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Title Page

METHAMPHETAMINE-LIKE DISCRIMINATIVE-STIMULUS EFFECTS OF NICOTINIC AGONISTS

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Running Title Page

Running title: Stimulant-like effects of nicotinic agonists

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Non-standard abbreviations:
ANOVA, Analysis of Variance;
DA, dopamine;
DHβE, dihydro-β-erythroidine hydrobromide;
ED_{50}, Effective $Dose_{50}$;
FR, Fixed-ratio;
LEDs, Light-emitting diodes;
MA, *d*-methamphetamine;
TO, Time-out;
nAChR, nicotinic acetylcholine receptors

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ABSTRACT

Nicotine recently was shown to engender methamphetamine (MA)-like discriminative-stimulus effects in rats, which may be indicative of shared psychomotor stimulant properties. To further investigate such overlapping discriminative-stimulus effects, nicotinic agonists varying in efficacy and selectivity were studied in squirrel monkeys that discriminated a moderate i.m. dose of MA (0.1 mg/kg) from vehicle. These included α4β2-selective ligands that may vary in efficacy from relatively high [nicotine, (+)- and (-)-epibatidine] to relatively low [isoarecolone, varenicline, (-)-cytisine, (-)-lobeline] and the α7-selective ligands anabaseine and anabasine. Results show that nicotine, (+)-epibatidine, and (-)-epibatidine substituted fully for MA, whereas the highest doses of other nicotinic agonists produced intermediate levels of MA-like effects (isoarecolone, anabaseine, anabasine, and varenicline) or did not [(-)-cytisine and (-)-lobeline] substitute for MA. The relative potencies of nicotinic agonists, based on ED_{50} values, corresponded better with their relative affinities at α4β2 than α7 receptors. Regardless of selectivity or efficacy, nicotinic agonists also were observed to produce untoward effects including salivation and emesis during or after experimental sessions. In pretreatment studies, the α4β2-selective antagonist dihydro-β-erythroidine hydrobromide (DHβE: 0.032 and 0.1 mg/kg) and the partial agonists varenicline (0.0032–0.1 mg/kg) and (-)-cytisine (0.032 and 0.1 mg/kg) surmountably antagonized (>10-fold rightward shift) nicotine's MA-like effects but were ineffective in blocking nicotine's emetic effects. Overall, our results show that: 1) MA-like discriminative-stimulus effects of nicotinic agonists likely are mediated through α4β2 nAChR actions and 2) nicotinic α4β2 partial agonists, like the nicotinic antagonist DHβE, can reduce MA-like behavioral effects of nicotine.

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INTRODUCTION

The discriminative-stimulus effects of nicotine have been widely characterized in laboratory animals (e.g., Jutkiewicz, 2011; Cunningham et al., 2012; see Smith and Stolerman, 2009 for review), and have been related to subjective effects that promote its persistent consumption among users of tobacco or other nicotine delivery devices (e.g., Smith and Stolerman, 2009; Benowitz, 2010). Pharmacological studies with selective agonists and antagonists additionally have identified likely mechanisms of action mediating the discriminative-stimulus effects of nicotine. For example, such effects of nicotine are readily mimicked by centrally acting nicotinic agonists with high affinity for the α4β2 nicotinic acetylcholine receptor (nAChR) subtype but not by drugs that act selectively at other subtypes of nAChR (e.g., α3β4 or α7) or by drugs from other pharmacological classes (e.g., muscarinic agents; see Smith and Stolerman, 2009 for review). Moreover, non-competitive (mecamylamine) and competitive (dihydro-β-erythroidine hydrobromide; DHβE)] antagonists that block α4β2 nAChRs in CNS attenuate nicotine's discriminative-stimulus effects, whereas peripherally-restricted antagonists or antagonists at other nAChR subtypes (e.g., nicotinic α7, muscarinic) are ineffective. In conjunction, such evidence strongly suggests that the discriminative-stimulus effects of nicotine are centrally-mediated, primarily via α4β2 nAChRs (e.g., Smith and Stolerman, 2009).

A growing body of evidence also indicates that, as with monoaminergic psychomotor stimulant drugs [e.g., cocaine, d-amphetamine, methamphetamine (MA)], the projection from ventral tegmental area (VTA) to the nucleus accumbens in the mesocorticolimbic dopamine (DA) system is a key element in brain circuitry that mediates the neurochemical and behavioral

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effects of nicotine. Accordingly, increases in DA neurotransmission have been proposed to mediate the reinforcing effects of nicotine and its consumption (e.g., Di Chiara, 2000; Smith and Stolerman, 2009). The involvement of common neural substrates also has led to the suggestion that nicotine and monoaminergic psychomotor stimulant drugs might engender overlapping subjective effects and, in laboratory animals, discriminative-stimulus effects (e.g., Smith and Stolerman, 2009). Data from some, but not all, previous studies in nicotine- and stimulant (cocaine- or d-amphetamine)-trained subjects have supported this suggestion (see Smith and Stolerman, 2009 for review), and have profitably advanced our understanding of the stimulant-like effects of nicotine and other nicotinic ligands. For example, using the psychomotor stimulant MA as a discriminative-stimulus in rats, our recent studies in rats suggest that: a) nicotinic agonists may vary in the extent to which they produce MA-like stimulant effects; b) α4β2 nAChR-mediated actions may play an important role in the MA-like stimulant effects of nicotinic agonists; and c) varenicline dose-dependently antagonized the MA-like stimulant actions of nicotine, consistent with the view that nicotinic partial agonists may help manage nicotine addiction and tobacco consumption (Rollema et al., 2007; Desai and Bergman, 2010).

The present research was conducted to further investigate the discriminative-stimulus effects of nicotine and related compounds. The goals of this work were to determine whether the discriminative-stimulus effects of MA and nicotine overlap in primate species and, if so, to examine the pharmacology of that overlap with a range of nAChR ligands. Using standard drug discrimination procedures, squirrel monkeys first were trained to distinguish a moderate dose of 0.1 mg/kg MA from saline. Next, the effects of monoamine uptake inhibitors (MA, cocaine), DA D_1- and D_2-like agonists [SKF82958, R-(-)-NPA], and a selective serotonin reuptake inhibitor (citalopram) were tested to confirm the role of dopaminergic mechanisms in these

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effects of MA (Tidey and Bergman, 1998). This provided a pharmacologically empirical basis for characterizing the effects of nicotinic ligands in MA-trained subjects. Subsequently, substitution tests with a wide range of nicotinic agonists were conducted to evaluate their ability to mimic MA's discriminative-stimulus effects. Drugs studied included α4β2 nAChR subtype-selective ligands previously characterized as either full agonists [nicotine, (+)-epibatidine, (-)-epibatidine] or partial agonists [(isoarecolone, varenicline, (-)-cytisine, (-)-lobeline); Anderson and Arenric, 1994; Baido and Daly, 1994; Hahn et al., 2003; Rollema et al., 2007]. Substitution tests also were conducted with the α7 nAChR subtype-selective agonists, anabasine and anabaseine (de Fiebre et al., 1995; Kem et al., 1997). Finally, drug interaction studies were conducted to compare modulation of the MA-like discriminative-stimulus effects of nicotine by the α4β2 competitive antagonist DHβE (Williams and Robinson, 1984) and the partial agonists varenicline and (-)-cytisine. Overall, results show overlap in the discriminative-stimulus effects of nicotinic agonists and MA in nonhuman primates and provide further support for the views that: a) MA-like stimulant effects of nicotinic agonists are primarily mediated through actions at α4β2 nAChRs; and b) nicotinic partial agonists that attenuate nicotine's stimulant-like discriminable effects may be useful pharmacotherapeutic adjuncts in the management of nicotine addiction.

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METHODS

Subjects

Four experimentally naïve adult male squirrel monkeys *(Saimiri sciureus)*, weighing 650 to 900 g were subjects in the present studies. All subjects were individually housed in a climate-controlled vivarium under an automated 12-hr light/dark cycle. Except during testing, monkeys had unlimited access to water and were fed a daily allotment of high protein monkey chow (Purina Monkey Chow, St. Louis, MO), supplemented with fruit and multivitamins in the home cage. All monkeys were weighed daily; food intake was not restricted, and diets were adjusted as needed to maintain recommended body weights. Behavioral experiments were conducted daily (Monday–Friday) between 08:00 AM and 06:00 PM, under protocols that were approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects were maintained in the McLean Animal Care Facility in accordance 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). This facility is licensed by the U.S. Department of Agriculture.

