 2010; 2014). Although latter findings suggest that the discriminative-stimulus effects of MA and nicotinic drugs share common features, the asymmetric pattern of cross-generalization also suggests that there are important differences between the stimulus complexes of these drugs. The reason for this asymmetric generalization between nicotinic drugs and MA remains unclear in the absence of additional data, but may be related to MA providing a weak cholinergic stimulus or in terms of nicotinic drugs acting indirectly as weak dopamine agonists.

As expected, the nonselective nAChR antagonist mecamylamine dose dependently blocked nicotine's (but not MA's) rate-decreasing effects on food-maintained behavior and (+)-epibatidine's discriminative-stimulus effects (e.g., Le Foll and Goldberg, 2009; Desai and Bergman, 2014). The effects of (+)-epibatidine also were moderately attenuated by the peripherally restricted nAChR antagonist hexamethonium, indicating that some peripheral nicotinic actions of epibatidine contributed to its discriminative-stimulus effects in the present studies. These findings contrast with previous studies (in both rodents and squirrel monkeys), in which hexamethonium did not alter the discriminative-stimulus effects of nicotine (Spealman et al., 1981; Stolerman et al., 1984). Although the particular aspect of epibatidine's peripheral actions that contributed to the present results are unknown, it is likely that the dissimilar results with hexamethonium across studies can be attributed to differences in the relative training doses of nicotine and epibatidine rather than

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TABLE 4

Doses of (+)-epibatidine alone and after pretreatment with mecamylamine, DHβE, MLA, hexamethonium, varenicline and cytisine that were required to produce 50% increase in (+)-epibatidine lever responding.

Shown are ED_{50} values ± S.E.M. (milligrams per kilogram); * represents significant effect of pretreatment drug vs. drug alone as determined by non-overlapping S.E.M. values. Values not determined were due to less than 50% (+)-epibatidine lever responding.

Treatment	ED_{50} value ± S.E.M.
mg/kg	*mg/kg*
(+)-Epibatidine alone	0.00040 ± 0.000053
+ 0.032 mg/kg Mecamylamine	0.00052 ± 0.000044*
+ 0.1 mg/kg Mecamylamine	0.00093 ± 0.00015*
+ 0.32 mg/kg Mecamylamine	N.D.
+ 0.032 mg/kg DHβE	0.00049 ± 0.000058
+ 0.1 mg/kg DHβE	0.00069 ± 0.00022*
+ 0.32 mg/kg DHβE	0.00068 ± 0.000081*
+ 0.56 mg/kg DHβE	N.D.
+ 0.01 mg/kg MLA	0.00037 ± 0.000088
+ 0.032 mg/kg MLA	0.00044 ± 0.000090
+ 0.056 mg/kg MLA	0.00042 ± 0.00010
+ 0.1 mg/kg MLA	0.00042 ± 0.000094
+ 1.0 mg/kg Hexamethonium	0.00042 ± 0.00010
+ 3.2 mg/kg Hexamethonium	0.00068 ± 0.00030
+ 5.6 mg/kg Hexamethonium	0.00077 ± 0.00017*
+ 0.001 mg/kg Varenicline	0.00058 ± 0.000012*
+ 0.0032 mg/kg Varenicline	0.00032 ± 0.00011
+ 0.01 mg/kg Varenicline	0.00027 ± 0.00013
+ 0.032 mg/kg Varenicline	0.00021 ± 0.000032
+ 0.1 mg/kg Varenicline	N.D.
+ 0.032 mg/kg Cytisine	0.00049 ± 0.000045
+ 0.1 mg/kg Cytisine	0.000095 ± 0.000043*
+ 1.0 mg/kg Cytisine	N.D.

MLA, methyllycaconitine; N.D., not determined.

