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Mecamylamine, dihydro-β-erythroidine, and dextromethorphan block conditioned responding evoked by the conditional stimulus effects of nicotine

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ABSTRACT

Current smokers express the desire to quit. However, the majority find it difficult to remain abstinent. As such, research efforts continually seek to develop more effective treatment. One such area of research involves the interoceptive stimulus effects of nicotine as either a discriminative stimulus in an operant drug discrimination task, or more recently as a conditional stimulus (CS) in a discriminated goal-tracking task. The present work investigated the potential role nicotinic acetylcholine receptors play in the CS effects of nicotine (0.4 mg/kg) using antagonists with differential selectivity for β2*, α7*, α6β2*, and α3β4* receptors. Methyllycaconitine (MLA) had no effect on nicotine-evoked conditioned responding. Mecamylamine and dihydro-β-erythroidine (DHβE) dose-dependently blocked responding evoked by the nicotine CS. In a time-course assessment of mecamylamine and DHβE, each blocked conditioned responding when given 5 min before testing and still blocked conditioned responding when administered 200 min before testing. Two novel *bis*-picolinium analogs (*N*, *N*′-(3, 3′-(dodecan-1,12-diyl)-*bis*-picolinium dibromide [bPiDDB], and *N*, *N*′-(decan-1,10-diyl)-*bis*-picolinium diiodide [bPiDI]) did not block nicotine-evoked conditioned responding. Finally, pretreatment with low dose combinations of mecamylamine, dextromethorphan, and/or bupropion was used to target α3β4* receptors. No combination blocked conditioned responding evoked by the training dose of nicotine. However, a combination of mecamylamine and dextromethorphan partially blocked nicotine-evoked conditioned responding to a lower dose of nicotine (0.1 mg/kg). These results indicate that β2* and potentially α3β4* nicotinic acetylcholine receptors play a role in the CS effects of nicotine and are potential targets for the development of nicotine cessation aids.

1. Introduction

Nicotine addiction is the number one preventable cause of death (Mackay and Eriksen, 2002). The majority of current smokers express a desire to quit (approximately 70%). However, few are able to remain abstinent longer than one month when treated with current smoking cessation therapies, with most relapsing within one week (National Institute on Drug Abuse (NIDA), 2006). A multifaceted approach to nicotine addiction is essential for successful treatment with a need to take into consideration genetic, neural, behavioral, and social factors (cf. Volkow and Li, 2005). As such, there is an increased interest in and need for basic research to further elucidate the underlying processes involved in nicotine addiction. Of primary interest in the present report are the interoceptive (subjective) effects of nicotine.

The interoceptive stimulus effects of nicotine are complex and a variety of preclinical tasks using rats have been developed to study the nicotine stimulus [for reviews see Smith and Stolerman (2009) and Wooters et al. (in press)]. The two-lever operant drug discrimination procedure has been the most widely used of these methods. In that task, nicotine serves as a discriminative stimulus (S^D) indicating which lever press will be reinforced. Alternatively, some recent studies have used a discriminated goal-tracking task in which nicotine serves as a conditional stimulus (CS) for intermittent access to the reinforcer (Bevins, 2009). There is precedent in the literature suggesting that the neuropharmacological processes mediating the subjective effects of a nicotine CS differ, in part, from those mediating the operant S^D effects. For example, using a two-lever drug discrimination task Zakharova et al. (2005) demonstrated that NMDA channel blockers play a minimal role in the S^D effects of nicotine (see also Kim and Brioni, 1995; Zaniewska et al., 2008). In contrast, Murray and Bevins (2007a) found that MK-801, a noncompetitive NMDA channel blocker, antagonized the CS effects of nicotine. More recently, our lab has demonstrated another dissociation between the CS and S^D effects of nicotine. The cannabinoid CB_1 receptor antagonist/inverse agonist rimonabant blocked the CS effects of nicotine (Murray et al., 2009), but has not been found to block the S^D effects of nicotine [e.g., Zaniewska et al. (2006); see Wooters et al.

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(in press) for a discussion of other potential differences]. Because these examples suggest that the neuropharmacological processes mediating the CS effects of nicotine differ somewhat from those of the S^D effects, it is important not to assume that findings regarding the S^D effects of nicotine will necessarily hold for the CS effects.

With this in mind, we sought to examine whether antagonists selective for different nicotinic acetylcholine receptor (nAChR) subtypes would block the CS effects of nicotine. Although there has been some research in this area with the nicotine S^D (e.g., Smith et al., 2007; Zaniewska et al., 2006), there is limited research on the role of nAChR subtypes in the CS effects of nicotine. To date, it has only been shown that the central and peripheral nAChR antagonist mecamylamine (see later) blocked the CS effects of nicotine, yet the mostly peripheral antagonist hexamethonium had no effect; a pattern that suggests a role for centrally located nAChRs in the CS effects of nicotine (Besheer et al., 2004). In the first experiment of this report, we determined whether antagonists with differential selectivity for the β2*, α7*, and α6β2* nAChR subtypes blocked the CS effects of nicotine. Compounds that were effective at blocking conditioned responding evoked by the nicotine CS (i.e., mecamylamine and DHβE) were then evaluated for their duration of action. Such an evaluation was conducted to provide much needed parametric information regarding these widely used antagonists.

The second experiment in this report targeted α3β4* nAChRs. Receptors containing these subunits are of interest because they are concentrated in the habenulointerpeduncular pathway. This pathway interacts with the mesolimbic system that has been widely implicated with the abuse potential of drugs (Taraschenko et al., 2007). There are no compounds currently available that preferentially target α3β4* receptors. Thus, we adopted the strategy of Glick et al. (2002) and used low dose combinations of drugs (mecamylamine, dextromethorphan, and bupropion) that overlap in antagonist action at the α3β4* subunit. With this strategy, Glick et al. (2002) found that such low dose combinations of drugs decreased intravenous nicotine self-administration. We determined whether a similar approach would block the CS effects of nicotine. Previous research from our laboratory had established dose–effect functions for mecamylamine or bupropion antagonism of the CS effects of nicotine (Besheer et al., 2004; Wilkinson et al., 2009). Thus, for the present study only a dose response curve for dextromethorphan alone was needed to identify low doses before the drug combination tests. To confirm this earlier research, however, we did test the dose of each drug utilized in this experiment for antagonism and substitution before any combination testing.

2. Materials and methods

2.1. Subjects

Thirty-two ($n = 16$/experiment) male Sprague–Dawley rats (weighing 269 ± 3 g at the start of the study) were obtained from Harlan (Indianapolis, IN USA). Rats were individually housed in clear polycarbonate cages lined with wood shavings in a temperature- and humidity-controlled room. Water was continuously available in the home cage. Food access was restricted to maintain rats at 85% of their free-feeding weight. Approximately every 30 days the target weight was increased by 2 g. All sessions were conducted during the light portion of a 12 h light: dark cycle. Experimental protocols were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee and followed the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

2.2. Apparatus

Eight conditioning chambers (Med-Associates, Georgia, VT, USA) were used in this study. Each chamber measuring 30.5 × 24.1 × 21 cm ($l \times w \times h$) was enclosed in a light and sound attenuating cubicle fitted with a fan to provide airflow and mask noise. The sidewalls were made of aluminum; the ceiling, front, and back walls were clear polycarbonate. Each chamber contained a recessed dipper receptacle (5.2 × 5.2 × 3.8 cm; $l \times w \times d$) in one aluminum sidewall. When the dipper arm was raised, it allowed access to 0.1 ml of 26% sucrose solution (w/v). An infrared emitter/detector unit located 1.2 cm inside the receptacle and 3 cm from the floor recorded head entries. A second infrared emitter/detector unit was mounted 14.5 cm from the sidewall containing the receptacle and 4 cm above the rod floor. Interruptions of this infrared unit provided a measure of general chamber activity. A personal computer with Med-Associates interface and software (Med-PC for Windows, version IV) timed the sessions, presented the sucrose, and recorded beam breaks for dipper entries and chamber crosses.