Apparatus

The apparatus and methodology were comparable to those employed previously (Kangas et al. 2013; Tidey and Bergman, 1998). During experimental sessions, monkeys sat in customized Plexiglas chairs (Kelleher and Morse, 1968) that were enclosed in ventilated, sound-attenuating chambers provided with white noise at all times to mask extraneous sounds. While seated, monkeys faced a panel containing two sets of colored stimulus lights. Two response levers extended into the chamber, one below each set of stimulus lights, and were comfortably within

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the subject's reach. The two response levers were set 15 cm apart. Depression of either lever with a force greater than 0.2 N produced an audible click and was recorded as a response. Prior to each behavioral session, a shaved portion of the monkey's tail was secured under brass electrodes by a small stock and was coated with electrode paste to ensure a low-resistance electrical contact between electrodes and tail. Brief, low-intensity stimuli (200 msec; 3 mA) could be delivered to the electrodes from a 60 Hz transformer. Experimental variables and data collection were controlled by PC computers with Med Associates interfacing equipment and operating software (MED-PC, MedState Notation, Med Associates Inc., St. Albans, VT, USA).

MA Discrimination

Subjects first were trained to press each of the two response levers under a 10-response fixed-ratio (FR10) schedule of stimulus-termination. Under this schedule, a brief, mild electric stimulus (200 ms; 3 mA) was programmed for delivery to the tail every 10 s during the illumination of red lights on the front panel. The completion of ten consecutive lever-press responses (FR10) on one lever within 10 s turned off the red lights and the associated program of current delivery. The completion of each FR10 also initiated a 50-sec timeout (TO) period, during which all lights in the chamber were extinguished and responding had no programmed consequences. The delivery of four electric stimuli prior to completion of the FR requirement also turned off all lights, terminated the program of stimulus delivery, and initiated the 50-sec TO period. Once performance was stable on both levers under the FR10 response requirement, subjects were trained to discriminate i.m. injections of 0.1 mg/kg MA from i.m. injections of saline (Tidey and Bergman, 1998). Previous studies have indicated that the generalization profiles for many drugs greatly depend on training dose, and that higher doses generally are more

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pharmacologically restrictive than lower doses. For example, this type of relationship previously has been exploited by Spealman and colleagues to study the role of different monoaminergic mechanisms in the discriminative-stimulus effects of cocaine (Spealman, 1995). Thus, in the present study, we lowered the training dose of MA from the highly restrictive dose of 0.32 mg/kg to 0.1 mg/kg to assess the possibility of overlapping discriminative-stimulus effects among compounds from different pharmacological classes. After MA injection, only responses on one lever were reinforced; after saline injection, only responses on the other lever were reinforced. The assignment of MA-associated and saline-associated levers was counterbalanced across monkeys. During all training sessions, responses on the inappropriate lever reset the FR response requirement on the injection-appropriate lever.

When discrimination performance was stable from day to day at or above criterion (90% accuracy), daily training sessions were extended to comprise one to four components. Each component, which consisted of 10 presentations of the FR10;TO 50-s schedule, was preceded by a 10-min TO period during which vehicle or MA could be administered. The number of components in daily training sessions varied in a pseudo-random manner, with the stipulation that MA was injected only before the final component of the session. Additionally, sessions in which only saline was administered in all components occurred periodically to avoid an invariant association between injection of MA and the final session component.

Drug testing was initiated when >90% of responses occurred on the injection-appropriate lever during the preceding training session and four of the last five training sessions. Test sessions comprised four components during which all schedule parameters and contingencies were identical to those in the training sessions, with the exception that 10 consecutive responses on either lever extinguished the red lights and terminated the associated program of current

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delivery. Testing was conducted once or twice per week with training sessions on intervening days. During test sessions, incremental doses of a test drug were administered at the beginning of the 10-min TO periods preceding components of the test session (cumulative dosing). This procedure allowed determination of the effects of up to four cumulative doses during a single test session.

After all drugs were studied for their ability to substitute for MA, drug interaction studies were conducted to determine how pretreatment with selected compounds [varenicline, (-)-cytisine, and DHβE] modified the MA-like effects of nicotine. Pretreatment times were based on data from preliminary experiments and published reports (Stolerman et al., 1995; 1997; Rollema et al., 2007; Desai and Bergman, 2010). Studies were conducted by administering single doses of varenicline (0.0032–0.1 mg/kg), (-)-cytisine (0.032–0.1 mg/kg) or DHβE (0.032–0.1 mg/kg) 5 min prior to re-determination of the cumulative dose-response function for nicotine (0.032–1.0 mg/kg), i.e., 15 min prior to the first session component. Cumulative doses of nicotinic ligands in substitution studies and pretreatment doses in drug interaction studies were selected on the basis of preliminary dose-ranging experiments. Doses ranged from those without effect to those that fully substituted for nicotine or produced untoward physiological effects that precluded further increase in either cumulative dose or, in drug interaction studies, pretreatment dose. In the latter case, observed effects were tabulated for presentation in a table.

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Drugs

Methamphetamine (MA) hydrochloride, cocaine hydrochloride, ± SKF82958 hydrobromide [(±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], R-(-)-NPA [R(−)-10,11-dihydroxy-N-*n*-propylnoraporphine

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hydrochloride], (-)-nicotine hydrogen tartrate, and (-) lobeline hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). Citalopram hydrobromide was generously supplied by Lundbeck (Valby, Denmark). (-)-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 anabasine hydrochloride [(*S*)-(+)-3-(2-piperidinyl)pyridine hydrochloride] were obtained from Tocris (Minneapolis, MN). Anabaseine dihydrochloride (3,4,5,6-tetrahydro-2,3′-bipyridine dihydrochloride), (+)-epibatidine [(2R)-2-(6-chloro-3-pyridinyl)-7-azabicyclo[2.2.1]heptane monohydrochloride], (-)-epibatidine [(2R)-2-(6-chloro-3-pyridinyl)-7-azabicyclo[2.2.1]heptane monohydrochloride], isoarecolone hydrochloride (1-methyl-4-acetyl-1,2,3,6-tetrahydropyridine hydrochloride), and dihydro-β-erythroidine hydrobromide (DHβE) [(2S,13bS)-2,3,5,6,8,9,10,13- octahydro-2-methoxy-1H,12H-benzo[i]pyrano[3,4-g]indolizin-12-one hydrobromide] were obtained from the National Institute on Drug Abuse (Bethesda, MD). Varenicline [6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine] was generously donated by Dr. Hans Rollema (Pfizer Global Research and Development). All drugs were dissolved in 0.9% saline or water, and were injected by the intramuscular route of administration. The pH of nicotine and varenicline was adjusted as needed to 7.0 with 0.1N sodium hydroxide. Doses of each drug are expressed in terms of the free base.

Data Analysis

Data from the test component immediately following injection were used to express the effects of the administered cumulative dose. The percentage MA-associated lever responding in each component of the session was calculated by dividing the number of responses on the injection lever by the total number of responses on both levers. Response rates were calculated

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for each component by dividing the total number of responses by the duration of the component minus timeout periods. If the mean response rate in a component was less than 0.2 responses per sec, data from that component 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 substitution with a dose of test drug alone or after pretreatment was defined for individual subjects and for the group of subjects as the allocation of ≥90% of total responses to the MA-associated lever. An intermediate level of responding (31-89%) on the MA-lever was defined as incomplete substitution, whereas the allocation of ≤30% of responses to the MA-lever was defined as no substitution.

