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Methylphenidate Enhances the Abuse-Related Behavioral Effects of Nicotine in Rats: Intravenous Self-Administration, Drug Discrimination, and Locomotor Cross-Sensitization

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Stimulant drugs, including D-amphetamine, cocaine, and methylphenidate, increase cigarette smoking in controlled human laboratory experiments. Although the mechanism(s) underlying this effect are unknown, it is possible that stimulants may enhance directly the abuse-related effects of nicotine. In the present study, we characterized the behavioral pharmacological interactions between methylphenidate and nicotine in the intravenous self-administration, drug discrimination, and locomotor cross-sensitization procedures. Adult male Sprague–Dawley rats were trained to respond for intravenous nicotine (0.01 or 0.03 mg/kg/infusion) or sucrose, and the acute effects of methylphenidate (1.25–10 mg/kg) were determined; in addition, separate groups of rats were treated with methylphenidate (2.5 mg/kg) or saline before 12 consecutive nicotine (0.03 mg/kg/infusion) self-administration sessions. Next, the discriminative stimulus effects of nicotine (0.03–0.3 mg/kg) and methylphenidate (1.25–10 mg/kg), alone and in combination with a low nicotine dose (0.056 mg/kg), were tested in nicotine-trained rats. Finally, the locomotor effect of repeated methylphenidate (2.5 mg/kg) was tested in rats previously treated with nicotine (0.2–0.8 mg/kg). Results indicated that acute methylphenidate increased the rate of nicotine self-administration at doses that reduced sucrose-maintained responding; furthermore, tolerance to this effect was not apparent following repeated methylphenidate. Methylphenidate, while not substituting for nicotine alone, dose-dependently enhanced the discriminative stimulus effect of a low nicotine dose. In addition, repeated nicotine exposure promoted the development of locomotor sensitization to methylphenidate. Taken together with recent clinical findings, these results suggest that methylphenidate may enhance the abuse-related behavioral effects of nicotine, perhaps increasing vulnerability to tobacco dependence.
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INTRODUCTION

Converging lines of evidence indicate that the use of stimulant drugs, including D-amphetamine and cocaine, is associated with tobacco cigarette smoking. Cigarette smoking is positively correlated with recent cocaine use among college students (Schorling *et al*, 1994), and drug abusers testing positive for recent cocaine use have elevated urinary levels of the nicotine metabolite cotinine (Roll *et al*, 1997). Results from controlled human laboratory experiments also indicate that acute administration of stimulant drugs can directly increase rates of spontaneous cigarette smoking. Relative to placebo, acute administration of oral D-amphetamine or bupropion, or intranasal administration of cocaine, has been shown to dose-dependently increase cigarette smoking (Cousins *et al*, 2001; Henningfield and Griffiths, 1981; Roll *et al*, 1997). In addition, D-amphetamine has been shown to increase choice of cigarettes over money (Tidey *et al*, 2000) and can increase progressive-ratio (PR) break points maintained by cigarettes (Sigmon *et al*, 2003). Taken together, these results suggest that stimulant drugs can promote cigarette smoking in the natural environment, as well as in controlled laboratory settings, perhaps by augmenting the reinforcing efficacy of nicotine. However, since cigarette smoking involves pharmacologic and non-pharmacologic factors in addition to nicotine (Bardo *et al*, 1999; Fowler *et al*, 2003; Rose and Levin, 1991), other interpretations cannot be ruled out.

Although the exact mechanism(s) underlying stimulant-induced increases in cigarette smoking is yet to be elucidated, evidence from preclinical animal studies suggests that the interactive effect of nicotine and stimulants on mesolimbic dopamine function may be involved. In a

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microdialysis study using rats, coadministration of cocaine with nicotine was shown to produce an additive increase in extracellular dopamine levels in the nucleus accumbens (NAcc) relative to administration of nicotine alone (Gerasimov *et al*, 2000a, b). Similarly, behavioral studies have shown that administration of indirect dopamine agonists, such as monoamine oxidase inhibitors (which prevent intracellular metabolism of dopamine) or bupropion (a dopamine reuptake inhibitor), increase nicotine self-administration in rats (Guillem *et al*, 2005, 2006; Rauhut *et al*, 2003). Thus, it is possible that stimulant drugs increase nicotine self-administration, at least in part, by augmenting nicotine-induced increases in extracellular dopamine levels in the NAcc.

Similar to the effects observed with cocaine and amphetamine, a recent report by Rush *et al* (2005) found that methylphenidate also increased cigarette smoking in humans tested in a controlled laboratory setting. In that study, methylphenidate (5–40 mg) dose-dependently increased both the total number cigarettes smoked, as well as positive subjective ratings of cigarette smoking relative to placebo. Specificity of the methylphenidate-induced facilitation of cigarette smoking was also evident, as methylphenidate dose-dependently decreased food consumption (Rush *et al*, 2005). Based on these findings, the current study sought to determine if there is a direct pharmacologic interaction between methylphenidate and nicotine using rat models of drug abuse. First, we assessed the dose effect of acute methylphenidate pretreatment on nicotine self-administration and sucrose-maintained responding. Second, we determined the effect of repeated methylphenidate pretreatment on nicotine self-administration. Third, we examined the discriminative stimulus effects of methylphenidate, both alone and in combination with a low nicotine dose, in rats trained to discriminate nicotine from saline. Finally, we examined the effect of repeated nicotine on subsequent methylphenidate-induced hyperactivity.

MATERIALS AND METHODS

Subjects

Adult male Sprague–Dawley rats (Harlan Industries Inc., Indianapolis, IN, USA), initially weighing 250–275 g, were used in all experiments. Rats used for operant conditioning experiments were housed individually in standard plastic cages, and rats used for the locomotor activity experiment were housed two per cage. For the self-administration and food-maintained responding experiments, rats were allocated 18–20 g of food per day (the daily food allotment for a given rat was held constant for the duration of each experiment). For the drug discrimination experiment, rats were provided sufficient food to maintain body weight at $\sim$350 g. For the locomotor activity experiment, rats were provided *ad lib* access to food. All rats had constant access to water in the home cage. Rats were housed in a temperature- and humidity-controlled colony set to a 14 : 10 light/dark cycle (lights on at 0600), and all experimental procedures were conducted during the light phase. Experimental protocols were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (1996) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

The drug self-administration, sucrose-maintained responding, and drug discrimination experiments were conducted in operant conditioning chambers (ENV-008, Med Associates, St Albans, VT, USA). Each chamber was housed in a sound-attenuating enclosure (ENV-018M, Med Associates) and was connected to a personal computer via an interface (SG-502, Med Associates). A 5×4.2 cm opening that allowed access to a recessed food tray was located on the front panel of the operant conditioning chamber. Two retractable metal response levers were mounted next to the food tray (one on each side) 7.3 cm above a metal-grid floor. A 28 V, 3-cm diameter, white cue light was centered 6 cm above each response lever. For the drug self-administration experiments, an infusion pump (PHM-100, Med Associates) delivered drug infusions via a silastic tube attached to a swivel mounted on the outside of the back wall.