2.3. Drugs

(−)-1-Methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt (nicotine), *N*,2,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine hydrochloride (mecamylamine), and (9*S*,13*S*,14*S*)-3-Methoxy-17-methylmorphinan hydrobromide (dextromethorphan) were purchased from Sigma (St. Louis, MO USA). (2S,13bS)-2-Methoxy-2,3,5,6,8,9,10,13-octahydro-1H,12H-benzo[i]pyrano[3,4-g]indolizin-12-one hydrobromide (dihydro-β-erythroidine [DHβE]) and [1a,4(S),6b,14a,16b]-20-Ethyl-1,6,14,16-tetramethoxy-4-[[[2-(3-methyl-2,5-dioxo-1-pyrrolidinyl)benzoyl]oxy]methyl]aconitane-7,8-d iol citrate (methyllycaconitine [MLA]) were purchased from Tocris (Ellisville, MO USA). 1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone hydrochloride (bupropion) was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Two *bis*-picolinium analogs *N*, *N*′-(3, 3′-(dodecan-1,12-diyl)-*bis*-picolinium dibromide (bPiDDB), and *N*, *N*′-(decan-1,10-diyl)-*bis*-picolinium diiodide (bPiDI) were synthesized according to previously established methods (Ayers et al., 2002), and obtained from the Department of Pharmaceutical Sciences, College of Pharmacy, at the University of Kentucky (Lexington, KY USA). All drugs were dissolved in 0.9% saline solution and injected subcutaneously (SC) at a volume of 1 ml/kg, except that bupropion was injected intraperitoneally (IP). Drug doses and injection-to-placement intervals were selected based on past research (Besheer et al., 2004; Shoaib et al., 2000; Glick et al., 2002; Motoshima et al., 2005; Neugebauer et al., 2006; Murray and Bevins, 2007a,b; Dwoskin et al., 2008). For each testing phase, a unique testing order was used for each rat. The pH of nicotine and dextromethorphan was adjusted to 7.0 ± 0.2 with a dilute NaOH solution. Nicotine doses are reported in the base form; all other drug doses are reported in the salt form.

2.4. Acquisition

Before the start of the experiment, all rats were handled for at least 2 min each for 3 days. For the 3 days immediately preceding acquisition, rats were treated daily with the training dose of nicotine (0.4 mg/kg) in the home cage to attenuate the initial locomotor suppressant effects of nicotine (Besheer et al., 2004; Bevins et al., 2001). Daily training sessions began the day following the last nicotine injection in the home cage. Training involved intermixed nicotine (0.4 mg/kg) and 0.9% saline sessions; injections occurred 5 min before placement in the conditioning chamber for a 20-min session. During nicotine sessions, rats had 4-s intermittent access to sucrose on 36 separate occasions. On saline sessions, sucrose deliveries were withheld. Four different computer programs were devised to vary sucrose timing of sucrose deliveries on nicotine sessions. Programs for saline sessions had 4-s 'empty' intervals inserted to equate comparisons with nicotine sessions. The average time before the first sucrose delivery (or equivalent time in saline sessions) across programs was 137 s with a range of 124 to 152 s. Time between subsequent deliveries within a program ranged from 4 to 80 s with a mean of 25 s. Nicotine and saline programs were randomly assigned to each rat with the condition that no more than two of the same session type occurred in a row.

2.5. Testing

Following training of the discrimination, rats entered a testing phase composed of repeating 5 day cycles. The first 4 days of each cycle were continued training sessions (2 nicotine and 2 saline) as described earlier. If the discrimination was maintained as defined by the testing criterion (see later), a 4-min extinction test session occurred in place of a normal training session on day 5; sucrose was withheld during test sessions. If the criterion was not met, the rat remained in the home cage on day 5.

2.5.1. Nicotine generalization

Nicotine generalization testing immediately followed acquisition of the discrimination. On test days, the assigned dose of nicotine (see Table 1) or saline was injected 5 min before a 4-min extinction test.

2.5.2. Antagonism and substitution

Following completion of nicotine generalization, testing cycles continued as previously described. For antagonism testing, the assigned antagonist(s) was injected at the prescribed time. As in training, nicotine was administered 5 min before testing. Substitution tests were similar except that saline replaced nicotine as the solution 5 min before testing. In these experiments, substitution tests were used to evaluate if there were any drug-alone effects. If there were no drug-alone effects, then conditioned responding would be different from nicotine, but not different from saline. Table 1 shows the progression of testing and key procedural details for Experiments 1 and 2.

2.6. Dependent measures and testing criterion

The main dependent measure was the rate per second of entries into the dipper receptacle before the first sucrose delivery on nicotine sessions or equivalent time for saline and test sessions. A rate measure was used because the time before the first sucrose delivery varied between sessions. Dipper entries before the first sucrose delivery were used so as to not confound any measure of conditioned responding with receiving sucrose in that session (cf. Besheer et al., 2004). In order to qualify to test, dipper entry rate on each nicotine session had to be at least 0.01 dipper entries per second higher than each saline session of that testing cycle (cf. Murray and Bevins, 2007a). To determine whether drug treatment affected motor activity, the number of chamber beam breaks per second was also analyzed. We used a rate measure from the exact time period as dipper entries to facilitate comparison across measures. The median effective dose (ED_{50}) for nicotine generalization was calculated using the linear portion of the ascending limb of the dose–effect curve.

2.7. Data analyses

For acquisition training, a paired-samples *t*-test was used to compare the mean of the last three days of training. For all testing phases evaluating dose or duration of antagonism, dipper entries and chamber activity were analyzed with separate one-way repeated measures ANOVAs. Significant one-way ANOVAs were followed with pair-wise comparisons using Fisher's $LSD_{\text{minimum mean difference (mmd)}}$ tests. When using an LSD follow-up comparison, if the difference between the two data points being compared is larger than the calculated LSD_{mmd} for the associated ANOVA then the two data points are significantly different from each other. On test days there were two injections—test compound and either nicotine or saline. As such, each testing phase had pretreatment injections of saline that were administered at each of the respective injection-to-placement intervals used before administration of the nicotine training dose (5-min before chamber placement). Relative to nicotine alone, pretreatment with saline did not affect responding evoked by nicotine. Thus, the mean of the two scores for each rat was used for analyses. Data for test phases that evaluated specific drug doses or drug combinations were

Table 1
Order of antagonism and substitution testing phases.

Type	Drug(s)	Doses (mg/kg)	*n*	Pretreatment time (min)	Nicotine dose (mg/kg)
Experiment 1					
Generalization	Nicotine	0.025, 0.05, 0.1, 0.2, 0.4	16	5	–
Antagonism	MLA	1, 2.5, 5	16	35	0.4
	DHβE	0.3, 1, 3, 10	16	25	0.4
	Mecamylamine	0.1, 0.5, 1, 2	16	25	0.4
Substitution	DHβE	3, 10	8	25	Vehicle
	Mecamylamine	1, 2	7	25	Vehicle
Antagonism	DHβE	3, 10	8	5, 10, 25, 50, 100, 200	0.4
(duration of action)	Mecamylamine	3, 10	7	5, 10, 25, 50, 100, 200	0.4
Antagonism	bPiDDB	0.3, 1, 3	8	15	0.4
	bPiDI	0.338, 1.13, 3.38	7	15	0.4
Experiment 2					
Generalization	Nicotine	0.025, 0.05, 0.1, 0.2, 0.4	16	5	–
Antagonism	Dextromethorphan	0.5, 1, 10, 20	15	20	0.4
	Mecamylamine	0.1	15	25	0.4
	Bupropion	5	15	15	0.4
Substitution	Dextromethorphan	0.5	15	20	Vehicle
	Mecamylamine	0.1	15	25	Vehicle
	Bupropion	5	15	15	Vehicle
Antagonism	Mec + Dex	0.1 + 0.5	14	25, 20	0.4
	Mec + Bup	0.1 + 5	14	25, 15	0.4
	Dex + Bup	0.5 + 5	14	20, 15	0.4
Antagonism	Mec + Dex	0.1 + 0.5	14	25, 20	0.1
	Mec + Bup	0.1 + 5	14	25, 15	0.1
	Dex + Bup	0.5 + 5	14	20, 15	0.1
Substitution	Dextromethorphan	1, 20	14	20	Vehicle
Antagonism	Dex + Mec	1 + 0.1	14	20, 25	0.1
	Dex + Bup	1 + 5	14	20, 15	0.1

+ indicates drugs/doses administered for combination tests. Drugs were administered individually at its respective injection-to-placement interval.