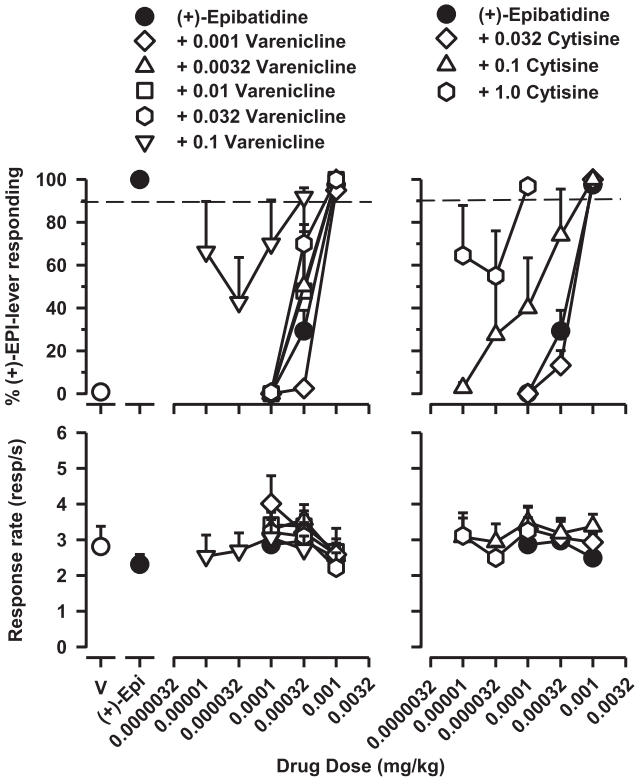

Fig. 5. Dose-effect functions of (+)-epibatidine alone or after pretreatment doses (10 minutes) of the partial nicotinic agonists varenicline (left panels) and cytisine (right panels). Effects on percent (+)-epibatidine-lever responding (top panels) and effects on response rates (bottom panels) are shown. Abscissa: drug dose (milligrams per kilogram). Ordinates: top panel: percentage (+)-epibatidine-lever responding; bottom panel: response rate.

receptor-mediated differences in their peripherally mediated actions on skeletal muscle or in the autonomic nervous system.

If the α4β2 nAChR subtype was exclusively involved in the behavioral effects of nicotinic ligands, pretreatment with a selective antagonist at this receptor would be expected to produce full blockade of (+)-epibatidine and/or nicotine's behavioral effects. However, in the present study the α4β2-selective nicotinic antagonist DHβE only moderately attenuated (+)-epibatidine's discriminative-stimulus effects and failed to antagonize the rate-decreasing effects of nicotine or (+)-epibatidine on food-reinforced behavior. These results are consistent with previous findings that the discriminative-stimulus effects of nicotine but not its rate-decreasing effects are antagonized by DHβE in nicotine-trained or MA-trained subjects (Stolerman et al., 1997; Gommans et al., 2000; Jutkiewicz et al., 2011; Desai and Bergman, 2014; also, see de Moura and McMahon, 2016). The limited and inconsistent antagonism by DHβE, in contrast to the robust and highly replicable antagonism produced by mecamylamine, strongly suggests that (+)-epibatidine, although characterized as an α4β2-selective agonist, produces its behavioral effects through other, or additional, subtypes of nAChR, i.e., heteromeric neuronal nAChR subunits containing β2 subunits or the 10-fold less-selective α3β4 nAChR subtype (Avalos et al., 2002; Wei et al., 2003). With regard to the α7 nAChR subtype, the inability of MLA to significantly alter the discriminative-stimulus effects of (+)-epibatidine adds to previous evidence indicating that the α7 nAChR subtype is not likely to play a major role in the stimulus effects of nicotinic agonists (Brioni et al., 1996).

Previous studies have suggested that varenicline and cytisine produce some nicotine-like stimulus effects but can attenuate nicotine's discriminative-stimulus effects in nicotine-trained and MA-trained subjects, presumably reflecting their partial agonist actions at the α4β2 nAChR subtype (e.g., LeSage et al., 2009; Jutkiewicz et al., 2011; Desai and Bergman, 2010, 2014). In the present studies, varenicline and cytisine, which are considered partial agonists, produced partial generalization for (+)-epibatidine and enhanced its stimulus effects and the rate-suppressant effects of nicotine. However, higher doses of these drugs did not antagonize the discriminative-stimulus effects of (+)-epibatidine or the response-rate decreasing effects of nicotine, as might be expected for partial agonists. In the absence of additional data, the reasons for the failure of these two drugs to display classic partial agonist activity when combined with full-nicotinic agonists remain unclear. Possibly, varenicline and cytisine do not have high enough affinity for nicotinic receptor subtypes to displace (+)-epibatidine or nicotine at the doses studied. Alternatively, these drugs also may be active at other nAChR subtypes (i.e., α3β4) that contributed to the behavioral effects of (+)-epibatidine and nicotine. Along these lines, the binding affinity profile of varenicline and cytisine at nAChR subtypes appears to differ from nicotine's affinity profile (see Table 2). For example, the relative affinity values for varenicline (0.08) and cytisine (0.28) compared with nicotine indicate

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that these drugs are approximately 12- and 4-fold more potent at $\alpha4\beta2$ nAChR subtypes, respectively (see Table 2). However, at $\alpha3\beta4$ nAChR subtypes, varenicline (2.51) appears to be about 2.5-fold less potent than nicotine, whereas cytisine (0.31) is approximately 3-fold more potent than nicotine (see Table 2).