Locomotor activity was monitored with an automated Digiscan animal activity monitoring system (AccuScan Instruments, Columbus, OH, USA). The system consisted of 12 clear Plexiglass chambers ($42 \times 42 \times 30$ cm) made opaque by attaching sheets of white plastic to each outer surface. Each chamber incorporated a horizontal 16×16 grid of photo beam sensors spaced 2.5 cm apart and 7.0 cm above the chamber floor. The activity monitors were interfaced to a personal computer operating Digipro System software (v. 1.40, AccuScan Instruments). Horizontal activity was measured as photo beam interruptions and expressed as distance traveled (cm).

Nicotine Self-Administration

For nicotine self-administration, rats were first trained briefly to lever press for sucrose reinforcement (45 mg BioServ pellets) by pressing an active lever (lever designations were counterbalanced across rats) in the two-lever operant conditioning chamber under a fixed-ratio (FR) 1 schedule of reinforcement in 15-min daily sessions. Over the next six sessions, the response requirement was incremented to an FR5. Training was considered complete following two sessions of responding under the FR5 schedule.

Following completion of the initial pre-training period, rats underwent intravenous catheterization surgery. Briefly, rats were anesthetized with injections of ketamine (60 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). The right jugular vein was then isolated, and one end of a silastic catheter was inserted. The other end of the catheter exited the skin and was secured to the skull by an acrylic head mount. During experimental sessions, a silastic leash protected by flexible metal tubing was used to attach the catheter to the drug infusion pump.

Nicotine self-administration commenced following a 5-day surgical recovery period, and was based on the general method of Corrigall and Coen (1989), with some modifications. Rats were reintroduced to the operant conditioning chamber during daily 60-min sessions. Responses on the active lever (FR1) were recorded and resulted in an infusion

of nicotine (0.01 or 0.03 mg/kg/infusion, delivered in a volume of 100 μl over 5.9 s); responses on the inactive lever were recorded but had no scheduled consequence. Completion of the FR requirement resulted in simultaneous activation of the infusion pump and the cue lights, which signaled a 20-s time-out (TO) period during which responding on either lever had no programmed consequence. Over the next six sessions, the FR1 schedule was gradually increased to a terminal FR5 20-s TO schedule. Rats were trained on the FR5 schedule until responding stabilized for three consecutive sessions according to the following criteria: (1) a minimum of 10 infusions per session, (2) less than 20% variability in the number of infusions earned, and (3) a minimum 2 : 1 active : inactive lever response ratio.

To assess the dose effect of methylphenidate on nicotine self-administration, separate groups of rats were pretreated with methylphenidate (0, 1.25, 2.5, 5, or 10 mg/kg, s.c.; random order within subjects) 10 min before a session in which the unit dose of nicotine was either 0.01 mg/kg/infusion ($n = 7$) or 0.03 mg/kg/infusion ($n = 7$). At least two maintenance sessions (no pretreatment) intervened between each pretreatment session in order to maintain stable nicotine self-administration behavior. To assess the effect of repeated methylphenidate on nicotine self-administration (0.03 mg/kg/infusion), separate groups of rats were pretreated 10 min before each of 12 consecutive sessions with methylphenidate (2.5 mg/kg, s.c.; $n = 8$) or saline ($n = 6$). Following drug pretreatment sessions, both groups of rats were pretreated with saline for three additional nicotine self-administration sessions.

Sucrose-Maintained Responding

In order to use procedures similar to the nicotine self-administration experiments, a separate group of rats ($n = 8$) was trained to lever press for sucrose reinforcement during daily 60-min sessions under an FR5 20-s TO and was allocated the same amount of daily food as the nicotine self-administration rats; however, these rats did not undergo surgery. In addition, since sucrose reinforcement maintains higher response rates than nicotine, these rats were given 25 sucrose pellets in the home cage 15 min before each experimental session in an attempt to reduce response rates by partial satiation. Rats were monitored to ensure that all pellets were consumed before each session. Although insertion of a long TO is sometimes used to decrease food reinforcement rate (Paterson *et al*, 2003), we did not use this procedure because it can impose an artificial ceiling effect which would not be sensitive to potential increases in the rate of responding. Training continued until stable responding was obtained, defined as (1) a minimum of 10 pellets earned per session, (2) less than 20% variability in the number of pellets earned, and (3) a minimum 2 : 1 active : inactive lever response ratio. The effect of methylphenidate (0, 1.25, 2.5, 5, or 10 mg/kg, s.c.; random order within subjects) given 10 min before the sessions was then determined. At least two maintenance sessions (no pretreatment) intervened between each pretreatment session in order to maintain stable sucrose-maintained behavior.

Nicotine Drug Discrimination

Rats ($n = 6$) were trained initially to lever press for sucrose reinforcement under an FR1 schedule. The FR requirement for reinforcement was subsequently increased over several sessions to a terminal FR10. In order to enhance acquisition of the nicotine–saline discrimination, only one lever (the saline-appropriate lever; counterbalanced across rats) was presented during these initial sessions. Once rats responded for two sessions under the FR10 schedule, nicotine discrimination training began. In this phase, nicotine (0.3 mg/kg, s.c.) or saline was administered before each session. Ten minutes after nicotine or saline was administered, rats were placed in the operant conditioning chamber, and the cue lights were illuminated to signal the beginning of the session and they remained illuminated for the duration of the session. When nicotine was administered, the nicotine-appropriate lever was presented and the saline-appropriate lever was removed. When saline was administered, the saline-appropriate lever was presented and the nicotine-appropriate lever was removed. For half of the rats, the left lever was designated the nicotine-appropriate lever and the right lever was designated the saline lever; the reverse was true for the remaining rats. Nicotine and saline were administered according to a double-alternation sequence (ie NNSSNN or SSNNSS, counterbalanced across rats) for eight consecutive sessions. Then, for the remainder of the study, both levers were presented each day, and responding on the injection-appropriate lever was reinforced according to the FR10 schedule; responses on the incorrect lever were recorded but had no programmed consequence. Training sessions were 15 min in duration. Training continued until the following criteria were met on eight consecutive sessions: (1) $\geqslant$85% of the total session responses occurred on the injection-appropriate lever and (2) first FR10 was completed on the injection-appropriate lever. Once these criteria were met, the test phase was initiated.