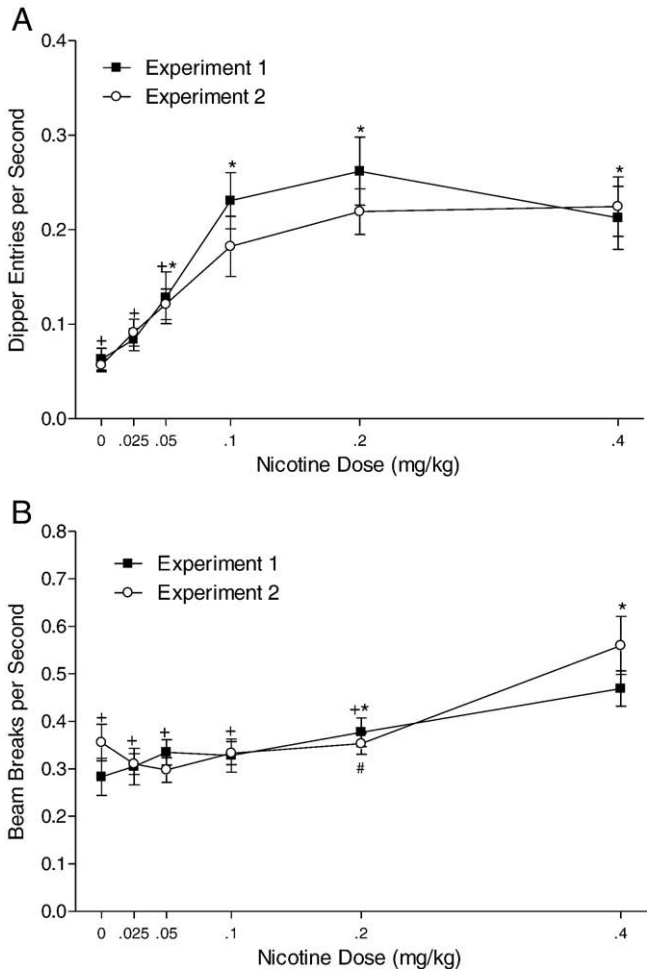

Fig. 1. Panel A shows the mean (±SEM) dipper entry rates during nicotine generalization testing. Panel B shows the mean (±SEM) beam breaks per second (activity) during nicotine generalization testing. For both experiments * denotes a significant difference ($p<0.05$) from saline; + denotes a significant difference from the training dose of nicotine (0.4 mg/kg). # denotes significant difference ($p<0.05$) from the training dose of nicotine (0.4 mg/kg) for Experiment 2 only.

analyzed using paired-samples *t*-tests for both dipper entries and chamber activity. For antagonist tests, contrasts were first limited to each drug or drug combination compared only with the training dose of nicotine. Any significant differences prompted a paired-samples *t*-test comparing that drug dose with saline. For substitution tests, each drug and dose was first compared to saline using paired-samples *t*-tests; a significant difference prompted a paired-samples *t*-test comparing that drug dose to nicotine. Statistical significance was declared using $p<0.05$ for all tests.

3. Results

One rat from Experiment 1 and two from Experiment 2 were removed from the experiments due to the inability to reliably maintain the nicotine-saline discrimination (see Table 1 for the number of rats in each testing phase).

3.1. Acquisition

In Experiments 1 and 2, rats readily acquired the drug discrimination (data not shown). The mean dipper entry rate across the last 3 nicotine sessions in Experiment 1 was significantly elevated compared to saline sessions, $t(15)=7.27$, $p<0.001$. The mean dipper entry rate across the last 3 nicotine sessions in Experiment 2 was significantly elevated compared to saline sessions, $t(15)=11.45$, $p<0.001$.

3.2. Testing

3.2.1. Nicotine generalization

Fig. 1A shows the mean dipper entry rate for the nicotine generalization phase for Experiment 1 (filled squares) and 2 (empty circles). Nicotine-evoked conditioned responding was sensitive to test dose [Dose main effect Experiment 1, $F(5,75)=16.24$, $p<0.001$; Experiment 2, $F(5,75)=15.38$, $p<0.001$]. In both experiments, dipper entry rate was higher at 0.05, 0.1, 0.2, and 0.4 mg/kg nicotine than saline (Experiment 1, $LSD_{mmd}=0.059$; Experiment 2, $LSD_{mmd}=0.05$). Furthermore, the rate of entries at 0.025 and 0.05 mg/kg was lower than the training dose of nicotine (0.4 mg/kg). As shown in Fig. 1B, chamber activity increased with nicotine dose [Dose main effect Experiment 1, $F(5,75)=4.91$, $p<0.001$; Experiment 2, $F(5,75)=11.81$, $p<0.001$]. For Experiment 1, activity was higher at 0.2 and 0.4 mg/kg nicotine than saline ($LSD_{mmd}=0.083$); only 0.4 mg/kg nicotine was higher than saline in Experiment 2 ($LSD_{mmd}=0.08$). Activity at the nicotine training dose was higher than all other nicotine doses. The ED_{50} for nicotine in Experiment 1 and 2 was 0.083 mg/kg and 0.08 mg/kg, respectively.

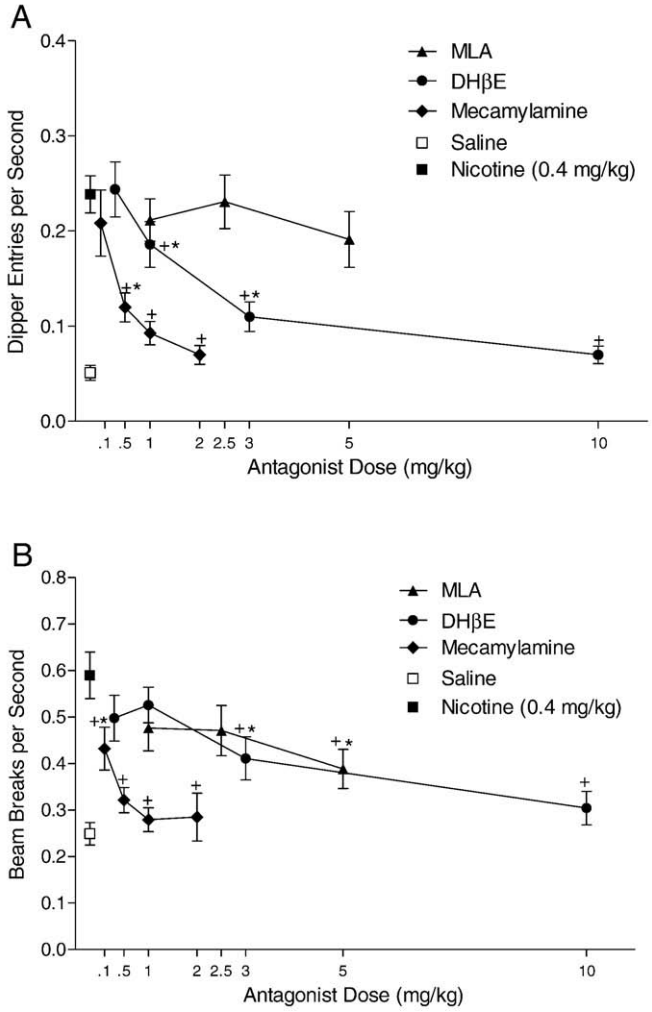

Fig. 2. Panel A shows the mean (±SEM) dipper entry rates during nicotinic acetylcholine receptor antagonism testing. Panel B shows the mean (±SEM) beam breaks per second (activity) during nicotinic acetylcholine receptor antagonism testing. * denotes a significant difference ($p<0.05$) from saline; + denotes a significant difference from the training dose of nicotine (0.4 mg/kg).

3.2.2. MLA, DHβE, and mecamylamine antagonism of nicotine

Fig. 2A shows conditioned responding evoked by nicotine after pretreatment of MLA, DHβE, or mecamylamine. MLA did not block nicotine-evoked conditioned responding (Dose main effect, $F<1$). In contrast, DHβE blocked nicotine-evoked conditioned responding [Dose main effect, $F(4,60)=17.80$, $p<0.001$]. More specifically, conditioned responding was fully antagonized to saline levels at the 10 mg/kg dose ($LSD_{mmd}=0.05$). Antagonism was only partial at 1 and 3 mg/kg DHβE with conditioned responding lower than the training dose of nicotine, but still higher than saline. Mecamylamine also blocked nicotine-evoked conditioned responding [Dose main effect, $F(4,60)=16.58$, $p<0.001$]. Conditioned responding was antagonized to saline levels by 1 and 2 mg/kg ($LSD_{mmd}=0.05$). Mecamylamine at 0.5 mg/kg partially blocked nicotine-evoked conditioned responding.

Fig. 2B shows nicotine-induced activity after pretreatment with MLA, DHβE, or mecamylamine. For MLA there was a significant main effect of Dose, $F(3,45)=3.44$, $p=0.025$. At 5 mg/kg MLA, activity was significantly lower than the nicotine training dose, but still higher than saline ($LSD_{mmd}=0.127$). For DHβE there was a main effect of Dose, $F(4,60)=10.23$, $p<0.001$. At 10 mg/kg DHβE, nicotine-evoked activity was decreased to saline levels ($LSD_{mmd}=0.097$). At 3 mg/kg DHβE, activity was lower than at the nicotine training dose, but higher than saline. For mecamylamine, there was a significant effect of Dose, $F(4,60)=16.07$, $p<0.001$. All doses of mecamylamine lowered activity evoked by nicotine; counts at 0.5, 1, and 2 mg/kg mecamylamine were comparable to saline ($LSD_{mmd}=0.092$).