Notwithstanding limited pharmacological support from antagonism studies, we found a robust correspondence between the relative potencies with which nicotinic drugs [including those studied in Desai et al. (2016a)] on the one hand decrease food-reinforced responding and produce (+)-epibatidine-like stimulus effects, and on the other hand inhibit [^{3}H]nicotine binding at $\alpha4\beta2$ nAChR subtypes (Fig. 6, left panels). These results suggest a prominent role for the $\alpha4\beta2$ nAChR in the behavioral effects of nicotine and other nicotinic drugs. Conversely, no correspondence was obtained between their relative behavioral potencies and relative potencies for inhibiting [^{125}I]-bungarotoxin binding at $\alpha7$ nAChRs (Fig. 6, middle panels). Although these results provide further evidence that the $\alpha7$ nAChR does not play a key role in the behavioral effects of nicotinic ligands, its involvement cannot be completely excluded since: 1) $\alpha7$ antagonists have been reported to either increase (Brunzell and McIntosh, 2012) or decrease (Markou and Paterson, 2001) nicotine self-administration in rats, 2) actions at $\alpha7$ nAChRs may be linked to the activation of other (e.g., $\beta2$*) nAChRs (Mameli-Engvall et al., 2006), and 3) $\alpha7$ nAChR subtypes may be involved in long-term maintenance of nicotine use rather than the initial acquisition of nicotine consumption (Levin et al., 2009).

The $\alpha3\beta4$ nAChR subtype also has received some attention regarding its possible role in the behavioral effects of nicotine (Toll et al., 2012). Activation of $\alpha3\beta4$ nAChRs outside the brain previously has been associated with off-target effects of varenicline or cytisine that may interfere with and/or mask their ability to effectively attenuate persistent use of nicotine (Peng et al., 2013). However, nAChRs (e.g., $\alpha3\beta4$) also have been identified in the medial habenula and ventral tegmental area, brain regions thought to be involved in nicotine's abuse-related effects (Gotti et al., 2006). Previously, we found that the relative potencies with which nicotine and the minor tobacco alkaloid anatabine produced reinforcing effects in monkeys corresponded better with their relative potencies for inhibiting binding to the $\alpha3\beta4$ than the $\alpha4\beta2$ nAChR subtype (Desai et al., 2016b). The close correspondence between the relative potencies of nAChR agonists in the present behavioral studies and in inhibiting [^{3}H]epibatidine binding to the $\alpha3\beta4$ nAChR subtype (Fig. 6, right panel) further suggests that this nAChR subtype may play a more significant role in the abuse-related effects of nicotine than once believed (Toll et al., 2012). Taken together, these data indicate the need for further critical evaluation of the role of the $\alpha3\beta4$ nAChR subtype in the abuse-related behavioral effects of nicotine and other nicotinic drugs. However, the current lack of $\alpha3\beta4$-selective agonists and antagonists precludes such evaluation. It is