During the test phase, sessions were 3 min in length; on these sessions, completion of an FR10 on either lever was reinforced with sucrose. Each test session was separated by at least two 15-min training sessions in which saline and the nicotine training dose were administered once each (random order). Test sessions were conducted only if baseline performance during the intervening training sessions remained stable (ie $\geqslant$85% of the total session responses occurred on the injection-appropriate lever and the first FR10 was completed on the injection-appropriate lever). Two dependent measures were collected during test sessions: (1) percentage of total responses occurring on the nicotine-appropriate lever (calculated as the number of responses on the nicotine-appropriate lever divided by the total number of responses on either lever) and (2) rate of responding (calculated as the total number of responses on either lever divided by 180 s). During test sessions, a dose–effect curve for nicotine (0.03, 0.056, 0.1, 0.17, and .3 mg/kg, s.c.; random order within subjects) was determined first, followed by determination of dose–effect curves for methylphenidate (1.25, 2.5, 5, and 10 mg/kg, s.c.; random order within subjects) administered alone or in combination with a subthreshold nicotine dose (0.056 mg/kg). Each drug dose that was tested alone was administered 10 min

before the test session; in the drug combination experiment, methylphenidate (1.25, 2.5, 5, and 10 mg/kg, s.c.; random order within subjects) was administered 15 min before the session, and nicotine (0.056 mg/kg) was administered 10 min before the start of the session.

Locomotor Effect of Methylphenidate in Nicotine-Sensitized Rats

Rats were placed individually for 60 min in the locomotor activity apparatus for a single habituation day. Each rat was then assigned randomly to receive repeated injections of a single dose of nicotine (0.2, 0.4, or 0.8 mg/kg, s.c.) or saline. Nicotine or saline injections were administered for 10 consecutive days (days 1–10), beginning on the day following the habituation session. After each injection, rats were placed immediately in the activity monitors for 60 min. Following the 10-day nicotine treatment period, rats remained in their home cages for a 14-day (days 11–24) drug-free period. Rats from each nicotine dose treatment group were then assigned randomly to receive challenge injections of methylphenidate (2.5 mg/kg) or saline immediately before placement in the activity monitors for 60 min for three consecutive sessions (days 25–27). The dependent measure was distance traveled (cm).

Drugs

S(−)-nicotine ditartrate (Sigma-Aldrich, St Louis, MO) and methylphenidate HCl (Mallinckrodt, St Louis, MO) were prepared in 0.9% NaCl (saline). Dilute NaOH was added to the nicotine solution until a pH of 7.4 was attained. The nicotine doses are expressed as the base weight and the methylphenidate doses are expressed as the salt weight.

Statistical Analyses

A one-way repeated-measures analysis of variance (ANOVA) (with dose as the within-subjects factor) was used to evaluate dose–effect curves in the acute nicotine self-administration, sucrose-maintained responding, and drug discrimination experiments. In cases where the dose effect attained statistical significance, Dunnett's *post hoc* tests were conducted to compare each dose to the corresponding saline control. Specifically, the dose effects of methylphenidate in rats self-administering either 0.01 or 0.03 mg/kg/infusion unit doses of nicotine were compared to the effect of saline pretreatment within the same groups. In the repeated nicotine self-administration and locomotor activity experiments, data were analyzed with mixed-factor ANOVA, with pretreatment dose serving as a between-subjects factor and session serving as a within-subjects factor. When data from experiments designed to test *a priori* hypotheses were analyzed, one-tailed tests were used. Where appropriate, the Newman–Keuls *post hoc* test was used to make multiple comparisons; in those instances, a conservative alpha level of $p \leqslant 0.01$ was used as the criteria for statistical significance to control for type I errors. In all other cases, $p \leqslant 0.05$ determined significance. In drug discrimination, the nicotine dose estimated to produce 50% nicotine-appropriate responding (ie the ED_{50} with 95% confidence intervals, expressed in mg/kg) was calculated using nonlinear regression of individual data points.

RESULTS

Dose Effect of Methylphenidate on Nicotine Self-Administration and Sucrose-Maintained Responding

Figure 1 illustrates the dose effect of methylphenidate on nicotine self-administration using two different unit doses (left panel) and on sucrose-maintained responding (right panel). Baseline responding for nicotine was higher in rats earning a unit dose of 0.01 mg/kg/infusion than in rats earning a unit dose of 0.03 mg/kg/infusion. At the 0.03 mg/kg/infusion unit dose, ANOVA revealed a significant main effect of methylphenidate dose ($F_{4,25} = 2.90$, $p < 0.05$). *Post hoc* tests indicated that 2.5 and 5 mg/kg of methylphenidate significantly increased the number of nicotine infusions

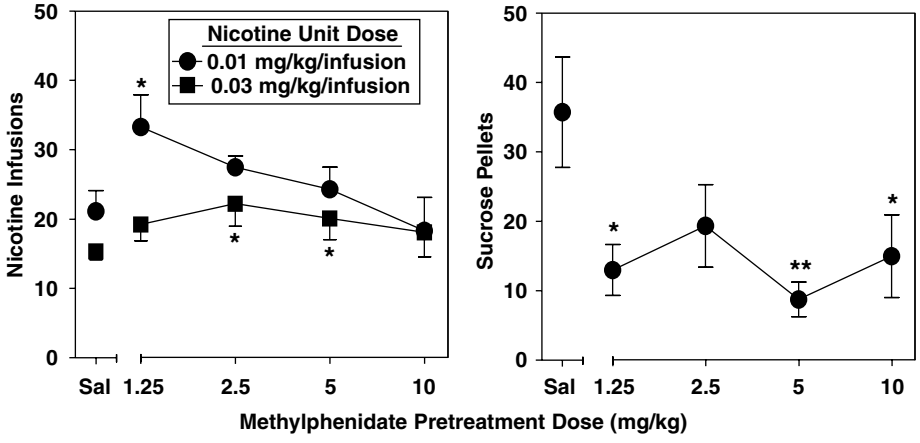

Figure 1 Dose effects of methylphenidate pretreatment on nicotine self-administration using two different unit doses (left panel; $n = 7$ per group) and on sucrose-maintained responding (right panel; $n = 8$). Data points represent the mean (±SEM) number of reinforcers earned per session as a function of methylphenidate pretreatment dose. *Indicates a significant difference from the effect of saline (Sal) on self-administration of the corresponding nicotine unit dose or on sucrose-maintained responding (*$p < 0.05$, **$p < 0.01$).