Data were further analyzed to compare potency and maximum effects among drugs, to evaluate drug interactions, and to examine correspondence between the effects of nicotinic drugs in the present experiments and their published affinities for different subtypes of the nicotinic receptor. As appropriate, ANOVA followed by Dunnett's *t*-test or a paired *t*-test was used to evaluate statistical significance of averaged data (defined at the 95% level of confidence; $p < 0.05$). When appropriate, interpolation or linear regression using Bioassay Software (Bioassay version Beta 6.2; MED Associates Inc.) was used to calculate ED_{50} values (S.E.M. for interpolation and 95% confidence limits for regression) from data points on the linear portions of the dose-response functions (Snedecor and Cochran, 1967). In experiments to evaluate varenicline-, (-)-cytisine-, and DHβE-nicotine interactions, ED_{50} values were determined for nicotine alone and in the presence of each drug; pairs of ED_{50} values were considered to be significantly different if their 95% confidence limits did not overlap. When significant differences in ED_{50} values were obtained, relative potency estimates were calculated using standard parallel-line bioassay techniques described by Finney (1964). Finally, correspondence

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between the effects of drugs in drug discrimination and 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 α4β2 and α7 nicotinic receptors *in vitro* (Ki values divided by the Ki value for nicotine alone). Relative affinity values for each drug were obtained from previously published radioligand binding experiments in rat brain. Data were taken from experiments using [^{3}H]-nicotine binding for the α4β2 receptor subtype and [^{125}I]-α-bungarotoxin (Bgt) binding for the α7 nicotinic receptor subtype, and affinities relative to nicotine were averaged across studies (see Table 1).

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RESULTS

MA Discrimination. The 0.1 mg/kg training dose of MA maintained reliable discriminative-stimulus control in all four subjects throughout the present studies (>18 months). During testing sessions, maximum responding on the MA-associated lever in all subjects occurred following a cumulative dose of 0.1 mg/kg MA (Fig. 1; top panel). MA also produced a dose-related elevation in response rate, which increased to approximately 200% of vehicle control values following the training dose or the cumulative test dose of 0.1 mg/kg; however, differences in the magnitude of effect among individual subjects precluded statistical significance for the averaged data ($F_{4,15} = 2.17$; $P > 0.05$). Neither the position and slope of the cumulative dose-response function for MA discrimination (0.0032–0.1 mg/kg) nor its apparent rate-increasing effects varied significantly over the course of the present studies. Consequently, discrimination and response rate data for MA at the beginning and end of the present studies were averaged for control values and graphic presentation.

Dopaminergic drugs. The administration of MA (0.0032–0.1 mg/kg) in test sessions produced dose-dependent substitution for the training dose of MA, with full substitution following the cumulative dose of 0.1 mg/kg MA (Fig. 1; top panel). Like MA, the nonselective monoamine transport blocker cocaine and the DA D_1-like and D_2-like agonists SKF82958 and R-(-)-NPA, respectively, produced dose-dependent and full substitution for 0.1 mg/kg MA, with maxima of 90–94% responding on the MA-associated lever following cumulative doses of 0.32 mg/kg cocaine, 0.1 mg/kg SKF82958, and 0.01 mg/kg R-(-)-NPA (Fig. 1; top panel). In contrast to MA, cocaine, and the DA D_1- and D_2-like agonists, the serotonin-selective reuptake inhibitor citalopram did not substitute for the training dose of MA (maximum: 2% drug-lever responding

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after a cumulative dose of 10 mg/kg; Fig. 1, top panel). Higher cumulative doses of citalopram were not studied to avoid untoward effects, e.g. convulsions, previously observed with high doses of serotonin-selective reuptake inhibitors (Spealman, 1995). Although cocaine, like MA, increased responding in a dose-related manner, response rates were not significantly changed from vehicle values by any of the drugs studied ($Fs_{4,15} \leq 1.23$; $Ps > 0.05$; Fig. 1; bottom panels).

Nicotinic agonists. Nicotine produced dose-dependent increases in responding on the MA-associated lever and fully substituted for the 0.1 mg/kg training dose of MA following cumulative doses of 0.1 (90%) and 0.32 mg/kg (100%) nicotine (Fig. 2, top left panel). The (+)- and (-)-enantiomers of epibatidine also produced dose-dependent increases on the MA-associated lever, and both isomers produced >85% responding on the MA-associated lever following the cumulative dose of 0.001 mg/kg [mean ± SEM: 86 ±14.3% for (+)-epibatidine and 96.8 ±3.33 for (-)-epibatidine; Fig. 2, top middle and right panels, respectively]. Neither nicotine nor the enantiomers of epibatidine significantly altered rates of responding from control values in the group of four monkeys (Fig. 2; bottom panels). However, the highest doses of each drug produced observable effects, including profuse salivation and emesis (see below and Table 2).

The nicotinic agonists isoarecolone, anabaseine, anabasine, and varenicline also produced dose-dependent increases in responding on the MA-associated lever (Fig. 3; top left panel). However, substitution was incomplete following the highest cumulative doses of these agonists, with mean values ranging from approximately 50 to 65% for responding on the MA-associated lever. Among these ligands, a clear plateau in MA-like effects indicative of partial agonist actions was observed only with varenicline: the mean MA-like effects of the highest cumulative pretreatment dose, 0.32 mg/kg, did not exceed those of the immediately preceding cumulative

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dose (0.18 mg/kg) and rates of responding were comparable to control values. The two highest doses of isoarecolone also produced comparable levels of responding on the MA-associated lever; however, the 0.5 log unit increase in dose from 3.2 to 10 mg/kg produced a small, albeit non-significant, increase in responding on the MA-associated lever and a decrease in response rate to 60% of control values, precluding a more definitive characterization of its efficacy. No indication of a plateau in MA-like effects was apparent with either anabasine or anabaseine. Anabasine did not alter mean rates of responding over the range of doses studied, whereas anabaseine reduced response rate in a dose-dependent manner and, following the highest cumulative dose (3.2 mg/kg) nearly or completely eliminated responding. As with nicotine and regardless of the presence or absence of effects on response rates, the highest cumulative doses of each of these nicotinic agonists produced untoward physiological signs that precluded further testing (see below and Table 2).

The rank order of potency with which nicotinic agonists produced MA-like effects was: (-)-epibatidine ≈ (+)-epibatidine > nicotine ≈ varenicline > anabaseine > anabasine > isoarecolone (Table 1). Based on ED_{50} estimates, isoarecolone and the two enantiomers of epibatidine were, respectively, the least and most potent nicotinic ligands in the present studies, and approximately 85- to 80-fold less and more potent than nicotine, respectively. The remaining drugs were approximately 3-fold (varenicline) and 10 to 20-fold (anabaseine, anabasine) less potent than nicotine in producing MA-like discriminative-stimulus effects (Table 1).

In contrast to the above ligands, (-)-cytisine and (-)-lobeline failed to substitute for the training dose of MA, producing ≤30% responding on the MA-associated lever over the range of doses that could be studied (Fig. 3; top right panel). Although response rates were not

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significantly altered following the highest cumulative doses of (-)-cytisine and (-)-lobeline, untoward observable effects precluded the administration of higher doses (see below; Table 2).

Observable effects of nicotinic agonists. The observable effects of nicotinic agonists in the present study included untoward physiological signs following one or more cumulative doses of each drug (Table 2). Regardless of the presence or absence of MA-like discriminative-stimulus effects, the highest cumulative doses of each agonist produced profuse salivation in all subjects that often was followed by emesis. In addition to producing excessive salivation and emesis, the highest cumulative doses of isoarecolone, anabasine, (-)-cytisine, and (-)-lobeline produced tremor in individual subjects (Table 2). Convulsions after cumulatively administered doses of 3.2 or 10 mg/kg lobeline also were noted in two subjects; these signs were quickly and completely attenuated by i.m. diazepam (1.0 mg/kg).

Antagonism of nicotine's MA-like effects. Pretreatment with the selective α4β2 nicotinic antagonist, DHβE (0.032 or 0.1 mg/kg) antagonized the MA-like discriminative-stimulus effects of cumulatively administered nicotine (0.01–1.0 mg/kg; Fig. 4, top panel). Both doses of DHβE produced a comparable level of antagonism, evident as an approximately 10-fold rightward shift in the dose-response curve and yielding an approximately 10-fold increase in the ED_{50} value for nicotine's MA-like effects (Fig. 4, top panel; Table 3). The highest cumulative dose of nicotine after treatment with DHβE (1.0 mg/kg) produced approximately 70% responding on the MA-associated lever and an approximately 30-40% decrease in response rates (Fig. 4; bottom right panel).