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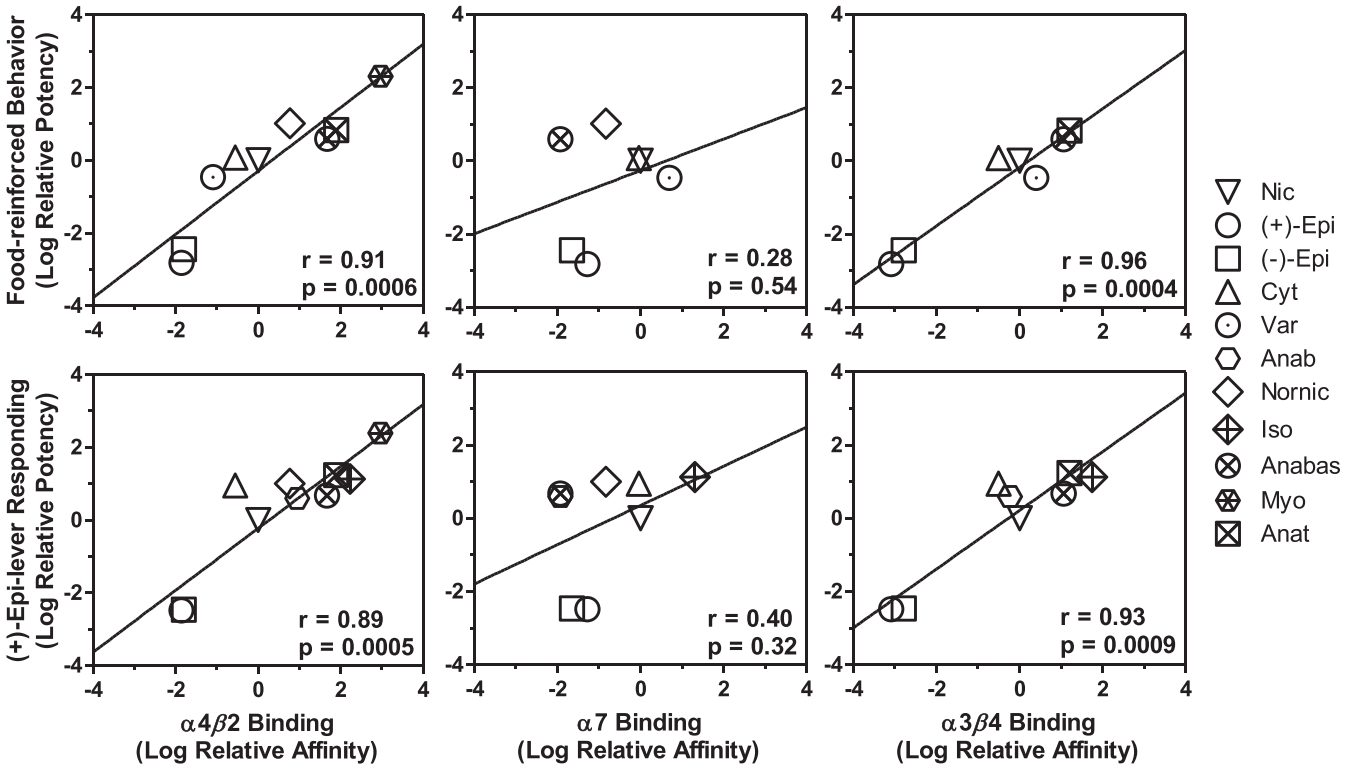

Fig. 6. Correlation between relative potencies of nicotinic drugs [nicotine: Nic; (+)-epibatidine: (+)-Epi; (−)-epibatidine: (−)-Epi; cytisine: Cyt; varenicline: Var; anabaseine: Anab; nornicotine: Nornic; isoarecolone: Iso; anabasine: Anabas; myosimine: Myo; and anatabine: Anat] in the present studies and their relative affinities at $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta4$ nAChRs in radioligand binding studies (see *Materials and Methods*). Abscissae: affinity relative to nicotine for inhibiting binding of radioligand at $\alpha4\beta2$ (left panels), $\alpha7$ (middle panels), and $\alpha3\beta4$ (right panels) nAChRs. Ordinates: potency relative to nicotine based on ED_{50} values for producing rate-decreasing effects on schedule-controlled behavior (Fig. 1; Table 1) and discriminative-stimulus effects (Fig. 3; Table 1). Data for the relative affinities of two nicotinic agonists at $\alpha7$ (anatabine, myosmine) and two at $\alpha3\beta4$ (nornicotine, myosmine) nAChRs are unavailable; therefore, these nicotinic agonists were excluded from correlational analysis at these nAChR subtypes.

noteworthy that other parameters (i.e., efficacy at nAChR subtypes) are also likely to play an important role in the behavioral profile of nicotinic agonists. Unfortunately, only limited in vitro efficacy information is currently available on a handful of nicotinic drugs at nAChR subtypes. Thus, it is not possible to meaningfully correlate in vitro efficacy data with our in vivo behavioral efficacy results.

In summary, there were three key findings. First, nicotinic agonists produced dose-related decreases in response rates for food-reinforced for behavior and, with the exception of lobeline, either fully or partially substituted for the $\alpha4\beta2$-selective nAChR agonist (+)-epibatidine without appreciably altering response rates under the stimulus-termination schedule. These results suggest important differences in the effects of nicotinic agonists on FR rates of responding under the two schedule conditions. Second, the nonselective nicotinic antagonist mecamylamine antagonized both the discriminative-stimulus effects of (+)-epibatidine and nicotine's rate-decreasing effects, whereas the $\alpha4\beta2$-selective nicotinic antagonist DhβE and the peripherally restricted nicotinic antagonist hexamethonium only slightly attenuated (+)-epibatidine discrimination and/or had no effect on the rate-altering effects of nicotine; the $\alpha7$-selective nicotinic antagonist MLA did not alter (+)-epibatidine discrimination. Third, the smoking cessation medications varenicline and cytisine, presumed to be $\alpha4\beta2$-selective partial agonists, only enhanced (+)-epibatidine discrimination and the rate-decreasing effects of nicotine. Taken together, these results suggest that the behavioral effects of nicotinic ligands might not be exclusively mediated by $\alpha4\beta2$ nAChR mechanisms and that a role for other nAChR subtypes deserves further consideration. Elucidating this role may lead to the identification of novel therapeutic targets and the development of candidate medications for smoking cessation.