earned relative to saline pretreatment. At the 0.01 mg/kg/infusion unit dose, ANOVA revealed a significant main effect of methylphenidate dose ($F_{4,25} = 2.55$, $p < 0.05$). *Post hoc* tests indicated that 1.25 mg/kg of methylphenidate significantly increased the number of nicotine infusions earned relative to saline pretreatment. For sucrose-maintained responding, ANOVA revealed a significant main effect of methylphenidate dose ($F_{4,28} = 3.41$, $p < 0.05$). *Post hoc* tests indicated that pretreatment with 1.25, 5, and 10 mg/kg of methylphenidate significantly decreased the number of pellets earned relative to saline pretreatment.

Effect of Repeated Methylphenidate on Nicotine Self-Administration

Figure 2 illustrates the effect of repeated pretreatment with methylphenidate (2.5 mg/kg) on nicotine self-administration (0.03 mg/kg/infusion). A mixed-factor, repeated-measures ANOVA across all sessions (3 baseline, 12 pretreatment, 3 saline alone) revealed a significant pretreatment × day interaction ($F_{17,204} = 1.969$; $p < 0.05$), indicating that pretreatment with methylphenidate, but not saline, increased nicotine self-administration. The effect of repeated methylphenidate was subsequently assessed by conducting both within-subject and between-subject analyses. *Post hoc* within-subject comparisons indicated that methylphenidate-treated rats self-administered significantly more nicotine infusions on pretreatment days 1, 2, 3, 5, 6, 7, 8, 9, and 12, as well as on the first saline pretreatment day (day 13), relative to their baseline levels. *Post hoc* within-subject comparisons of saline-treated rats revealed a significant decrease in the number of nicotine infusions earned on pretreatment day 12, relative to their baseline levels. *Post hoc* between-group comparisons also indicated that methylphenidate-treated rats self-administered significantly more nicotine infusions than saline-treated rats on pretreatment days 1, 2, 3, 7, 8, 11, and 12, as well as on the first saline pretreatment day (day 13).

In order to examine whether the methylphenidate-induced increase in nicotine self-administration was due to a general increase in lever pressing, a mixed-factor, repeated-measures ANOVA was also conducted on the inactive lever response data (results not shown). This analysis revealed no significant main effects or interactions, indicating that the methylphenidate-induced increase in responding was specific to the active lever.

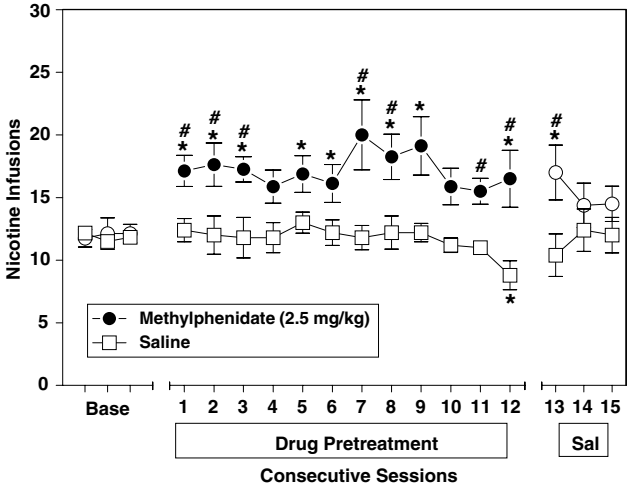

Figure 2 Effect of repeated pretreatment with methylphenidate (2.5 mg/kg; $n = 8$) or saline ($n = 6$) on nicotine self-administration at a unit dose of 0.03 mg/kg/infusion. Data points represent the mean (±SEM) number of reinforcers earned across 3 baseline (Base), 12 drug pretreatment, and 3 saline (Sal) sessions. *Indicates a significant difference from the number of infusions earned during the respective final baseline session (*$p < 0.01$). #Indicates a significant difference from the number of infusions earned by saline-treated rats on the corresponding session (#$p < 0.01$).

Discriminative Stimulus Effects of Methylphenidate in Nicotine-Trained Rats

Figure 3 illustrates the dose effects of nicotine (left panels) and methylphenidate, alone or in combination with nicotine (right panels), on responding in rats trained to discriminate nicotine from saline. In substitution testing, nicotine (0.03–0.3 mg/kg) produced dose-dependent and full generalization, with an ED_{50} of 0.08 (0.05–0.12) mg/kg. The main effect of nicotine dose attained statistical significance ($F_{5,35} = 21.88$, $p < 0.001$), with *post hoc* tests indicating that 0.1, 0.17, and 0.3 mg/kg of nicotine elicited significantly greater nicotine-appropriate responding than saline control values; response rates were not disrupted significantly by any nicotine dose. Conversely, methylphenidate did not elicit nicotine-appropriate responding at any dose when administered alone. However, when methylphenidate was coadministered 5 min before a subthreshold dose of nicotine that did not elicit nicotine-appropriate responding by itself (0.056 mg/kg), a significant dose-dependent increase in responding on the nicotine-appropriate lever was obtained ($F_{4,29} = 5.50$, $p < 0.01$). *Post hoc* tests indicated that, relative to saline, significant increases in nicotine-appropriate responding were obtained following administration of 5 and 10 mg/kg of methylphenidate in combination with 0.056 mg/kg of nicotine. Coadministration of nicotine with the 5 and 10 mg/kg doses of methylphenidate also produced significantly greater levels of nicotine-appropriate responding relative to these doses alone. Response rates were not disrupted significantly by any methylphenidate dose, whether administered alone or in combination with nicotine.