3.2.3. DHβE, and mecamylamine substitution

Mecamylamine and DHβE alone did not alter general chamber activity (data not shown). Neither dose of DHβE (3 and 10 mg/kg) substituted for the CS effects of nicotine [Dose main effect, $F(2,14)=57.50$, $p<0.001$; ($LSD_{mmd}=0.058$)]. For activity, there was a main effect of DHβE Dose, $F(2,14)=12.82$, $p<0.001$. Activity was below nicotine, but did not differ from saline ($LSD_{mmd}=0.147$). Neither dose of mecamylamine (1 and 2 mg/kg) substituted for the CS effects of nicotine [Dose main effect, $F(2,14)=33.37$, $p<0.001$; ($LSD_{mmd}=0.058$)]. For activity, there was a main effect of mecamylamine Dose, $F(2,14)=8.07$, $p=0.005$. Activity did not differ from saline ($LSD_{mmd}=0.191$).

3.2.4. DHβE and mecamylamine antagonism of nicotine: duration of effects

Fig. 3A and B shows the duration of antagonism of the CS effects of nicotine with DHβE and mecamylamine, respectively. At 3 mg/kg DHβE, there was a main effect of injection-to-placement interval (IPI), $F(6,42)=12.08$, $p<0.001$. Specifically, there was full antagonism at the 25 and 50 min intervals ($LSD_{mmd}=0.055$). For 10 mg/kg DHβE, there was a main effect of IPI, $F(6,42)=12.01$, $p<0.001$. There was full antagonism at the 25, 50, and 100 min intervals; partial antagonism was observed at the 5, 10, and 200 min ($LSD_{mmd}=0.063$). At 1 mg/kg mecamylamine, there was a main effect of IPI, $F(6,36)=6.92$, $p<0.001$. There was full antagonism from 10 to 200 min; partial antagonism was observed at 5 min ($LSD_{mmd}=0.069$). At 2 mg/kg mecamylamine, there was a main effect of IPI, $F(6,36)=17.28$,

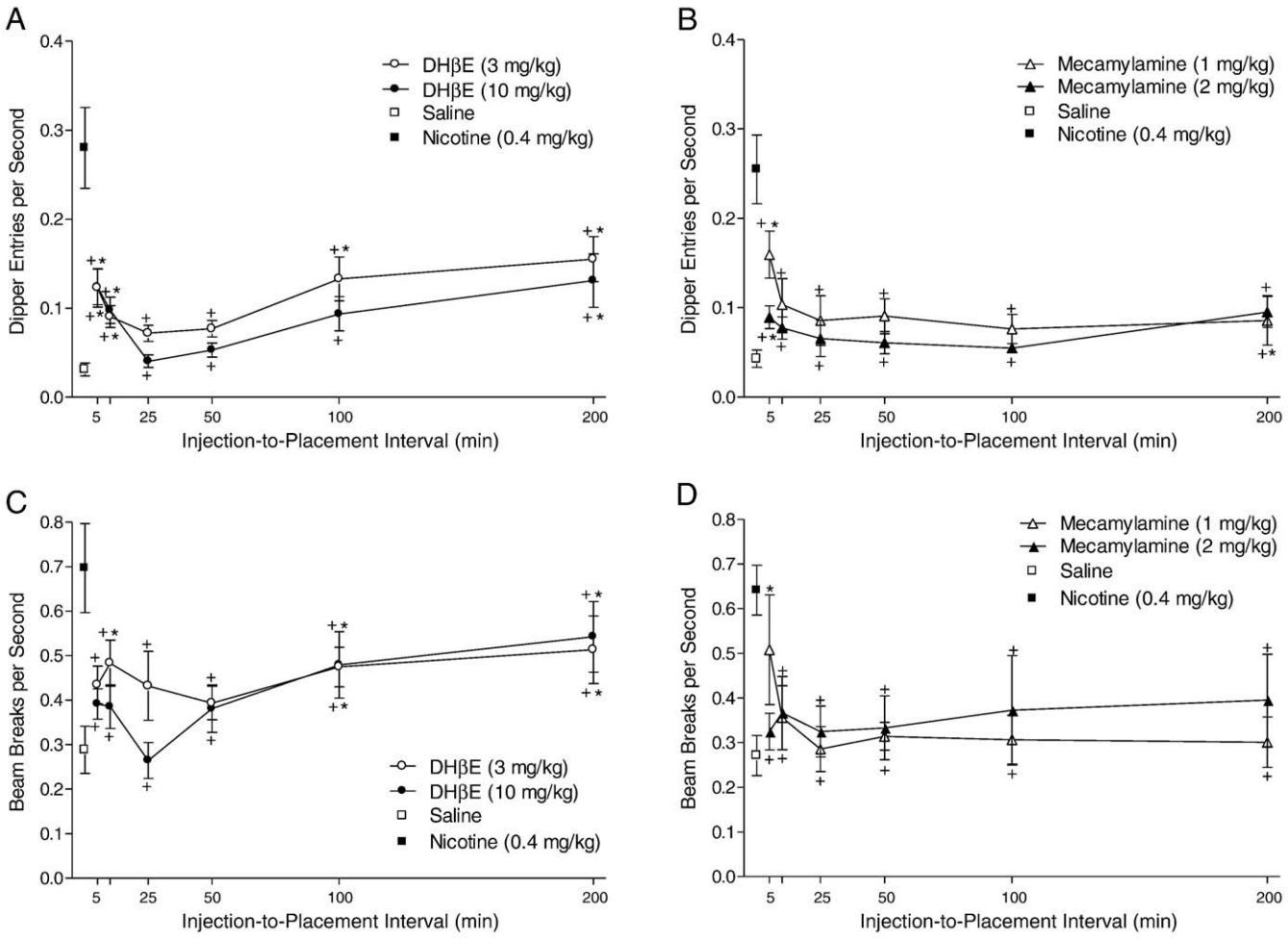

Fig. 3. Panel A shows the mean (±SEM) dipper entry rates for the temporal dynamics of nAChR antagonism testing of DHβE. Panel B shows the mean (±SEM) dipper entry rates during the temporal dynamics of nAChR antagonism testing of mecamylamine. Panel C shows the mean (±SEM) beam breaks per second (activity) during the temporal dynamics of nAChR antagonism testing of DHβE. Panel D shows the mean (±SEM) beam breaks per second (activity) during the temporal dynamics of nAChR antagonism testing of mecamylamine. * denotes a significant difference ($p<0.05$) from saline; + denotes a significant difference from the training dose of nicotine (0.4 mg/kg).

$p<0.001$. There was full antagonism from 10 to 100 min; partial antagonism was seen at 5 and 200 min ($LSD_{mmd}=0.048$).

Fig. 3C and D shows the duration of antagonism of nicotine-induced hyperactivity with DHβE and mecamylamine, respectively. At 3 mg/kg DHβE, there was a main effect of IPI, $F(6,42)=3.09$, $p=0.014$. Activity at 5, 25, and 50 min was at saline levels ($LSD_{mmd}=0.162$). At the 10, 100, and 200 min IPIs, activity was lower than nicotine, but higher than saline. For 10 mg/kg DHβE, there was a main effect of IPI, $F(6,42)=5.11$, $p=0.001$. From 5 to 50 min activity was at saline levels ($LSD_{mmd}=0.177$). At 100 and 200 min activity was lower than nicotine, but still higher than saline. For 1 mg/kg mecamylamine, there was a main effect of IPI, $F(6,36)=6.11$, $p<0.001$. Activity was comparable to saline from the 10 to the 200 min IPI; the 5-min interval was different from nicotine and saline ($LSD_{mmd}=0.158$). At 2 mg/kg mecamylamine, there was a main effect of IPI, $F(6,36)=5.01$, $p=0.001$. Activity was at saline levels at time points ($LSD_{mmd}=0.146$).

3.2.5. Bis-picolinium analog antagonism of nicotine

Fig. 4A and B shows nicotine-evoked conditioned responding in the *bis*-picolinium analogue antagonism phase. The main effect of Dose for bPiDDB was not statistically significant, $F(3,21)=2.79$, $p=0.066$. There was a main effect of Dose for bPiDI, $F(3,18)=5.02$, $p=0.011$. The 3.38 mg/kg dose of bPiDI fully antagonized the CS effects of nicotine ($LSD_{mmd}=0.093$). Fig. 4C and D shows the activity for the *bis*-picolinium analogue antagonism phase. There was a main effect of Dose for bPiDDB, $F(3,18)=4.68$, $p=0.012$, with activity lowered to saline levels at 3 mg/kg ($LSD_{mmd}=0.093$). There was a main effect of Dose for bPiDI, $F(3,18)=13.60$, $p<0.001$. At 3.38 mg/kg bPiDI, activity was significantly lower than nicotine and saline ($LSD_{mmd}=0.158$). Thus, the highest dose of bPiDI impaired motor abilities.