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Authorship Contributions

Participated in research design: Withey, Doyle, Desai.

Conducted experiments: Withey, Doyle.

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Wrote or contributed to the writing of the manuscript: Withey, Doyle, Bergman, Desai.

References

Anderson DJ and Arneric SP (1994) Nicotinic receptor binding of [^{3}H]cytisine, [^{3}H]-nicotine and [^{3}H]methylcarbamylcholine in rat brain. *Eur J Pharmacol* **253**: 261–267.

Avalos M, Parker MJ, Maddox FN, Carroll FI, and Luetje CW (2002) Effects of pyridine ring substitutions on affinity, efficacy, and subtype selectivity of neuronal nicotinic receptor agonist epibatidine. *J Pharmacol Exp Ther* **302**:1246–1252.

Badio B and Daly JW (1994) Epibatidine, a potent analgetic and nicotinic agonist. *Mol Pharmacol* **45**:563–569.

Barrett JE (1976) Effects of alcohol, chlordiazepoxide, cocaine and pentobarbital on responding maintained under fixed-interval schedules of food or shock presentation. *J Pharmacol Exp Ther* **196**:605–615.

Beckmann JS, Meyer AC, Pivavarchyk M, Horton DB, Zheng G, Smith AM, Wooters TE, McIntosh JM, Crooks PA, Bardo MT, et al. (2015) r-bPiDI, an $\alpha6\beta2$* nicotinic receptor antagonist, decreases nicotine-evoked dopamine release and nicotine reinforcement. *Neurochem Res* **40**:2121–2130.

Benowitz NL (2010) Nicotine addiction. *N Engl J Med* **362**:2295–2303.

Bergman J and Warren PH (1989) Effects of kappa opioids on schedule-controlled behavior of squirrel monkeys. *J Pharmacol Exp Ther* **248**:1102–1108.

Brioni JD, Kim DJ, and O'Neill AB (1996) Nicotine cue: lack of effect of the $\alpha7$ nicotinic receptor antagonist methyllycaconitine. *Eur J Pharmacol* **301**:1–5.

Brunzell DH and McIntosh JM (2012) Alpha7 nicotinic acetylcholine receptors modulate motivation to self-administer nicotine: implications for smoking and schizophrenia. *Neuropsychopharmacology* **37**:1134–1143.

Cunningham CS, Javors MA, and McMahon LR (2012) Pharmacologic characterization of a nicotine-discriminative stimulus in rhesus monkeys. *J Pharmacol Exp Ther* **341**:840–849.

Cunningham CS and McMahon LR (2011) The effects of nicotine, varenicline, and cytisine on schedule-controlled responding in mice: differences in $\alpha4\beta2$ nicotinic receptor activation. *Eur J Pharmacol* **654**:47–52.

Damaj MI, Creasy KR, Grove AD, Rosecrans JA, and Martin BR (1994) Pharmacological effects of epibatidine optical enantiomers. *Brain Res* **664**:34–40.

Damaj MI, Patrick GS, Creasy KR, and Martin BR (1997) Pharmacology of lobeline, a nicotinic receptor ligand. *J Pharmacol Exp Ther* **282**:410–419.

Decker MW, Brioni JD, Bannon AW, and Arneric SP (1995) Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. *Life Sci* **56**:545–570.

de Fiebre CM, Meyer EM, Henry JC, Muraskin SI, Kem WR, and Papke RL (1995) Characterization of a series of anabaseine-derived compounds reveals that the 3-(4)-dimethylaminocinnamylidine derivative is a selective agonist at neuronal nicotinic alpha 7/125I-alpha-bungarotoxin receptor subtypes. *Mol Pharmacol* **47**: 164–171.

de Moura FB and McMahon LR (2016) Differential antagonism and tolerance/cross-tolerance among nicotinic acetylcholine receptor agonists: scheduled-controlled responding and hypothermia in C57BL/6J mice. *Behav Pharmacol* **27**:240–248.

de Moura FB and McMahon LR (2017) The contribution of $\alpha4\beta2$ and non-$\alpha4\beta2$ nicotinic acetylcholine receptors to the discriminative stimulus effects of nicotine and varenicline in mice. *Psychopharmacology (Berl)* **234**:781–792.

Desai RI and Bergman J (2010) Drug discrimination in methamphetamine-trained rats: effects of cholinergic nicotinic compounds. *J Pharmacol Exp Ther* **335**: 807–816.