Effect of Repeated Nicotine on the Development of Methylphenidate Locomotor Sensitization

Figure 4 illustrates the dose effect of repeated nicotine on locomotor activity. Across the repeated treatment phase, nicotine produced progressive increases in locomotor activity, an effect indicative of sensitization. A mixed-factor, repeated-measures ANOVA of these data revealed a significant nicotine dose × day interaction ($F_{27,441} = 11.07$, $p < 0.001$). On day 1, 0.8 mg/kg of nicotine produced significant hypoactivity relative to saline. Across treatment days, 0.4 mg/kg of nicotine produced a significant increase in activity by day 3 and all nicotine doses elicited a significant increase in locomotor activity on days 5–10 relative to saline.

Figure 5 illustrates the dose effect of repeated nicotine on subsequent challenge injections of either saline (upper

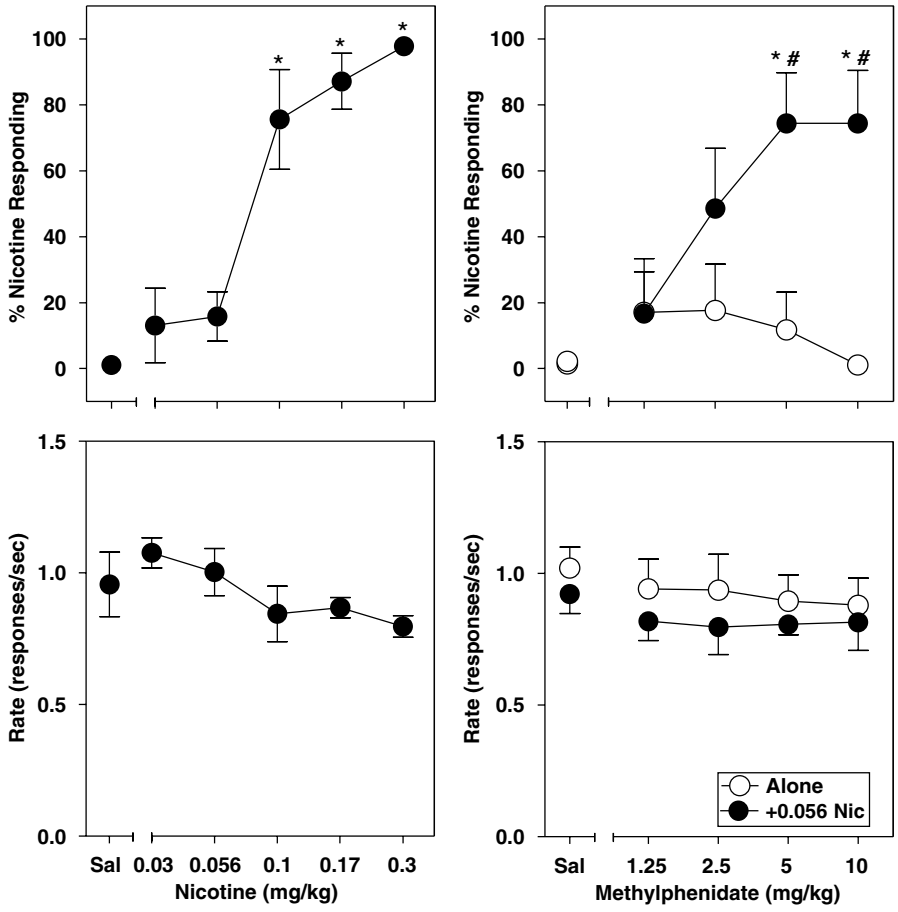

Figure 3 Discriminative stimulus and rate effects of nicotine (0.03–0.3 mg/kg, left panel) and methylphenidate (1.25–10 mg/kg, right panel) in rats ($n = 6$) trained to discriminate nicotine (0.3 mg/kg) from saline. Data points in the upper panels represent the mean (±SEM) percentage of responses occurring on the nicotine-appropriate lever as a function of dose, whereas data points in the lower panels represent the mean (±SEM) rate of responding (responses per second) as a function of dose. *Indicates a significant difference from the saline (Sal) control values (*$p < 0.01$). #Indicates a significant difference relative to the same methylphenidate dose given alone (#$p < 0.05$).

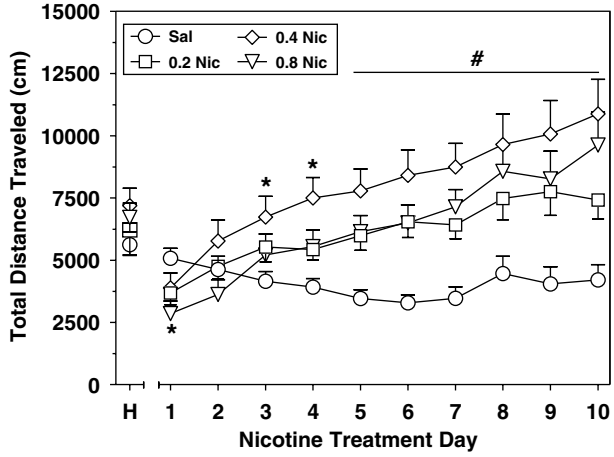

Figure 4 Dose effect of nicotine on locomotor activity across the 10-day repeated treatment phase ($n = 16$ rats per dose). Rats were given 1 habituation day (designated as 'H') before the 10 treatment days. Data are expressed as the mean (±SEM) total distance traveled (cm) per day. *Indicates a significant difference from the saline control value on the corresponding treatment day (*$p < 0.01$). #Indicates a significant difference from saline for each nicotine dose across days 5–10 (#$p < 0.01$).

panel) or 2.5 mg/kg of methylphenidate (lower panel). A mixed-factor ANOVA (using prior nicotine treatment and challenge drug as between-subjects factors and challenge day as the within-subjects factor) revealed a significant challenge drug × day interaction ($F_{2,100} = 9.3$, $p < 0.001$). Subsequent analysis of the saline challenge data indicated that prior nicotine treatment did not alter activity in response to saline on any challenge day. Analysis of the methylphenidate challenge data indicated that prior nicotine treatment also did not alter significantly the initial response to methylphenidate on day 1. However, by day 3, ANOVA revealed a significant main effect of prior nicotine treatment ($F_{3,25} = 4.02$, $p < 0.05$), indicating that exposure to nicotine promoted the induction of locomotor sensitization to methylphenidate. *Post hoc* Dunnett's tests indicated that rats treated previously with 0.8 mg/kg of nicotine exhibited significantly greater activity following methylphenidate relative to rats treated previously with saline ($p < 0.01$). To explore this latter finding further, the time course effect of methylphenidate on challenge days 1 and 3 in the rats treated previously with saline or nicotine (0.8 mg/kg) was compared (Figure 6); note that these data points reflect the