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Pretreatment with the nicotinic α4β2 partial agonist varenicline (0.0032, 0.032, or 0.1 mg/kg) also antagonized MA-like discriminative-stimulus effects engendered by cumulatively-administered nicotine (0.01–1.0 mg/kg). Like DHβE, all doses of varenicline shifted the nicotine dose-response curve rightward to a similar extent (Fig. 5, top left panel). Based on estimated ED_{50} values, the potency of nicotine for producing MA-associated responding decreased approximately 12-fold in the presence of 0.032 mg/kg of varenicline, and approximately 16-fold following a 30-fold higher pretreatment dose of varenicline (0.1 mg/kg; Table 3). As in experiments with DHβE, the highest cumulative dose of nicotine (1.0 mg/kg) after treatment with the two highest doses of varenicline (0.032 and 0.1 mg/kg) produced a moderate (approximately 30-40%) but statistically non-significant decrease in response rates (Fig. 5; bottom left panel).

Like DHβE and varenicline, the nicotinic α4β2 partial agonist (-)-cytisine (0.032 or 0.1 mg/kg) antagonized the MA-like discriminative-stimulus effects of cumulatively-administered nicotine (0.01–1.0 mg/kg) by shifting its dose-effect curve rightward (Fig. 5, top right panel). Based on ED_{50} values, the effects of nicotine were displaced approximately 7- and 14-fold rightward by pretreatment with 0.032 mg/kg and 0.1 mg/kg (-)-cytisine, respectively (Table 3). Like varenicline, pretreatment doses of (-)-cytisine did not substantively alter nicotine's effects on response rates, and only moderate (<50%) decreases from vehicle-control rates of responding were observed following their combination (Fig. 5, bottom right panel).

Although not studied separately, neither the α4β2 antagonist DHβE nor the α4β2 partial agonists varenicline and (-)-cytisine appeared to attenuate the emetic effects of cumulative doses of nicotine that produced full substitution for MA. As described above, tremor or frank convulsions following treatment with several nicotinic ligands was evident in the present studies,

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and higher doses of DHβE, like α4β2 full and partial agonists, previously have been reported to produce seizure activity (Damaj et al. 1999; Dobelis et al. 2003). Consequently, higher pretreatment doses of DHβE, varenicline, or (-)-cytisine or higher cumulative doses of nicotine after pretreatment with these drugs were not studied so as to avoid further untoward effects in the present studies.

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DISCUSSION

The main objective of the present studies was to characterize discriminative-stimulus effects of nicotine and nicotinic ligands in monkeys that discriminated a moderate training dose of the monoaminergic stimulant MA (0.1 mg/kg). Initial experiments indicated that, as in previous studies with a higher MA training dose (0.3 mg/kg), indirect monoaminergic agonists (MA, cocaine) and direct DA D_1- and D_2-like receptor agonists (SKF82958 and R-(-)-NPA, respectively) engendered dose-dependent and full substitution for MA (Tidey and Bergman, 1998). These results, supporting the view that DA-related mechanisms play a prominent role in the discriminative-stimulus effects of MA, provide a pharmacologically empirical basis for evaluating behavioral overlap in the effects of drugs that act via different (dopaminergic vs. nicotinic) receptor mechanisms.

Nicotine and the enantiomers of epibatidine also produced dose-dependent increases in MA-associated responding, and fully (or, in the case of (+)-epibatidine, nearly fully) substituted for MA without greatly altering response rates. The comparable effects of these nicotinic full agonists are consistent with their similar nicotinic α4β2 subtype selectivity (see Table 1) and with previous drug discrimination data from nicotine-trained rodents (Reavill et al., 1987; Damaj et al., 1994). They contrast somewhat with data from MA-trained rats in which only nicotine fully substituted for the training dose of MA (Desai and Bergman, 2010). In those studies, 0.001 mg/kg of both (+)-epibatidine and (-)-epibatidine produced approximately 60-70% responding on the MA-associated lever, whereas a 3-fold increase in the dose of both enantiomers completely eliminated responding, precluding further testing. Differences in the two studies may reflect species-related differences in vulnerability to the rate-decreasing effects of the epibatidine enantiomers or, alternatively, differences in the resistance of responding maintained by stimulus-

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termination (present studies) and food presentation (previous studies) to their behaviorally disruptive effects. Notwithstanding these considerations, the present findings clearly show that the discriminative-stimulus effects of nicotine and the enantiomers of epibatidine overlap substantively with those of monoaminergic stimulants like MA in primate species.

The nicotinic receptor ligand varenicline also engendered dose-related MA-like effects but, in contrast to nicotine and epibatidine, the highest doses produced only intermediate levels of substitution. Varenicline previously has been characterized as a nicotinic partial agonist at the α4β2 receptor subtype (Rollema et al., 2007; 2010) and, depending on experimental conditions, may substitute partially or fully for nicotine in nicotine-trained rats and monkeys (Rollema et al., 2007; Smith et al., 2007; LeSage et al., 2009; Jutkiewicz et al., 2011; Cunningham et al., 2012). The plateau in the dose-effect function for varenicline at an intermediate level of responding on the MA-associated lever in the present experiments, in conjunction with its ability to antagonize the stimulant effects of nicotine in MA-trained rodents (Desai and Bergman, 2010), is consistent with its characterization as a nicotinic partial agonist.

Like varenicline, isoarecolone is characterized as an α4β2-selective ligand and can fully reproduce the discriminative-stimulus effects of nicotine in nicotine-trained rats (Reavill et al., 1987; Damaj et al., 1994). Based upon other behavioral and biochemical findings, however, isoarecolone has been forwarded as a nicotinic partial agonist (Reavill et al., 1987; Mirza et al. 1996; Whiteaker et al., 1995, Hahn et al. 2003; Shoaib, 2006). The present findings that the highest doses of isoarecolone produced only an intermediate level of substitution for MA might be considered supporting evidence for that view. However, this interpretation remains speculative in the absence of a more definitive characterization of isoarecolone's efficacy, e.g., varenicline-like antagonism of nicotine's behavioral effects.

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The minor tobacco alkaloids anabasine and anabaseine produced dose-related increases in responding on the MA-associated lever without nicotine-like full substitution or a varenicline-like plateau in MA-like effects. Both drugs previously have displayed α7 and, with lower efficacy, α4β2 receptor-mediated agonist actions (Arendash et al., 1995; Kem et al., 1997; Stevens et al., 1998). Possibly, the limited nicotine-like effects of anabasine and anabaseine in the present experiments reflect relatively low efficacy at the α4β2 receptor (e.g., Takada et al., 1989; Brioni et al., 1994; de Fiebre et al., 1995; Stolerman et al., 1995; Desai and Bergman, 2010). Alternatively, α7-mediated actions of anabasine and anabaseine may have obscured the full expression of their MA-like effects. Although such explanations are speculative in the absence of further information, the present and previous findings in monkeys and rats (Desai and Bergman, 2010) show that anabasine and anabaseine can produce MA-like effects. Although limited, such stimulant-like effects of minor tobacco alkaloids, like those of nicotine, may contribute to the maintenance of tobacco consumption (Clemens et al., 2009; see Hoffman and Evans, 2012 for review).

(-)-Cytisine and (-)-lobeline, which have high nAChR affinity and α4β2 subtype-selectivity (see Table 1), failed to engender MA-like discriminative-stimulus effects in the present studies. Previously, (-)-cytisine was shown to both partially substitute for nicotine in rats and block its discriminative-stimulus effects, consistent with its characterization as a nicotinic partial agonist (Stolerman et al., 1984; Reavill et al., 1990; Brioni et al., 1994; Jutkiewicz et al., 2011; Cunningham et al., 2012). The absence of MA-like effects in the present studies suggest that (-)-cytisine may have less of a stimulant action in primate species than other α4β2 partial agonists such as varenicline. (-)-Lobeline, like (-)-cytisine, is considered a partial agonist at the α4β2 nAChR but, in addition, appears to act through multiple mechanisms, including

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monoamine uptake inhibition (Damaj et al., 1997; Dwoskin and Crooks, 2002). Thus, (-)-lobeline has been shown to substitute for a low training dose of cocaine, yet attenuate the effects of a higher training dose of cocaine or methamphetamine (Miller et al., 2001; Harrod et al., 2003; Desai et al., 2003; Cunningham et al., 2006). Further indicative of its poorly-understood actions, (-)-lobeline has been reported to produce nicotine-like effects in studies of locomotor activity, but not in place conditioning, self-administration, or drug discrimination studies (Fudala and Iwamoto, 1986; Corrigall and Coen, 1989; Reavill et al., 1990; Stolerman et al., 1995; Harrod et al., 2003). Although the absence of MA-like effects in the present study is not inconsistent with its characterization as a nicotinic partial agonist, additional data showing that (-)-lobeline, like varenicline, can antagonize such effects of nicotine would strengthen this categorization.