3.2.6. Dextromethorphan antagonism of nicotine

Fig. 5A shows nicotine-evoked conditioned responding after dextromethorphan pretreatment. Dextromethorphan blocked the CS effects of nicotine [Dose main effect, $F(4,56)=8.02$, $p<0.001$]; conditioned responding was partially antagonized at 10 and 20 mg/kg ($LSD_{mmd}=0.057$). Fig. 5B shows nicotine-induced activity after dextromethorphan pretreatment. There was a significant decrease in activity with increased dextromethorphan dose [Dose main effect, $F(4,56)=6.84$, $p<0.001$]. At the 10 and 20 mg/kg dextromethorphan doses, activity was lowered to saline levels ($LSD_{mmd}=0.116$).

3.2.7. Mecamylamine and bupropion antagonism of nicotine

Table 2 shows conditioned responding and activity for 0.4 mg/kg nicotine after pretreatment with 0.1 mg/kg mecamylamine or 5 mg/kg bupropion. Mecamylamine had no effect on responding to the training dose of nicotine, $t<1$. However, mecamylamine decreased nicotine-induced activity to saline levels, $t(14)=2.18$, $p=.047$. Bupropion increased conditioned responding to the training dose of

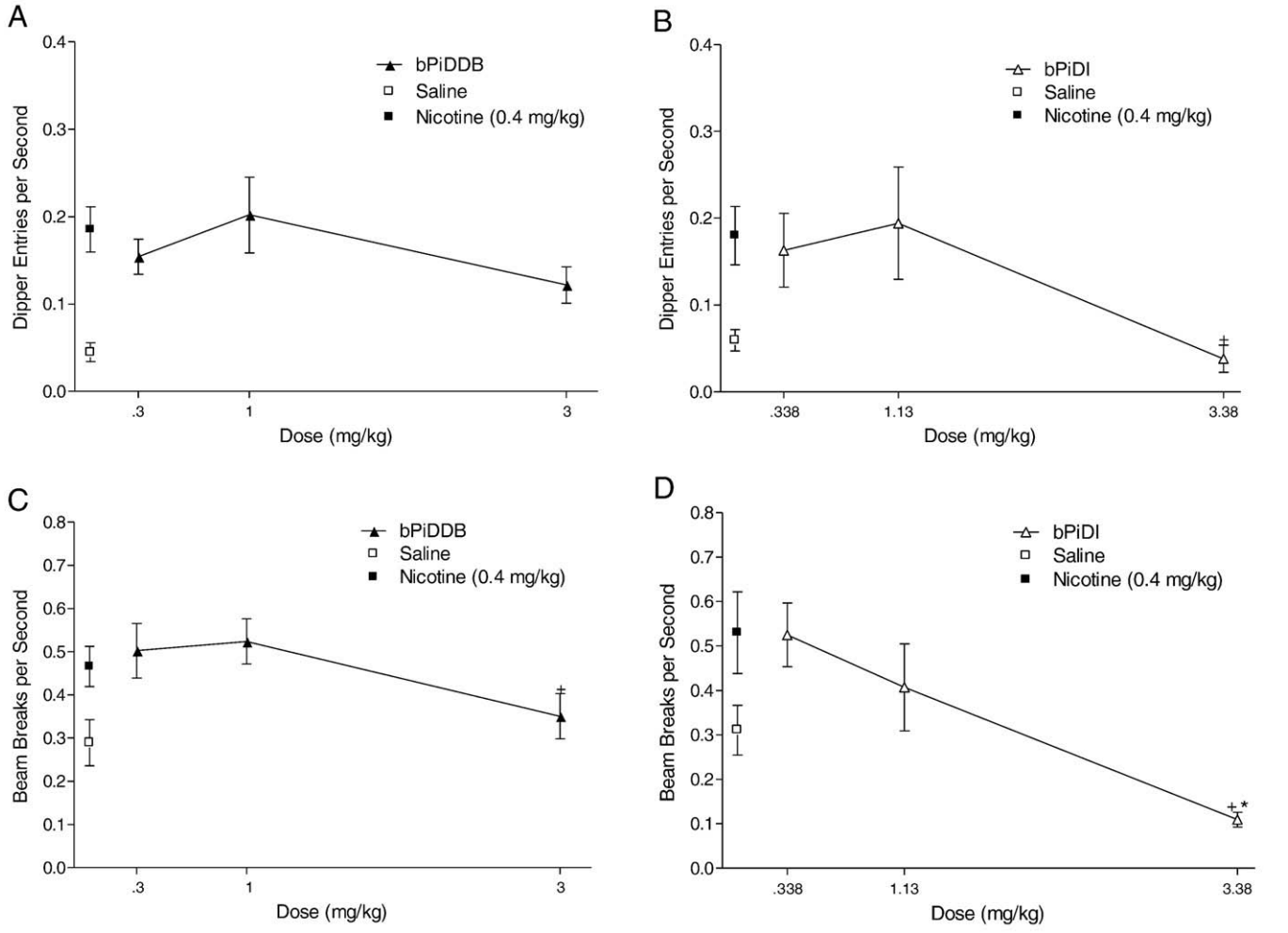

Fig. 4. Panel A shows the mean (±SEM) dipper entry rates for the *bis*-picolinium (bPiDDB) antagonism testing. Panel B shows the mean (±SEM) dipper entry rates for the *bis*-picolinium (bPiDI) antagonism testing. Panel C shows the mean (±1 SEM) beam breaks per second (activity) for the *bis*-picolinium (bPiDDB) antagonism testing. Panel D shows the mean (±SEM) beam breaks per second (activity) for the *bis*-picolinium (bPiDI) antagonism testing. * denotes a significant difference ($p<0.05$) from saline; + denotes a significant difference from the training dose of nicotine (0.4 mg/kg).

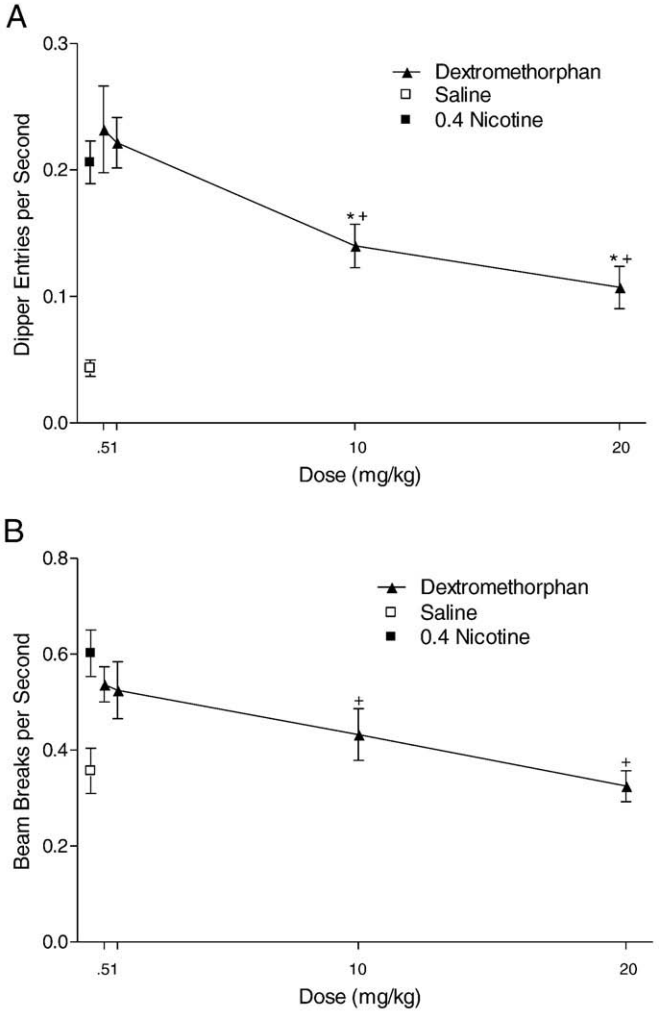

Fig. 5. Panel A shows the mean (±SEM) dipper entry rates during dextromethorphan antagonism testing. Panel B shows the mean (±SEM) beam breaks per second (activity) during dextromethorphan antagonism testing. * denotes a significant difference ($p<0.05$) from saline; + denotes a significant difference from the training dose of nicotine (0.4 mg/kg).

nicotine, $t(14)=-2.87$, $p=0.014$, but did not alter nicotine-induced activity, $t<1$.