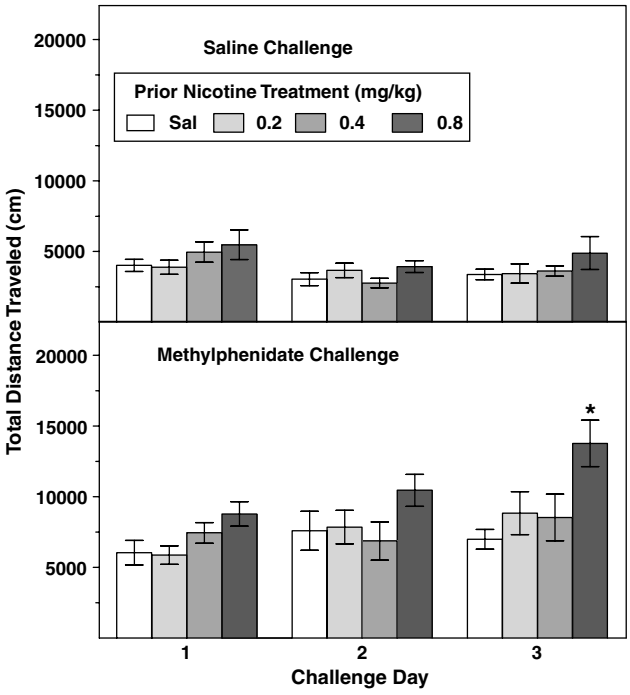

Figure 5 Effects of three consecutive challenge administrations of saline (upper panel) and methylphenidate (2.5 mg/kg; lower panel) on locomotor activity in separate groups of rats treated previously with nicotine (0.2–0.8 mg/kg) or saline ($n = 8$ per group). Data are expressed as the mean (±SEM) distance traveled (cm) during each challenge day as a function of prior nicotine treatment dose. *Indicates a significant difference from the saline control value on the corresponding challenge day ($*p < 0.05$).

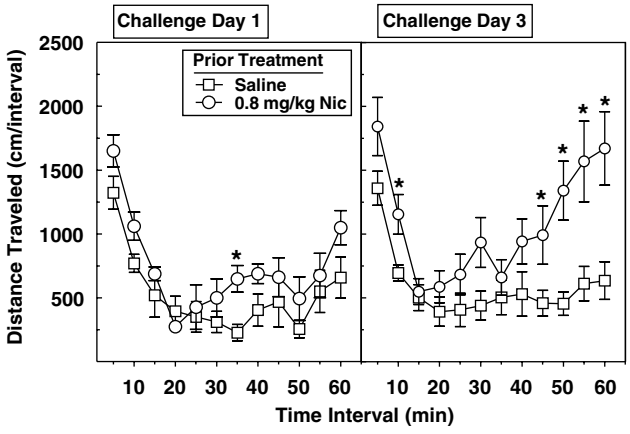

Figure 6 Time course effect of a challenge injection of methylphenidate (2.5 mg/kg) on day 1 (left panel) and day 3 (right panel) in rats treated previously with saline ($n = 8$) or nicotine (0.8 mg/kg; $n = 8$). Data are expressed as the mean (±SEM) distance traveled (cm) during each 5-min time interval. *Indicates a significant difference from saline-treated rats during the corresponding time interval ($*p < 0.01$).

time course effect of the total activity data of rats treated previously with saline and 0.8 mg/kg of nicotine during challenge days 1 and 3 presented in the lower portion of Figure 5. A mixed-factor ANOVA revealed a significant nicotine treatment × time interval × challenge day interaction, $F_{11,132} = 2.03$, $p < 0.05$. *Post hoc* analysis indicated that, whereas nicotine-treated rats were significantly more active following methylphenidate at only the 35-min time point relative to saline-treated rats on day 1 (Figure 6, left panel), nicotine-treated rats were significantly more active at the 10 and 45–60 min time points on day 3 (Figure 6, right panel). Thus, although the data from day 1 provided little evidence of cross-sensitization to the initial methylphenidate challenge, prior exposure to 0.8 mg/kg of nicotine promoted the induction of sensitization following repeated methylphenidate injections.

DISCUSSION

The results of the present preclinical investigation demonstrate that methylphenidate enhances the abuse-related behavioral effects of nicotine in rats as assessed in the intravenous self-administration, drug discrimination, and locomotor cross-sensitization procedures. Acute pretreatment with methylphenidate (1.25–10 mg/kg) increased nicotine self-administration, although this effect differed depending on the nicotine unit dose available. In rats self-administering a low unit dose of nicotine (0.01 mg/kg/infusion), an increase in the number of infusions earned was obtained following pretreatment with 1.25 mg/kg of methylphenidate, but not following pretreatment with higher methylphenidate doses (2.5–10 mg/kg). In rats self-administering a higher unit dose of nicotine (0.03 mg/kg/infusion), an increase in the number of infusions earned was obtained only following 2.5 and 5 mg/kg of methylphenidate. These results indicate that as the unit dose of nicotine is increased, a greater pretreatment dose of methylphenidate is needed to enhance responding. However, since there were no differences in the effect of pretreatment with 10 mg/kg of methylphenidate between rats self-administering either nicotine unit dose, it is possible that high doses of stimulants can alter nicotine self-administration nonspecifically. While the precise reason(s) for this biphasic pattern of results is unknown, Rauhut *et al* (2003) found similar biphasic dose effects of acute bupropion or methamphetamine pretreatment on nicotine self-administration in rats. Prada and Goldberg (1985) also reported that caffeine pretreatment increased nicotine self-administration in squirrel monkeys at low doses (3–10 mg/kg), while higher doses (60–100 mg/kg) decreased response rates. It is possible that the descending limb of the biphasic dose effect curve for stimulant drugs on nicotine self-administration is due to nonspecific behavioral impairment or to the induction of stereotypic behavior that is incompatible with lever pressing. Thus, the nonspecific effect of 10 mg/kg of methylphenidate may have masked the change in responding that normally occurs when the unit dose of nicotine is altered, thereby eliminating the difference between the two nicotine self-administration groups.