Although only isoarecolone and anabaseine decreased response rates, the highest cumulative doses of all nicotinic agonists produced untoward physiological signs (emesis, tremor, or convulsions) that precluded the study of higher doses (see Table 2). However, profuse salivation and emesis alone did not appear to interfere with discrimination behavior, e.g., nicotine and epibatidine fully substituted for MA despite profuse salivation and emesis in all subjects. Although the precise mechanism responsible for these adverse physiological signs remains unclear, it is notable that they can be produced by both α4β2 nAChR agonists and antagonists as well as by α7-selective ligands (Damaj et al. 1999; Dobelis et al. 2003). Thus, it is unlikely that these signs reflect actions at a single subtype of nicotinic receptor.

Pretreatment with the competitive antagonist DHβE and the α4β2-selective partial agonists varenicline and (-)-cytisine shifted the dose-effect function for nicotine's MA-like effects rightward, complementing similar results in nicotine-trained rats (Stolerman et al., 1997; Jutkiewicz et al., 2011). Although each drug served as a surmountable antagonist, the range of

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antagonism was surprisingly limited, i.e., an approximately 1-1.25 log unit rightward shift in the nicotine dose-effect function. The limited range of antagonist actions of DHβE and (-)-cytisine might reflect the use of two antagonist doses spanning only a 0.5 log unit range; higher pretreatment doses might have led to additional antagonism. In the case of varenicline, however, the lowest pretreatment dose (0.003 mg/kg) increased nicotine's ED_{50} value approximately 10-fold, whereas 10- and 30-fold increases in pretreatment dose produced only a <2-fold further increase in nicotine's ED_{50} value. The reasons for such limited dose dependence in the nicotine-antagonist effects of varenicline are uncertain but may be partly related to training dose. In previous studies of the same three antagonists in nicotine-trained rats, dose dependence was more evident in subjects that discriminated a low, rather than high, dose of nicotine (Jutkiewicz et al., 2011). Possibly, a lower training dose of MA and a concomitant increase in nicotine's potency might also have revealed greater antagonist dose dependence in the present studies.

Comparison of the potencies of nicotinic agonists with their reported binding affinities at α4β2 and α7 nicotinic receptor subtypes reveals a good correspondence between their relative behavioral potencies and their relative potencies for inhibiting [^{3}H]-nicotine binding at the α4β2 receptors ($r^2 = 0.83$, $p = 0.005$) but not [^{125}I]-α-Bgt binding at α7 receptors ($r^2 = 0.01$, $p = 0.83$; Fig. 6; Table 1). These observations parallel a similar analysis in MA-trained rats, and are consistent with the ability of the α4β2 receptor blocker DHβE, but not the α7 receptor blocker MLA, to antagonize nicotine's discriminative-stimulus effects (Brioni et al., 1996; Desai and Bergman, 2010). In concert with the antagonist effects of DHβE and the partial agonists varenicline and cytisine in the present experiments, such correspondence provides added support for the idea that the stimulant-like effects of nicotine and other nicotinic agonists are

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predominantly mediated by their actions at the α4β2 nAChR (Reavill et al., 1987; 1988; Stolerman et al., 1995; Desai and Bergman, 2010).

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AUTHORSHIP CONTRIBUTION

Participated in research design: Desai, Bergman

Conducted experiments: Desai

Performed data analysis: Desai

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

Other: Desai acquired funding for the research.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Effects of cumulative administration of the indirect monoamine agonists MA and cocaine, the direct DA D_1- and D_2-like agonists SKF82958 and *R*-(-)-NPA, respectively, and the selective serotonin reuptake inhibitor, citalopram, in squirrel monkeys trained to discriminate 0.1 mg/kg MA from saline under a fixed-ratio schedule of stimulus termination. *Ordinates:* percentage MA-associated responding (*top panel*), response rates (*bottom panel*). *Abscissae:* cumulative drug dose in mg/kg (log scale). Each data point represents all four subjects tested at each dose. The percentage of responses emitted on the MA-associated lever was not plotted if fewer than half of the subjects responded at that dose. During control sessions, the training dose of MA produced 97.3% ± 1.8 (S.E.M.) responding on the MA-associated lever and injections of saline produced 1.1% ± 0.9 (S.E.M.) responding on the saline-associated.

Figure 2. Effects of the cumulatively administered nicotinic agonists, nicotine and the enantiomers of epibatidine, in squirrel monkeys trained to discriminate 0.1 mg/kg MA from saline. *Ordinates for the top and bottom panels and abscissae* are as in Fig. 1. See Figure 1 for other details. Each data point represents all four subjects tested (n = 4) at each dose.

Figure 3. *Left panels:* Effects of the cumulatively administered nicotinic agonists, isoarecolone, anabaseine, anabasine, and varenicline in squirrel monkeys trained to discriminate 0.1 mg/kg MA from saline. *Right panels:* Effects of the cumulatively administered nicotinic agonists, (-)-cytisine and (-)-lobeline in squirrel monkeys trained to discriminate 0.1 mg/kg MA from saline. *Ordinates for the top and bottom panels and abscissae* are as in Fig. 1. See Figure 1 for other details. Each data point represents all four subjects tested (n = 4) at each dose.

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Figure 4. Effects of pretreatment with the selective competitive α4β2 nicotinic antagonist, DHβE on MA-like responding produced by nicotine in squirrel monkeys trained to discriminate 0.1 mg/kg MA from saline. *Ordinates for top and bottom panels as in Fig. 1. Abscissae:* dose of cumulatively administered nicotine (mg/kg; log scale). See Fig. 1 for other details. Each data point represents all subjects tested (n = 4) at each dose.

Figure 5. Effects of pretreatment with the partial nicotinic agonists varenicline and (-)-cytisine on MA-like responding produced by nicotine in squirrel monkeys trained to discriminate 0.1 mg/kg MA from saline. *Ordinates for top and bottom panels as in Fig. 1. Abscissae:* dose of cumulatively administered nicotine (mg/kg; log scale). See Fig. 1 for other details. Each data point represents all subjects tested (n = 4) at each dose.

Figure 6. Relationship between the relative potencies of nicotinic drugs in the present MA-discrimination studies and their relative affinities at α4β2 and α7 nicotinic receptors in radioligand binding studies (see Methods). *Abscissa:* affinity relative to nicotine for inhibiting binding of radioligand to α4β2 (top panel) and α7 (bottom panel) nicotinic receptors; *ordinates:* potency of nicotinic drugs relative to nicotine, based on ED_{50} values, for engendering MA-associated lever responding (from Table 1). Isoarecolone was excluded from this correlation analysis at the α7 nicotinic receptor subtypes because affinity values obtained at this site are not clearly defined (see Table 1).

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TABLES

Table 1: ED_{50} values (± S.E.M) and relative potencies with which nicotinic agonists produce MA-like effects in squirrel monkeys (nicotine=1) and their affinity and relative affinity at α4β2 and α7 nACh receptors (nicotine=1). The *in vitro* values for inhibiting [^{3}H] nicotine binding at α4β2 nicotinic receptors and [^{125}I]-α-Bgt binding at α7 nicotinic receptors are the average of values taken from the references cited below.