Desai RI and Bergman J (2014) Methamphetamine-like discriminative-stimulus effects of nicotinic agonists. *J Pharmacol Exp Ther* **348**:478–488.

Desai RI and Bergman J (2015) Effects of the nanoparticle-based vaccine, SEL-068, on nicotine discrimination in squirrel monkeys. *Neuropsychopharmacology* **40**: 2207–2216.

Desai RI, Doyle MR, Withey SL, and Bergman J (2016a) Nicotinic effects of tobacco smoke constituents in nonhuman primates. *Psychopharmacology (Berl)* **233**: 1779–1789.

Desai RI, Sullivan KA, Kohut SJ, and Bergman J (2016b) Influence of experimental history on nicotine self-administration in squirrel monkeys *Psychopharmacology (Berl)*. **233**(12):2253–2263.

D'Souza MS and Markou A (2013) The "stop" and "go" of nicotine dependence: role of GABA and glutamate. *Cold Spring Harb Perspect Med* **3**:a012146 .

Dukat M and Glennon RA (2003) Epibatidine: impact on nicotinic receptor research. *Cell Mol Neurobiol* **23**:365–378.

Dwoskin LP and Crooks PA (2002) A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. *Biochem Pharmacol* **63**:89–98.

Epping-Jordan MP, Picciotto MR, Changeux JP, and Pich EM (1999) Assessment of nicotinic acetylcholine receptor subunit contributions to nicotine self-administration in mutant mice. *Psychopharmacology (Berl)* **147**:25–26.

Etter JF, Lukas RJ, Benowitz NL, West R, and Dresler CM (2008) Cytisine for smoking cessation: a research agenda. *Drug Alcohol Depend* **92**:3–8.

Faure P, Tolu S, Valverde S, and Naudé J (2014) Role of nicotinic acetylcholine receptors in regulating dopamine neuron activity. *Neuroscience* **282**:86–100.

Ferretti G, Dukat M, Giannella M, Piergentili A, Pigini M, Quaglia W, Damaj MI, Martin BR, and Glennon RA (2003) Binding of nicotine and homoazanicotine analogues at neuronal nicotinic acetylcholinergic (nACh) receptors. *Bioorg Med Chem Lett* **13**:733–735.

Fowler CD, Tuesta L, and Kenny PJ (2013) Role of $\alpha5$* nicotinic acetylcholine receptors in the effects of acute and chronic nicotine treatment on brain reward function in mice. *Psychopharmacology (Berl)* **229**:503–513.

Glick SD, Maisonneuve IM, and Kitchen BA (2002) Modulation of nicotine self-administration in rats by combination therapy with agents blocking $\alpha3\beta4$ nicotinic receptors. *Eur J Pharmacol* **448**:185–191.

Goldberg SR, Risner ME, Stolerman IP, Reavill C, and Garcha HS (1989) Nicotine and some related compounds: effects on schedule-controlled behaviour and discriminative properties in rats. *Psychopharmacology (Berl)* **97**:295–302.

Gommans J, Stolerman IP, and Shoaib M (2000) Antagonism of the discriminative and aversive stimulus properties of nicotine in C57BL/6J mice. *Neuropharmacology* **39**:2840–2847.

Gotti C, Fornasari D, and Clementi F (1997) Human neuronal nicotinic receptors. *Prog Neurobiol* **53**:199–237.

Gotti C, Zoli M, and Clementi F (2006) Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci* **27**:482–491.

Hahn B, Sharples CG, Wonnacott S, Shoaib M, and Stolerman IP (2003) Attentional effects of nicotinic agonists in rats. *Neuropharmacology* **44**:1054–1067.

Harrod SB, Dwoskin LP, Green TA, Gehrke BJ, and Bardo MT (2003) Lobeline does not serve as a reinforcer in rats. *Psychopharmacology (Berl)* **165**:397–404.

Hays JT, Ebbert JO, and Sood A (2008) Efficacy and safety of varenicline for smoking cessation. *Am J Med* **121** (Suppl 1):S32–S42.

Hughes JR, Shiffman S, Callas P, and Zhang J (2003) A meta-analysis of the efficacy of over-the-counter nicotine replacement. *Tob Control* **12**:21–27.

Jensen AA, Mikkelsen I, Frølund B, Bräuner-Osborne H, Falch E, and Krogsgaard-Larsen P (2003) Carbamoylcholine homologs: novel and potent agonists at neuronal nicotinic acetylcholine receptors. *Mol Pharmacol* **64**:865–875.