Interestingly, the dose-dependent increase in nicotine self-administration following acute methylphenidate did not appear to undergo tolerance, as repeated administration of 2.5 mg/kg of methylphenidate continued to increase self-administration of nicotine (0.03 mg/kg/infusion) across 12 consecutive sessions. Nicotine self-administration returned to control levels across a subsequent 3-day period of saline

administration, indicating that the effect of methylphenidate was reversible. The lack of tolerance to repeated methylphenidate is comparable to studies demonstrating that tolerance does not develop to the effects of repeated administration of bupropion on nicotine self-administration. Specifically, Shoaib *et al* (2003) demonstrated that repeated administration of 30 mg/kg of bupropion continued to increase nicotine self-administration across a 28-day treatment period and Rauhut *et al* (2005) reported that the acute decrease in nicotine self-administration produced by administration of 70 mg/kg of bupropion did not undergo tolerance across a 14-day treatment period.

Although these results indicate that low doses of methylphenidate can specifically increase nicotine self-administration under an FR schedule of reinforcement, the exact mechanisms underlying this effect are not known. One potential interpretation is that methylphenidate attenuated the reinforcing effect of nicotine, thus leading to an increase in nicotine intake in an attempt to surmount the attenuation in reinforcing effectiveness (Yokel, 1987). However, given that prior work has demonstrated that selective nicotinic acetylcholine receptor antagonists such as mecamylamine and dihydro-β-erythriodine generally fail to increase nicotine self-administration (Corrigall and Coen, 1989; Watkins *et al*, 1999; but see Fattore *et al* (2002) for evidence of a mecamylamine-induced increase in nicotine self-administration), it seems unlikely that methylphenidate attenuated the reinforcing effect of nicotine. Although an FR schedule was used in the present study, assessing the effects of methylphenidate pretreatment on nicotine self-administration under a PR schedule could help to clarify the mechanisms underlying the rate-increasing effect of methylphenidate on nicotine self-administration observed using an FR schedule (Arnold and Roberts, 1997; Richardson and Roberts, 1996). Alternatively, since nicotine infusions were signaled by illumination of cue lights, methylphenidate may have augmented the response to the visual stimuli known to play an important role in nicotine self-administration behavior in rats (Caggiula *et al*, 2001, 2002; Palmatier *et al*, 2006). Consistent with this notion, Files *et al* (1989) reported that methylphenidate (5–20 mg/kg) increased response rates during extinction (ie when food reinforcement was no longer available) if responding resulted in presentation of conditioned stimuli paired previously with food delivery, but not when responding occurred without the presentation of food-paired stimuli. Likewise, methylphenidate has been shown to selectively increase response rates maintained by a water-paired conditioned stimulus, while decreasing response rates in the absence of a water-paired stimulus (Robbins, 1978). Regardless of the interpretation, however, given that methylphenidate selectively increased responding for nicotine, but not sucrose, it appears that methylphenidate can differentially alter the incentive motivation for a drug and nondrug reinforcer. This conclusion parallels the recent clinical work of Rush *et al* (2005) showing that oral methylphenidate increases tobacco smoking in humans tested in a controlled laboratory environment, while decreasing food consumption.

To our knowledge, this is the first report to examine the discriminative stimulus effect of methylphenidate in nicotine-trained rats. Although nicotine produced dose-dependent substitution, methylphenidate did not elicit appreciable levels of nicotine-appropriate responding at any dose. However, when coadministered with a subthreshold dose of nicotine (0.056 mg/kg), methylphenidate produced a dose-dependent increase in nicotine-appropriate responding, although the maximal effect of methylphenidate did not meet criteria used traditionally for declaring full substitution (ie $\geqslant$80% nicotine-appropriate responding). This augmentation in the effect of a low nicotine dose by methylphenidate is similar to results reported by Gasior *et al* (2002), who examined the discriminative stimulus effects of caffeine in nicotine-trained rats. In that report, rats were trained to discriminate nicotine (0.4 mg/kg) from saline, and dose–effect curves were generated for nicotine and caffeine, both alone and in combination with a subthreshold dose of nicotine (0.05 mg/kg). Similar to the present results, caffeine administered alone did not elicit nicotine-appropriate responding at any dose; however, when combined with a low dose of nicotine, caffeine dose-dependently generalized to the nicotine cue (Gasior *et al*, 2002). The fact that methylphenidate did not substitute for nicotine when given alone contrasts with other reports showing that the discriminative stimulus effects of other classical stimulant drugs overlap, at least partially, with the nicotine cue. Bardo *et al* (1997) reported that nicotine substituted partially for D-amphetamine, and Desai *et al* (1999, 2003) reported that nicotine substituted fully for cocaine; in addition, both amphetamine and cocaine substitute partially for the nicotine cue (Mansbach *et al*, 1998). Regarding bupropion, some reports have found that bupropion substitutes partially (Desai *et al*, 2003) or fully (Wiley *et al*, 2002; Young and Glennon, 2002) for nicotine in generalization tests; however, a range of bupropion pretreatment doses failed to shift the nicotine dose–response curve (Shoaib *et al*, 2003), and mecamylamine pretreatment blocks the discriminative stimulus effects of nicotine, but not bupropion (Wiley *et al*, 2002). Thus, while the discriminative stimulus effects of nicotine and stimulant drugs, including methylphenidate, appear to overlap to some extent, there is sufficient evidence to conclude that the cues produced by these drugs are dissociable.

Consistent with other reports (Dwoskin *et al*, 1999; LeSage *et al*, 2006), the results of the locomotor activity experiment revealed that nicotine initially induced hypoactivity, followed by hyperactivity across repeated injections. More important, although there was little evidence indicative of cross-sensitization to the initial methylphenidate challenge following nicotine exposure, nicotine pre-exposure promoted the induction of locomotor sensitization following three repeated methylphenidate injections. The failure to find cross-sensitization between repeated nicotine and acute methylphenidate contrasts with evidence of cross-sensitization between nicotine and other stimulant drugs. Nicotine pre-exposure enhances the subsequent locomotor stimulant effect of acute amphetamine (Birrell and Balfour, 1998), bupropion (Wilkinson *et al*, 2006), cocaine (Collins and Izenwasser, 2004), and methamphetamine (Kuribara, 1999). While the explanation for these dissociable effects among stimulants is not known, the nicotine-induced enhancement in locomotor sensitization with repeated methylphenidate indicates at least a partial overlap in the

mechanisms underlying both nicotine and methylphenidate sensitization.