Drug	Doses mg/kg	ED_{50} ± S.E.M mg/kg (μmol/kg)	Relative Potency (MA-like S[d])	In Vitro Affinity (Ki values) at α4β2 (nM)	Relative Affinity at α4β2 receptors	In Vitro Affinity (Ki values) at α7 (nM)	Relative Affinity at α7 receptors
Nicotine	0.01–0.32	0.032 ± 0.014 (0.198)	1	3.4 [a-e, g-j, l]	1	4895 [i]	1
(+)-Epibatidine	0.0001–0.001	0.0005 ± 0.00003 (0.0024)	0.015	0.05 [h, j]	0.015	255 [f]	0.052
(-)-Epibatidine	0.0001–0.001	0.0005 ± 0.00002 (0.0024)	0.015	0.06 [h, j]	0.018	109 [f]	0.022
Isoarecolone	0.32–10	2.8 ± 0.73 (19.3)	87.5	611 [i]	179.7	>100,000 [i]	20.43
Anabaseine	0.1–1.0	0.53 ± 0.17 (3.31)	16.6	32 [m]	9.41	58 [m]	0.012
Anabasine	0.1–1.0	0.74 ± 0.09 (4.31)	23.1	260 [m]	76.5	58 [m]	0.012
Varenicline	0.032–0.18	0.11 ± 0.025 (0.52)	3.44	0.17 [k, l]	0.05	620 [k]	0.127
(-)-Cytisine	0.032–1.0	ND	ND	0.012–1.5 [a-e]	0.004–0.44	260–15000 [a, d, n]	0.05–3.06
(-)-Lobeline	0.1–3.2	ND	ND	1.5–16 [b, o, p]	0.44– 4.71	11600–13100 [n, p]	2.37–2.68

[a] Jensen et al., 2003, [b] Anderson & Arenric, 1994, [c] Xiao and Kellar, 2004, [d, e] Marks et al., 1986, 1996, [f] Sullivan et al., 1994, [g] Decker et al., 1995, [h] Badio and Daly, 1994, [i] Hahn et al., 2003, [j] Damaj et al., 1994, [k,l] Rollema et al., 2007, 2010, [m] Kem et al., 1997; [n] de Fiebre et al., 1995; [o] Damaj et al., 1997; [p] Miller et al., 2003 ND = Not Determined

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Table 2: Cumulative i.m. doses (mg/kg) of nicotinic agonist that produced observable untoward effects (excessive salivation, emesis, tremor, or convulsions) during test sessions (n=4 Ss). One or more effects were observed following injection of the listed cumulative dose either prior to or following the session component in which that dose was studied. + represents the observation of each effect in an individual subject.

Drug	Doses (mg/kg)	Salivation/Foam	Emesis	Tremor	Convulsions
Vehicle	0	-	-	-	-
Nicotine	0.32	+ + + +	+ + + +	-	-
(+)-Epibatidine	0.001	+ + + +	+ + + +	-	-
(-)-Epibatidine	0.001	+ + + +	+ + + +	-	-
	1.0	+	+	-	-
	3.2	+ + +	+ + +	-	-
Isoarecolone	10.0	+ + + +	+ + + +	+ + +	-
	1.0	+ + +	+ + +	-	-
Anabaseine	3.2	+ + + +	+ + + +	-	-
Anabasine	1.0	+ +	+ +	+ +	-
	0.1	+ + + +	+ + + +	-	-
	0.18	+ + + +	+ +	-	-
Varenicline	0.32	+ + + +	+ + +	-	-
	0.32	+ + + +	+ + + +	-	-
(-)-Cytisine	1.0	+ + + +	+ + +	+ + +	-
	3.2	+ + + +	+ +	+	+
(-)-Lobeline	10.0	+	+	+	+

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Table 3: Doses of nicotine alone and after pretreatment with DHβE, varenicline, or (-)-cytisine that are calculated to produce 50% responding on the MA-associated lever (ED_{50} values with 95% confidence intervals) and relative potencies of nicotine after pretreatment [ED_{50} nicotine / ED_{50} nicotine after pretreatment). Data were obtained in squirrel monkeys trained to discriminate i.m. injections of 0.1 mg/kg (0.67 μmol/kg) MA from saline.

Pretreatment Drug	Doses (mg/kg)	ED_{50} (95% CL) mg/kg (μmol/kg)	Relative Potency
	Nicotine 0.01–0.32	0.03 (0.02–0.05) (0.20)	1
DHβE	0.032	0.39 (0.19–1.90) (2.39)	0.08 (0.03–0.20)
	0.1	0.30 (0.12–2.76) (1.85)	0.11 (0.03–0.27)
Varenicline	0.0032	0.39 (0.19–2.55) (2.40)	0.09 (0.02–0.23)
	0.032	0.75 (0.45–1.69) (4.63)[a]	0.06 (0.01–0.14)
	0.1	0.53 (0.26–3.02) (3.25)[a]	0.07 (0.02–0.16)[a]
(-)-Cytisine	0.032	0.23 (-0.19–0.02)[b] (1.39)	0.13 (0.03–0.36)
	0.1	0.44 (0–0.13)[b] (2.72)	0.08 (0.02–0.23)

[a] Significant deviation from linearity; [b] Estimate due to non-significant regression