3.2.8. Mecamylamine, bupropion, and dextromethorphan substitution

Table 3 shows dipper entries and activity for the substitution tests with mecamylamine (0.1 mg/kg), bupropion (5 mg/kg), and dextromethorphan (0.5, 1, 20 mg/kg). Mecamylamine did not substitute for the nicotine CS and activity levels were not different from saline $ts<1$. Bupropion partially substituted for nicotine with dipper entries higher than saline, $t(14)=2.26$, $p=0.041$, but not as high as nicotine, $t(14)=-7.48$, $p<0.001$. Activity levels for bupropion were higher than saline

Table 2
Dipper entries and activity for the antagonism tests in Experiment 2.

Drug	Dipper entries/s (mean ± SEM)	Beam breaks/s (activity) (mean ± SEM)
Saline	.043 ± .007	.357 ± .047
Nicotine (0.4 mg/kg)	.206 ± .017[a]	.602 ± .049[a]
Mecamylamine (0.1 mg/kg)	.223 ± .024[a]	.493 ± .055[b]
Bupropion (5 mg/kg)	.289 ± .029[a,b]	.557 ± .052[a]

[a] Significant difference from saline.
[b] Significant difference from 0.4 mg/kg nicotine.

Table 3
Dipper entries and activity for substitution tests in Experiment 2.

Drug	Dipper entries/s (mean ± SEM)	Beam breaks/s (activity) (mean ± SEM)
Saline	.064 ± .011	.297 ± .033
Nicotine (0.4 mg/kg)	.246 ± .032[a]	.639 ± .064[a]
Mecamylamine (0.1 mg/kg)	.067 ± .011[b]	.304 ± .039[b]
Saline	.055 ± .008	.270 ± .023
Nicotine (0.4 mg/kg)	.262 ± .025[a]	.610 ± .056[a]
Bupropion (5 mg/kg)	.080 ± .008[a,b]	.371 ± .045[a,b]
Saline	.053 ± .006	.305 ± .040
Nicotine (0.4 mg/kg)	.276 ± .033[a]	.626 ± .053[a]
Dextromethorphan (0.5 mg/kg)	.056 ± .008[b]	.379 ± .085[b]
Saline	.050 ± .012	.263 ± .045
Nicotine (0.4 mg/kg)	.264 ± .039[a]	.541 ± .054[a]
Dextromethorphan (1 mg/kg)	.054 ± .012[b]	.247 ± .039[b]
Saline	.061 ± .007	.266 ± .034
Nicotine (0.4 mg/kg)	.223 ± .025[a]	.545 ± .061[a]
Dextromethorphan (20 mg/kg)	.045 ± .009[b]	.249 ± .022[b]

[a] Significant difference from saline.
[b] Significant difference from 0.4 mg/kg nicotine.

[$t(14)=2.47$, $p=0.027$], but not as high as nicotine, $t(14)=-4.47$, $p=0.001$. Dextromethorphan did not alter dipper entries or chamber activity, $ts\leq1.2$. In sum, mecamylamine (0.1 mg/kg) and dextromethorphan (0.5 mg/kg) had no drug-alone effects, however, bupropion (5 mg/kg) did have an effect on its own.

3.2.9. Drug combination antagonism testing with 0.4 mg/kg nicotine

Table 4 shows the results for combination testing with 0.4 mg/kg nicotine. No drug combination (0.1 mg/kg mecamylamine + 0.5 mg/kg dextromethorphan, 0.1 mg/kg mecamylamine + 5 mg/kg bupropion, or 0.5 mg/kg dextromethorphan + 5 mg/kg bupropion) altered conditioned responding to the training dose of nicotine, $ts<1$. Further, no drug combination lowered the nicotine-induced hyperactivity, $ts<1$.

3.2.10. Drug combination antagonism testing with 0.1 mg/kg nicotine

Table 4 also shows the results for combination testing with 0.1 mg/kg nicotine. No drug combination (0.1 mg/kg mecamylamine + 0.5 mg/kg dextromethorphan, 0.1 mg/kg mecamylamine + 5 mg/kg bupropion, or 0.5 mg/kg dextromethorphan + 5 mg/kg bupropion) altered conditioned responding to 0.1 mg/kg nicotine, $ts<1$. Further, activity with the drug combination pretreatment or just saline was comparable to this low dose of nicotine, $ts\leq1.21$.

Table 4
Dipper entries and activity for drug combination tests in Experiment 2.

Drug	Dipper entries/s (mean ± SEM)	Beam breaks/s (activity) (mean ± SEM)
Saline	.065 ± .010	.355 ± .039
Nicotine (0.4 mg/kg)	.254 ± .039[a]	.586 ± .077[a]
Mec (0.1 mg/kg)–Dex (0.5 mg/kg)	.201 ± .026	.466 ± .040
Mec (0.1 mg/kg)–Bup (5 mg/kg)	.206 ± .029	.482 ± .034
Dex (0.5 mg/kg)–Bup (5 mg/kg)	.251 ± .033	.463 ± .048
Saline	.075 ± .010	.359 ± .032
Nicotine (0.1 mg/kg)	.158 ± .021[a]	.411 ± .028
Mec (0.1 mg/kg)–Dex (0.5 mg/kg)	.121 ± .021	.374 ± .026
Mec (0.1 mg/kg)–Bup (5 mg/kg)	.169 ± .030	.411 ± .028
Dex (0.5 mg/kg)–Bup (5 mg/kg)	.170 ± .025	.454 ± .036
Saline	.057 ± .009	.305 ± .034
Nicotine (0.1 mg/kg)	.176 ± .026[a]	.439 ± .050
Mec (0.1 mg/kg)–Dex (1 mg/kg)	.108 ± .020[a,b]	.366 ± .035
Dex (1 mg/kg)–Bup (5 mg/kg)	.162 ± .020	.406 ± .061

Mec = mecamylamine; Dex = dextromethorphan; Bup = bupropion.
[a] Significant difference from saline.
[b] Significant difference from 0.4 mg/kg nicotine.

The bottom portion of Table 4 shows the dipper entry and activity results for the combination tests with the higher dose of dextromethorphan (1 mg/kg). The dextromethorphan + bupropion drug combination did not decrease conditioned responding to 0.1 mg/kg nicotine, $t<1$. In contrast, the dextromethorphan + mecamylamine combination decreased conditioned responding to the 0.1 mg/kg nicotine dose, $t(12)=-2.85$, $p=0.015$, but was significantly higher than saline, $t(12)=3.50$, $p=0.004$. These results indicate partial antagonism with this combination. For activity, the drug combinations and saline alone did not differ from 0.1 mg/kg nicotine, ts < 1.

4. Discussion

As detailed in the Introduction, a majority of smokers desire to quit, but only a very small portion of them reach sustained abstinence (National Institute on Drug Abuse (NIDA), 2006). This outcome indicates that chronic tobacco use and accompanying nicotine dependence is a tenacious and complex problem. Sustained abstinence remains low even with the current pharmacotherapies available (nicotine, bupropion, and varenicline) prompting the need for more research into nicotine and its effects that may contribute to chronic tobacco use (Dwoskin et al., 2009). The discovery that nicotine has CS effects suggests that it may acquire additional appetitive properties that could affect the progression to addiction, the tenacity of the addiction, as well as to greater difficulty in quitting and increased relapse [see Bevins (2009) for a more in depth discussion]. As such, elucidating the neuropharmacological mechanisms underlying the CS effects of nicotine could aid at identifying new targets for more effective pharmacotherapies.

The main purpose of the present study was to further elucidate the neuropharmacological mechanisms mediating the CS effects of nicotine using antagonists that have differential selectivity for nAChR subunits. The noncompetitive nAChR antagonist mecamylamine has been shown to block the reinforcing (Glick et al., 2002), the discriminative stimulus (Zaniewska et al., 2006), and the locomotor effects of nicotine (Bevins and Besheer, 2001). The present research found that 1 and 2 mg/kg mecamylamine fully blocked nicotine-evoked conditioned responding replicating a previous work from our laboratory (Besheer et al., 2004). Although mecamylamine is considered to be a broad-spectrum antagonist at nAChRs, a work by Papke et al. (2001) indicates that mecamylamine has greater selectivity for $\alpha3\beta4^*$ receptors. This latter finding suggests a need to further investigate $\alpha3\beta4^*$ receptors in the behavioral effects of nicotine.

DHβE is a widely used antagonist with selectivity for $\beta2^*$ nAChRs (Williams and Robinson, 1984). In the present study, it fully antagonized nicotine-evoked conditioned responding at 10 mg/kg. This receptor is the most abundant neuronal nAChR (Benowitz, 1996). Nicotine has a high affinity for $\alpha4\beta2^*$ receptors and they are thought to play a major role in the psychostimulant effects of nicotine (Wonnacott et al., 2005). In rodents, $\alpha4\beta2^*$ receptors upregulate with chronic nicotine administration (Gentry and Lukas, 2002); this upregulation is also seen in the brains of postmortem human smokers (Benwell et al., 1988; Breese et al., 1997). DHβE has also been shown to block the S^D effects of nicotine in a two-lever operant drug discrimination task (Shoaib et al., 2000). Our results extend the conclusions of this research to the CS effects of nicotine as indexed in a discriminated goal-tracking task.