Jutkiewicz EM, Brooks EA, Kynaston AD, Rice KC, and Woods JH (2011) Patterns of nicotinic receptor antagonism: nicotine discrimination studies. *J Pharmacol Exp Ther* **339**:194–202.

Kem WR, Mahnir VM, Papke RL, and Lingle CJ (1997) Anabaseine is a potent agonist on muscle and neuronal alpha-bungarotoxin-sensitive nicotinic receptors. *J Pharmacol Exp Ther* **283**:979–992.

Le Foll B and Goldberg SR (2009) Effects of nicotine in experimental animals and humans: an update on addictive properties. *Handb Exp Pharmacol* **192**:335–367.

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LeSage MG, Shelley D, Ross JT, Carroll FI, and Corrigall WA (2009) Effects of the nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. *Pharmacol Biochem Behav* **91**:461–467.

Levin ED, Petro A, Rezvani AH, Pollard N, Christopher NC, Strauss M, Avery J, Nicholson J, and Rose JE (2009) Nicotinic α7- or β2-containing receptor knockout: effects on radial-arm maze learning and long-term nicotine consumption in mice. *Behav Brain Res* **196**:207–213.

Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, and Faure P (2006) Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron* **50**:911–921.

Markou A and Paterson NE (2001) The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. *Nicotine Tob Res* **3**:361–373.

Marks MJ, Stitzel JA, Romm E, Wehner JM, and Collins AC (1986) Nicotinic binding sites in rat and mouse brain: comparison of acetylcholine, nicotine, and alpha-bungarotoxin. *Mol Pharmacol* **30**:427–436.

Matera C, Quadri M, Sciaccaluga M, Pomè DY, Fasoli F, De Amici M, Fucile S, Gotti C, Dallanoce C, and Grazioso G (2016) Modification of the anabaseine pyridine nucleus allows achieving binding and functional selectivity for the $\alpha3\beta4$ nicotinic acetylcholine receptor subtype. *Eur J Med Chem* **108**:392–405.

McGehee DS, Heath MJ, Gelber S, Devay P, and Role LW (1995) Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* **269**:1692–1696.

Miller DK, Harrod SB, Green TA, Wong MY, Bardo MT, and Dwoskin LP (2003) Lobeline attenuates locomotor stimulation induced by repeated nicotine administration in rats. *Pharmacol Biochem Behav* **74**:279–286.

Mukhin AG, Gündisch D, Horti AG, Koren AO, Tamagnan G, Kimes AS, Chambers J, Vaupel DB, King SL, Picciotto MR, et al. (2000) 5-Iodo-A-85380, an $\alpha4\beta2$ subtype-selective ligand for nicotinic acetylcholine receptors. *Mol Pharmacol* **57**:642–649.

National Academy of Sciences (2011) Guide for the Care and Use of Laboratory Animals, 8th edn. The national academies press: Washington, D.C. www.nap.edu.

Parker MJ, Beck A, and Luetje CW (1998) Neuronal nicotinic receptor $\beta2$ and $\beta4$ subunits confer large differences in agonist binding affinity. *Mol Pharmacol* **54**: 1132–1139.

Peng C, Stokes C, Mineur YS, Picciotto MR, Tian C, Eibl C, Tomassoli I, Guendisch D, and Papke RL (2013) Differential modulation of brain nicotinic acetylcholine receptor function by cytisine, varenicline, and two novel bispidine compounds: emergent properties of a hybrid molecule. *J Pharmacol Exp Ther* **347**:424–437.

Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, Fuxe K, and Changeux JP (1998) Acetylcholine receptors containing the $\beta2$ subunit are involved in the reinforcing properties of nicotine. *Nature* **391**:173–177.

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, Changeux JP, Maskos U, and Fratta W (2008) Crucial role of $\alpha4$ and $\alpha6$ nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* **28**:12318–12327.

Quarta D, Ciruela F, Patkar K, Borycz J, Solinas M, Lluis C, Franco R, Wise RA, Goldberg SR, Hope BT, et al. (2007) Heteromeric nicotinic acetylcholine-dopamine autoreceptor complexes modulate striatal dopamine release. *Neuropsychopharmacology* **32**:35–42.

Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, et al. (2007) Pharmacological profile of the $\alpha4\beta2$ nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology* **52**:985–994.

Rollema H, Shrikhande A, Ward KM, Tingley FD, III, Coe JW, O'Neill BT, Tseng E, Wang EQ, Mather RJ, Hurst RS, et al. (2010) Pre-clinical properties of the $\alpha4\beta2$ nicotinic acetylcholine receptor partial agonists varenicline, cytisine and dianicline translate to clinical efficacy for nicotine dependence. *Br J Pharmacol* **160**:334–345.