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Figure 1

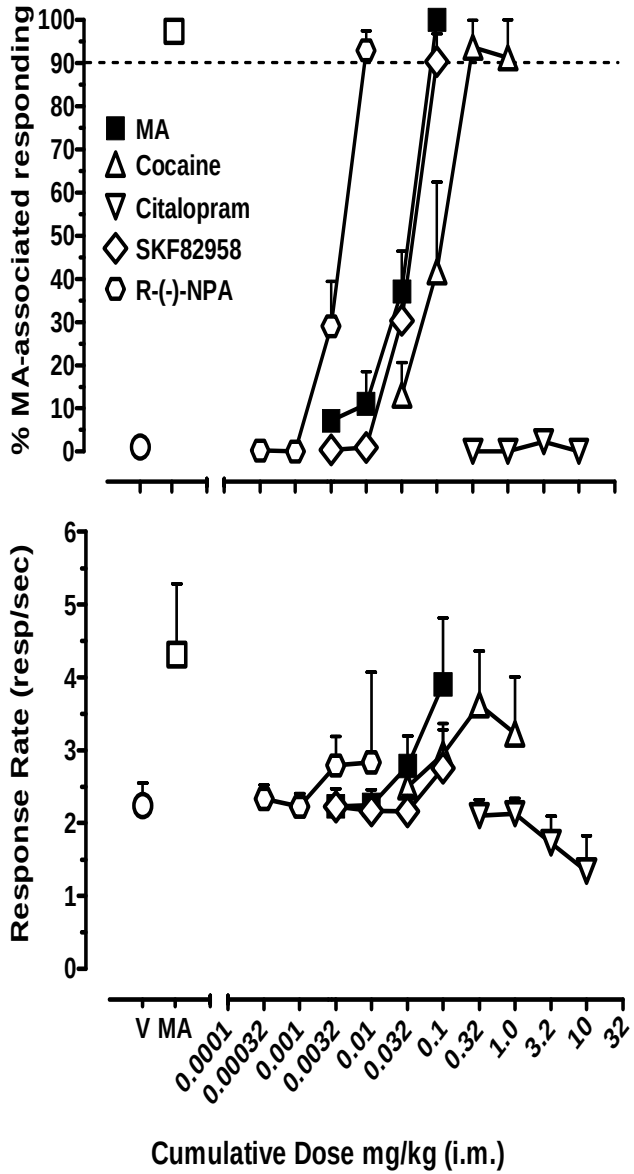

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Figure 2

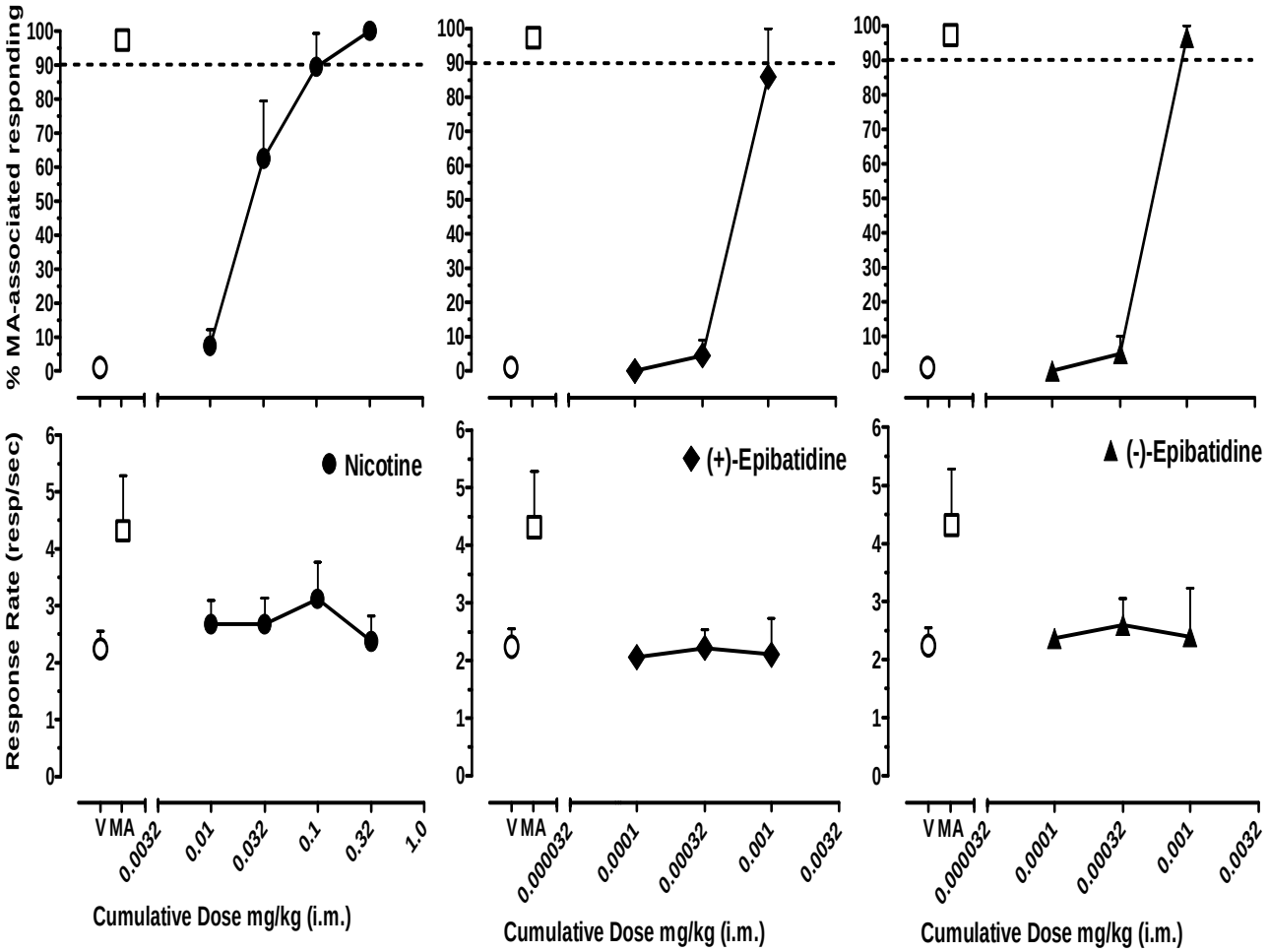

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Figure 3

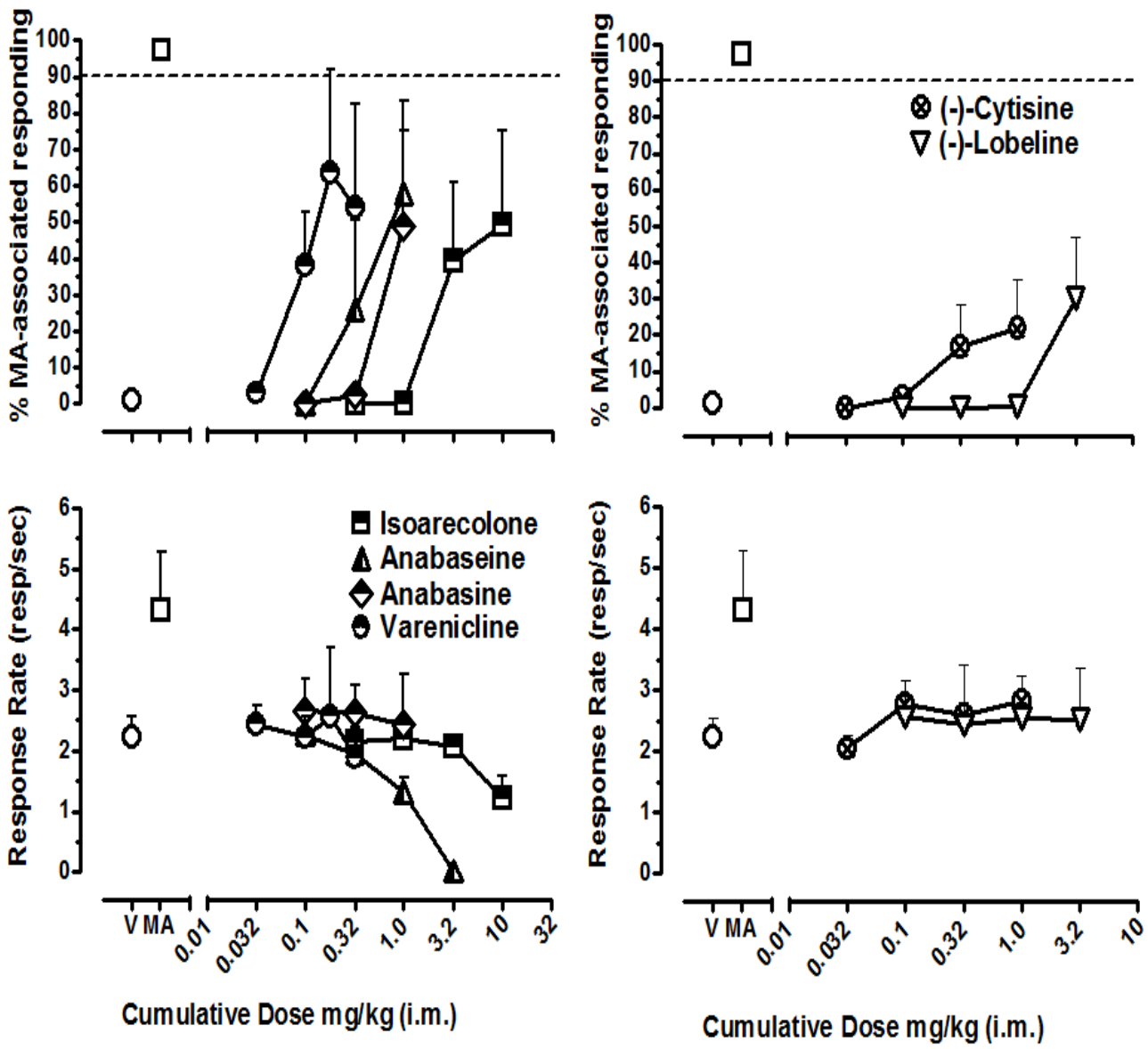