Mecamylamine and DHβE pretreatment also decreased nicotine-induced hyperactivity in the present study. Subsequent tests with the two highest doses of each antagonist alone (i.e., no nicotine) did not affect baseline activity. This finding suggests that blockade of nicotine-evoked conditioned responding does not reflect non-specific motor impairment of these compounds. Thus, pretreatment with DHβE and mecamylamine appears to block the CS effects of nicotine along with nicotine-induced hyperactivity. Such results indicate that these drugs potentially prevent the expression of nicotine sensitization (DiFranza and Wellman, 2007); sensitization is thought to contribute to nicotine's addictive properties (DiFranza and Wellman, 2007; Schoffelmeer et al., 2002). In animal studies, nicotine sensitization is expressed behaviorally as increased locomotor activity resulting from repeated administrations of nicotine. Miller et al. (2001) found that nicotine sensitization developed after only four nicotine exposures even with each exposure being separated by one week. That study also showed that mecamylamine blocked the expression of nicotine-induced behavioral sensitization. We extend these results to show that mecamylamine can block nicotine-induced hyperactivity in a Pavlovian drug discrimination task with chronic nicotine exposure.

Using a two-lever drug discrimination procedure where rats were trained to discriminate 0.2 mg/kg nicotine from vehicle Shoaib et al. (2000) found that 5 mg/kg DHβE blocked the S^D effects of nicotine for up to 45 min. Our study found that a higher and lower dose of DHβE (3 or 10 mg/kg), and mecamylamine (1 or 2 mg/kg) partially antagonized the CS effects of nicotine when administered 5 min before testing. Mecamylamine still fully antagonized the nicotine CS at 200 min, whereas DHβE only partially antagonized at this latest time point. These results indicate that mecamylamine is behaviorally effective longer than DHβE. We have also shown that systemic administration of mecamylamine can fully block the stimulus effects of intravenous administration of nicotine for a 2 h session (Murray and Bevins, 2009). Knowing that mecamylamine and DHβE have a long duration of behavioral effectiveness allows the use of these drugs in lengthy behavioral tasks.

MLA did not antagonize nicotine-evoked conditioned responding at any dose. Research by Turek et al. (1995) found that systemic administration of MLA crosses the blood–brain barrier in the rat, and we showed a significant decrease in general chamber activity at the 5 mg/kg dose. Thus, it seems unlikely that the inability of MLA to affect the conditioned stimulus effects of nicotine reflects an inability of this drug to cross the blood–brain barrier. Consistent with the present research, Brioni et al. (1996) tested MLA systemically and intra-cerebroventricularly in a two-lever drug discrimination task with nicotine and found no substitution or antagonism with either route of administration. Overall, it appears that $\alpha7^*$ nAChRs do not play a role in the interoceptive stimulus effects of nicotine.

We also evaluated the effects of two novel compounds, bPiDDB and bPiDI, to antagonize the conditional stimulus effects of nicotine. These compounds appear to block nAChR subtypes involved in mediating nicotine-evoked dopamine release (e.g., $\alpha6\beta2$; Dwoskin et al., 2004, 2008). In vivo microdialysis in the nucleus accumbens has demonstrated that bPiDDB attenuated nicotine-evoked dopamine overflow (Rahman et al., 2007). Neugebauer et al. (2006) showed that bPiDDB dose-dependently decreased nicotine self-administration. Dwoskin et al. (2008), however, found that bPiDDB had no effect on the nicotine S^D. Our results are consistent with those of the operant drug discrimination task in that bPiDDB did not block nicotine-evoked conditioned responding. The differential effects seen between self-administration and drug discrimination suggest that nAChR subtypes that mediate nicotine-evoked dopamine release do not play a significant role in the neuropharmacological mechanisms involved in the conditional or the discriminative stimulus effects of nicotine. This interpretation is consistent with Murray and Bevins (2007a), in which the two dopamine antagonists SCH-23390 and eticlopride did not block the CS effects of nicotine. The highest bPiDI dose significantly decreased nicotine-evoked conditioned responding. However, at that same dose chamber activity dropped significantly below saline indicating a locomotor impairment effect.

Glick et al. (2002) used low dose combinations of drugs that have common action at $\alpha3\beta4^*$ nAChRs (mecamylamine, dextromethorphan, bupropion, and another compound synthesized in his laboratory [18-methoxycoronaridine]) on nicotine self-administration. In that study, rats were trained to self-administer nicotine (0.028 mg/kg/infusion) and then tested with each drug alone (0.1 mg/kg mecamylamine, 0.5 mg/kg dextromethorphan, 5 mg/kg bupropion, 0.5 mg/kg 18-methoxycoronaridine) and with each low dose combination. No drug had any effect on self-administration behavior when administered alone. However, all low dose drug combinations significantly decreased the number of nicotine infusions per hour. These authors

concluded that there was additive antagonism at α3β4* receptors indicating its importance to the underlying neural mechanisms in nicotine self-administration behavior. Given that Experiment 1 showed full antagonism with mecamylamine and that mecamylamine has a somewhat higher affinity for α3β4* nAChRs (cf. Papke et al., 2001), we adopted the same strategy as Glick et al. (2002) in Experiment 2 and used low dose combinations of mecamylamine, dextromethorphan, and bupropion. Although each drug acts at non-nAChRs, there was no overlap in action except at α3β4* nAChR subtype.

Before any low dose combination testing we evaluated dextromethorphan antagonism; mecamylamine and bupropion antagonism had previously been established using our discrimination procedures (Besheer et al., 2004; Wilkinson et al., 2009). Dextromethorphan partially blocked nicotine-evoked conditioned responding at the 10 and 20 mg/kg doses. Higher doses were not tested because of cutaneous toxicity. These results differ from those of Zakharova et al. (2005) in which dextromethorphan had no effect on the S^D effects nicotine. When tested alone, bupropion (5 mg/kg) increased nicotine-evoked conditioned responding in substitution and antagonism tests, but not when bupropion was tested in combination with mecamylamine and dextromethorphan. Perhaps, the mecamylamine and dextromethorphan blocked bupropion's increase in nicotine-evoked conditioned responding. Although combinations that included bupropion did not decrease nicotine-evoked conditioned responding, the 0.1 mg/kg mecamylamine and 1 mg/kg dextromethorphan combination did partially block nicotine-evoked conditioned responding. This finding may reflect a partial role for α3β4* nAChRs on the CS effects of nicotine. However, further research investigating other drug dose combinations, or more selective antagonists, is needed before fully accepting this interpretation.

In sum, these data indicate that β2* and α3β4* nAChRs potentially play a role in the CS effects of nicotine. However, it is important to note that our data also suggests that the contribution of α3β4* nAChRs may be minor and less than that of β2* nAChRs. These results suggest that β2* and α3β4* nAChR subtypes are potential targets of subsequent pharmacotherapy development for the treatment of nicotine addiction. Given the noncompetitive binding action of mecamylamine, the fact that it has greater selectivity at α3β4* nAChRs, and that it has longer lasting effects than DHβE, may be a drug of interest for the treatment of nicotine addiction at low doses. Research has shown that mecamylamine blocks the physiological effects of nicotine at low doses without precipitating withdrawal (Shytle et al., 2002; Siu and Tyndale, 2007). Rose et al. (1994) has demonstrated that a mecamylamine–nicotine patch combination was more effective in the treatment of nicotine addiction than the nicotine patch alone. Given that low dose drug combinations targeting α3β4* nAChRs significantly reduced the rewarding effects of nicotine (e.g., nicotine self-administration) and partially blocked the conditional stimulus effects of nicotine, there is a need to develop better ligands for this receptor subtype.

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References

Ayers JT, Dwoskin LP, Deaciuc AG, Grinevich VP, Zhu J, Crooks PA. *bis*-Azaaromatic quaternary ammonium analogues: ligands for alpha4beta2* and alpha7* subtypes of neuronal nicotinic receptors. Bioorg Med Chem Lett 2002;12:3067–71.

Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol 1996;36:597–613.

Benwell ME, Balfour DJ, Anderson JM. Evidence that tobacco smoking increases the density of (−)-[3H]nicotine binding sites in human brain. J Neurochem 1988;50:1243–7.

Besheer J, Palmatier MI, Metschke DM, Bevins RA. Nicotine as a signal for the presence or absence of sucrose reward: a Pavlovian drug appetitive conditioning preparation in rats. Psychopharmacology (Berl) 2004;172:108–17.