Sanjakdar SS, Maldoon PP, Marks MJ, Brunzell DH, Maskos U, McIntosh JM, Bowers MS, and Damaj MI (2015) Differential roles of $\alpha6\beta2$* and $\alpha4\beta2$* neuronal nicotinic receptors in nicotine- and cocaine-conditioned reward in mice. *Neuropsychopharmacology* **40**:350–360.

Schmitt JD, Sharples CG, and Caldwell WS (1999) Molecular recognition in nicotinic acetylcholine receptors: the importance of π-cation interactions. *J Med Chem* **42**: 3066–3074.

Shoaib M (2006) Effects of isoarecolone, a nicotinic receptor agonist in rodent models of nicotine dependence. *Psychopharmacology (Berl)* **188**:252–257.

Smith JW and Stolerman IP (2009) Recognising nicotine: the neurobiological basis of nicotine discrimination. *Handb Exp Pharmacol* **192**:295–333.

Spealman RD, Goldberg SR, and Gardner ML (1981) Behavioral effects of nicotine: schedule-controlled responding by squirrel monkeys. *J Pharmacol Exp Ther* **216**: 484–491.

Stolerman IP, Chandler CJ, Garcha HS, and Newton JM (1997) Selective antagonism of behavioural effects of nicotine by dihydro-beta-erythroidine in rats. *Psychopharmacology (Berl)* **129**:390–397.

Stolerman IP, Garcha HS, and Mirza NR (1995) Dissociations between the locomotor stimulant and depressant effects of nicotinic agonists in rats. *Psychopharmacology (Berl)* **117**:430–437.

Stolerman IP, Garcha HS, Pratt JA, and Kumar R (1984) Role of training dose in discrimination of nicotine and related compounds by rats. *Psychopharmacology (Berl)* **84**:413–419.

Sullivan JP, Decker MW, Brioni JD, Donnelly-Roberts D, Anderson DJ, Bannon AW, Kang CH, Adams P, Piattoni-Kaplan M, Buckley MJ, et al. (1994) (+/-)-Epibatidine elicits a diversity of in vitro and in vivo effects mediated by nicotinic acetylcholine receptors. *J Pharmacol Exp Ther* **271**:624–631.

Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, and Lester HA (2004) Nicotine activation of $\alpha4$* receptors: sufficient for reward, tolerance, and sensitization. *Science* **306**: 1029–1032.

Toll L, Zaveri NT, Polgar WE, Jiang F, Khroyan TV, Zhou W, Xie XS, Stauber GB, Costello MR, and Leslie FM (2012) AT-1001: a high affinity and selective $\alpha3\beta4$ nicotinic acetylcholine receptor antagonist blocks nicotine self-administration in rats. *Neuropsychopharmacology* **37**:1367–1376.

Vierck CJ, Hansson PT, and Yezierski RP (2008) Clinical and pre-clinical pain assessment: are we measuring the same thing? *Pain* **135**:7–10.

Walker N, Howe C, Glover M, McRobbie H, Barnes J, Nosa V, Parag V, Bassett B, and Bullen C (2014) Cytisine versus nicotine for smoking cessation. *N Engl J Med* **371**:2353–2362.

Watkins SS, Epping-Jordan MP, Koob GF, and Markou A (1999) Blockade of nicotine self-administration with nicotinic antagonists in rats. *Pharmacol Biochem Behav* **62**:743–751.

Wei ZL, Xiao Y, George C, Kellar KJ, and Kozikowski AP (2003) Functionalization of the alicyclic skeleton of epibatidine: synthesis and nicotinic acetylcholine receptor binding affinities of epibatidine analogues. *Org Biomol Chem* **1**:3878–3881.

Xiao Y and Kellar KJ (2004) The comparative pharmacology and up-regulation of rat neuronal nicotinic receptor subtype binding sites stably expressed in transfected mammalian cells. *J Pharmacol Exp Ther* **310**:98–107.

Xiao Y, Meyer EL, Thompson JM, Surin A, Wroblewski J, and Kellar KJ (1998) Rat $\alpha3/\beta4$ subtype of neuronal nicotinic acetylcholine receptor stably expressed in a transfected cell line: pharmacology of ligand binding and function. *Mol Pharmacol* **54**:322–333.

Yenugonda VM, Xiao Y, Levin ED, Rezvani AH, Tran T, Al-Muhtasib N, Sahibzada N, Xie T, Wells C, Slade S, et al. (2013) Design, synthesis and discovery of picomolar selective $\alpha4\beta2$ nicotinic acetylcholine receptor ligands. *J Med Chem* **56**: 8404–8421.

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