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Figure 4

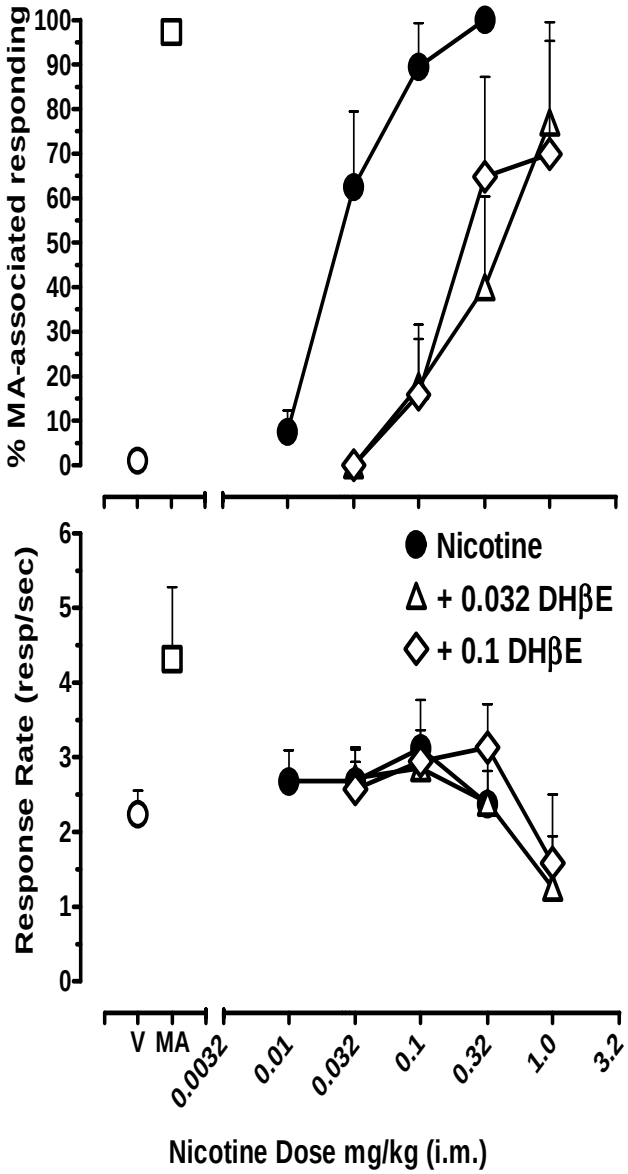

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Figure 5

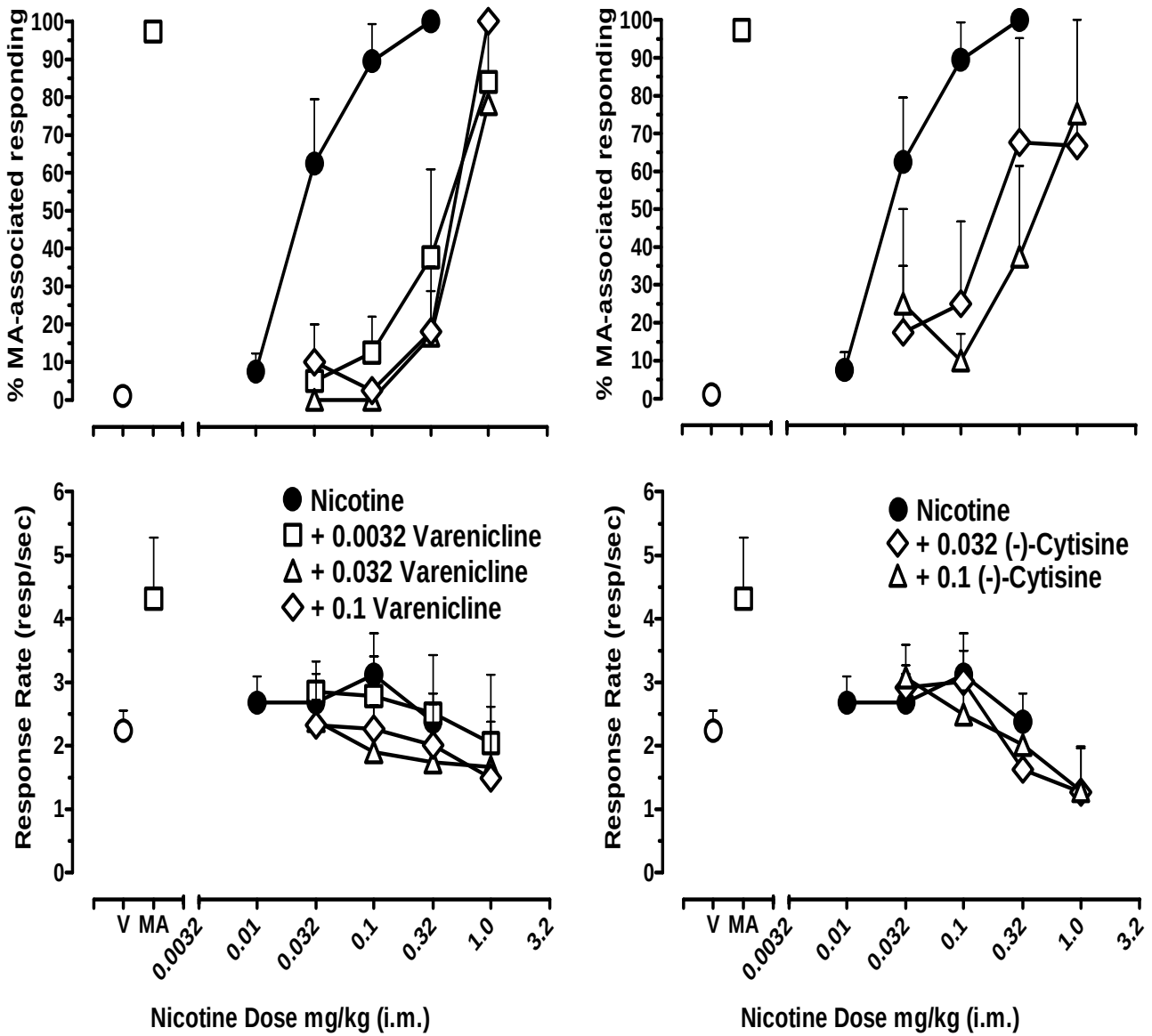

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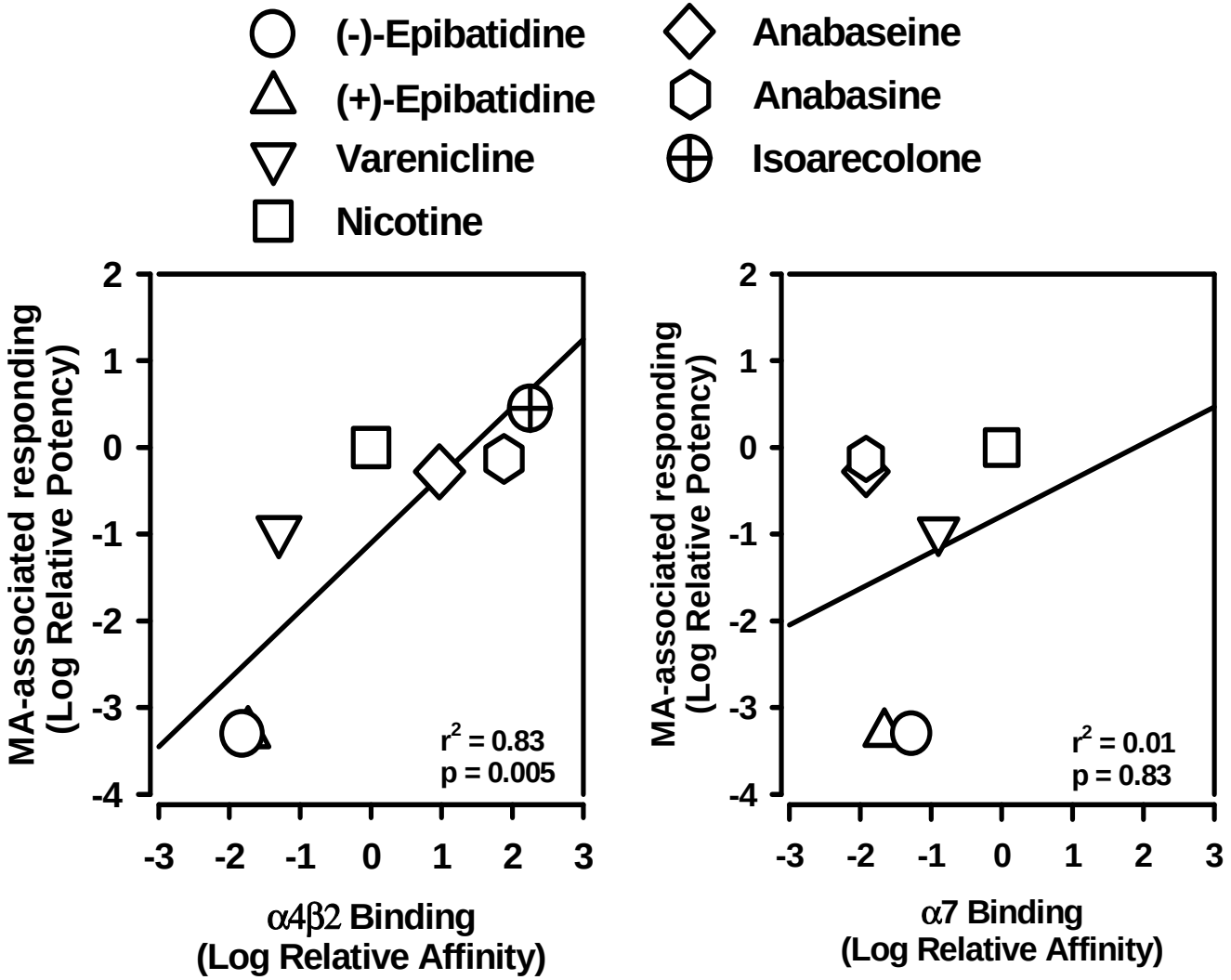
Figure 6



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