Bevins RA. Altering the motivational function of nicotine through conditioning processes. In: Bevins RA, Caggiula AR, editors. The motivational impact of nicotine and its role in tobacco use: the 55th Nebraska Symposium on Motivation. New York, NY: Springer; 2009. p. 111–29.

Bevins RA, Besheer J. Individual differences in rat locomotor activity are diminished by nicotine through stimulation of central nicotinic acetylcholine receptors. Physiol Behav 2001;72:237–44.

Bevins RA, Besheer J, Pickett KS. Nicotine-conditioned locomotor activity in rats: dopaminergic and GABAergic influences on conditioned expression. Pharmacol Biochem Behav 2001;68:135–45.

Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, et al. Effect of smoking history on [3H]nicotine binding in human postmortem brain. J Pharmacol Exp Ther 1997;282:7-13.

Brioni JD, Kim DJ, O'Neill AB. Nicotine cue: lack of effect of the alpha 7 nicotinic receptor antagonist methyllycaconitine. Eur J Pharmacol 1996;301:1–5.

DiFranza JR, Wellman RJ. Sensitization to nicotine: how the animal literature might inform future human research. Nicotine Tob Res: J Soc Res Nicotine Tob 2007;9:9-20.

Dwoskin LP, Sumithran SP, Zhu J, Deaciuc AG, Ayers JT, Crooks PA. Subtype-selective nicotinic receptor antagonists: potential as tobacco use cessation agents. Bioorg Med Chem Lett 2004;14:1863–7.

Dwoskin LP, Wooters TE, Sumithran SP, Siripurapu KB, Joyce BM, Lockman PR, et al. N, N′-Alkane- diyl-*bis*-3-picoliniums as nicotinic receptor antagonists: inhibition of nicotine-evoked dopamine release and hyperactivity. J Pharmacol Exp Ther 2008;326:563–76.

Dwoskin LP, Smith AM, Wooters TE, Zhang Z, Crooks PA, Bardo MT. Nicotinic receptor-based therapeutics and candidates for smoking cessation. Biochem Pharmacol 2009;78(7):732–43.

Gentry CL, Lukas RJ. Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. Curr drug targets CNS Neurol Disord 2002;1:359–85.

Glick SD, Maisonneuve IM, Kitchen BA. Modulation of nicotine self-administration in rats by combination therapy with agents blocking alpha 3 beta 4 nicotinic receptors. Eur J Pharmacol 2002;448:185–91.

Kim DJB, Brioni JD. Modulation of the discriminative stimulus properties of (−)-nicotine by diazepam and ethanol. Drug Dev Res 1995;34:47–54.

Mackay J, Eriksen M. Tobacco atlas. World Health Organization. London: Hanway Press; 2002.

Miller DK, Wilkins LH, Bardo MT, Crooks PA, Dwoskin LP. Once weekly administration of nicotine produces long-lasting locomotor sensitization in rats via a nicotinic receptor- mediated mechanism. Psychopharmacology (Berl) 2001;156:469–76.

Motoshima S, Suemaru K, Kawasaki Y, Jin C, Kawasaki H, Gomita Y, et al. Effects of alpha4beta2 and alpha7 nicotinic acetylcholine receptor antagonists on place aversion induced by naloxone in single-dose morphine-treated rats. Eur J Pharmacol 2005;519:91–5.

Murray JE, Bevins RA. Behavioral and neuropharmacological characterization of nicotine as a conditional stimulus. Eur J Pharmacol 2007a;561:91-104.

Murray JE, Bevins RA. The conditional stimulus effects of nicotine vary as a function of training dose. Behav Pharmacol 2007b;18:707–16.

Murray JE, Bevins RA. Acquired appetitive responding to intravenous nicotine reflects a Pavlovian conditioned association. Behav Neurosci 2009;123:97-108.

Murray JE, Wells NR, Lyford GD, Bevins RA. Investigation of endocannabinoid modulation of conditioned responding evoked by a nicotine CS and the Pavlovian stimulus effects ofCP 55, 940 in adult male rats. Psychopharmacology 2009;205:655–65.

National Institute on Drug Abuse (NIDA). Tobacco addiction. Research Report Series; 2006. July, Available from: www.drugabuse.gov/researchreports/nicotine/nicotine.html.

National Research Council. Guide for the care and use of laboratory animals. Washington (DC: National Academy Press; 1996.

Neugebauer NM, Zhang Z, Crooks PA, Dwoskin LP, Bardo MT. Effect of a novel nicotinic receptor antagonist, N,N′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide, on nicotine self-administration and hyperactivity in rats. Psychopharmacology (Berl) 2006;184:426–34.

Papke RL, Sanberg PR, Shytle RD. Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. J Pharmacol Exp Ther 2001;297:646–56.

Rahman S, Neugebauer NM, Zhang Z, Crooks PA, Dwoskin LP, Bardo MT. The effects of a novel nicotinic receptor antagonist N,N-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB) on acute and repeated nicotine-induced increases in extracellular dopamine in rat nucleus accumbens. Neuropharmacology 2007;52:755–63.

Rose JE, Behm FM, Westman EC, Levin ED, Stein RM, Ripka GV. Mecamylamine combined with nicotine skin patch facilitates smoking cessation beyond nicotine patch treatment alone. Clin Pharmacol Ther 1994;56:86–99.

Schoffelmeer AN, De Vries TJ, Wardeh G, van de Ven HW, Vanderschuren LJ. Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. J Neurosci: J Soc Neurosci 2002;22:3269–76.

Shoaib M, Zubaran C, Stolerman IP. Antagonism of stimulus properties of nicotine by dihydro-beta-erythroidine (DHbetaE) in rats. Psychopharmacology (Berl) 2000;149:140–6.

Shytle RD, Penny E, Silver AA, Goldman J, Sanberg PR. Mecamylamine (Inversine): an old antihypertensive with new research directions. J Hum Hypertens 2002;16:453–7.

Siu EC, Tyndale RF. Non-nicotinic therapies for smoking cessation. Annu Rev Pharmacol Toxicol 2007;47:541–64.

Smith JW, Stolerman IP. Recognising nicotine: the neurobiological basis of nicotine discrimination. In: Henningfield JE, London ED, Pogun S, editors. Handbook of experimental pharmacology. New York, NY: Springer; 2009. p. 295–333.
Smith JW, Mogg A, Tafi E, Peacey E, Pullar IA, Szekeres P, et al. Ligands selective for alpha4beta2 but not alpha3beta4 or alpha7 nicotinic receptors generalize to the nicotine discriminative stimulus in the rat. Psychopharmacology (Berl) 2007;190:157–70.
Taraschenko OD, Shulan JM, Maisonneuve IM, Glick SD. 18-MC acts in the medial habenula and interpeduncular nucleus to attenuate dopamine sensitization to morphine in the nucleus accumbens. Synapse 2007;61:547–60.
Turek JW, Kang CH, Campbell JE, Arneric SP, Sullivan JP. A sensitive technique for the detection of the alpha 7 neuronal nicotinic acetylcholine receptor antagonist, methyllycaconitine, in rat plasma and brain. J Neurosci Methods 1995;61:113–8.
Volkow ND, Li TK. Drugs and alcohol: treating and preventing abuse, addiction and their medical consequences. Pharmacol Ther 2005;108:3-17.
Wilkinson JL, Carroll FI, Bevins RA. An investigation of bupropion substitution for the interoceptive effects of nicotine in rats. J Psychopharmacol (Oxf) 2009. doi:10.1177/0269881109102518.
Williams M, Robinson JL. Binding of the nicotinic cholinergic antagonist, dihydro-beta-erythroidine, to rat brain tissue. J Neurosci: J Soc Neurosci 1984;4:2906–11.
Wonnacott S, Sidhpura N, Balfour DJ. Nicotine: from molecular mechanisms to behaviour. Curr Opin Pharmacol 2005;5:53–9.
Wooters TE, Bevins RA, Bardo MT. Neuropharmacology of the interoceptive stimulus properties of nicotine. Curr Drug Abuse Rev.
Zakharova ES, Danysz W, Bespalov AY. Drug discrimination analysis of NMDA receptor channel blockers as nicotinic receptor antagonists in rats. Psychopharmacology (Berl) 2005;179:128–35.
Zaniewska M, McCreary AC, Przegalinski E, Filip M. Evaluation of the role of nicotinic acetylcholine receptor subtypes and cannabinoid system in the discriminative stimulus effects of nicotine in rats. Eur J Pharmacol 2006;540:96-106.
Zaniewska M, McCreary AC, Sezer G, Przegaliński E, Filip M. Effects of agmatine on nicotine-evoked behavioral responses in rats. Pharmacol Rep 2008;60:645–54.