Although the present study does not address the neuropharmacological mechanisms underlying the interactive behavioral effects of methylphenidate and nicotine, it is possible that the mesolimbic dopamine pathway, a brain circuit implicated in drug reward (Bardo, 1998; Leshner and Koob, 1999; Wise, 1998), is involved. Nicotine increases dopaminergic neurotransmission by activation of high-affinity $\beta 2$ subunit-containing nicotinic cholinergic receptors localized on dopamine cell bodies in the ventral tegmental area (VTA), as well as by altering the tone of γ-amino butyric acid and glutamate inputs to the VTA (Mansvelder and McGehee, 2002; Mansvelder *et al*, 2003); the net effect is an increase in extracellular dopamine levels in the NAcc (Pontieri *et al*, 1996). In contrast, methylphenidate, a dopamine and norepinephrine transport inhibitor, does not release dopamine directly, but rather prevents dopamine clearance in the NAcc and other corticolimbic structures, thus yielding a net increase in extracellular dopamine in those terminal fields (Grace, 2001). As these mechanisms would suggest, it has been shown that coadministration of methylphenidate augments nicotine-induced increases in extracellular dopamine content in the NAcc (Gerasimov *et al*, 2000a, b), suggesting that the interactive effects of these drugs on mesolimbic dopamine transmission underlies the behavioral interactions noted in the present study. Regarding self-administration specifically, blockade of central dopamine receptors reduces nicotine self-administration (Corrigall and Coen, 1991; Corrigall *et al*, 1992), whereas administration of monoamine oxidase inhibitors or the dopamine reuptake inhibitor bupropion increases nicotine self-administration (Guillem *et al*, 2005, 2006; Rauhut *et al*, 2003). One caveat to this interpretation is that methylphenidate and bupropion are relatively nonselective inhibitors of the norepinephrine and dopamine transporters (Dwoskin *et al*, 2006; Han and Gu, 2006). While this suggests that both dopaminergic and noradrenergic mechanisms may be involved, a role for norepinephrine seems unlikely given that reboxetine, a selective norepinephrine uptake inhibitor, does not increase nicotine self-administration in rats (Rauhut *et al*, 2002). Thus, it appears that an enhanced response to nicotine-evoked dopamine release in reward-relevant limbic terminal fields following methylphenidate administration may underlie the increase in nicotine self-administration.

Similarly, locomotor sensitization may also reflect alterations in dopamine transmission, as an enhanced dopamine response following repeated administration of psychostimulant drugs, including nicotine, is linked with the expression of behavioral sensitization (Benwell and Balfour, 1992; Pierce and Kalivas, 1997; Vezina, 2004). Interestingly, nicotine can augment dopamine transporter function (Middleton *et al*, 2004) and density (Harrod *et al*, 2004), suggesting that enduring nicotine-induced alterations in dopamine transporter function and/or density may play a role in the enhanced effect of repeated methylphenidate following nicotine treatment.

In drug discrimination, methylphenidate pretreatment may have also enhanced the discriminative stimulus effect of a low nicotine dose by enhancing the dopaminergic effect of nicotine. Caffeine, which augments extracellular dopamine levels, has been reported to potentiate the discriminative stimulus effect of a low nicotine dose (Gasior *et al*, 2002), suggesting that a similar mechanism may be implicated in the present drug discrimination results. However, evidence of a primary role for dopamine in mediating the nicotine cue is lacking, as selective dopamine antagonists generally fail to alter nicotine discrimination or attenuate nicotine discrimination only at doses that suppress response rates (Brioni *et al*, 1994; Corrigall and Coen, 1994; Le Foll *et al*, 2005). Since methylphenidate also elevates extracellular levels of acetylcholine in addition to dopamine (Tzavara *et al*, 2006), it may be that methylphenidate enhances nicotine's discriminative stimulus effects via a primarily nondopaminergic mechanism.

From a clinical perspective, it is interesting to note that methylphenidate (Ritalin®) is the most commonly prescribed pharmacotherapy for attention-deficit/hyperactivity disorder (ADHD), a behavioral disorder characterized by excessive levels of activity, impulsivity, and inattention (Arnsten, 2006; Solanto *et al*, 2001; Volkow *et al*, 2005). Methylphenidate is an effective treatment for the behavioral and cognitive deficits associated with ADHD when administered orally at therapeutic doses (Greenhill, 2001; Volkow and Swanson, 2003; Biederman *et al*, 2004). Thus, one potential caveat of the present study is that methylphenidate was administered via subcutaneous injections, which may have produced a more rapid onset of action than orally administered methylphenidate as used in clinical settings. A study by Gerasimov *et al* (2000a, b) demonstrated that orally administered methylphenidate in rats is less potent in stimulating locomotor activity and increasing DA levels in the NAcc relative to the same methylphenidate doses administered via intraperitoneal injection; whether the same holds true for subcutaneous injections is yet to be determined. Another potential caveat is that the dose range of methylphenidate used in the present study included doses higher than those used clinically. Kuczenski and Segal (2005) advocated for the use of lower doses of methylphenidate (ie $\leqslant 3$ mg/kg) in preclinical studies in order to approximate clinically relevant plasma levels of methylphenidate. While some of the methylphenidate doses used in the present study (ie 5 and 10 mg/kg) likely exceeded clinical doses, the lower doses evaluated (ie 1.25 and 2.5 mg/kg) are thought to fall within the therapeutic dose range. Importantly, these lower doses produced the greatest increase in nicotine self-administration, suggesting that the present results have clinical relevance.

In addition to the present results showing that methylphenidate alters the behavioral effects of nicotine in adult rats, developmental exposure to methylphenidate has been shown to alter the response to cocaine in adult rats. Administration of methylphenidate to periadolescent rats produces changes in subsequent cocaine self-administration (Brandon *et al*, 2001), cocaine-conditioned place preference (Andersen *et al*, 2002; Carlezon *et al*, 2003), and cocaine-induced hyperactivity (Torres-Reveron and Dow-Edwards, 2005) in adulthood. While the present findings demonstrate that methylphenidate can increase nicotine self-administration in adult rats, there is evidence that the behavioral effects of methylphenidate can differ between adolescent and adult rats (Andersen, 2005; Torres-Reveron and Dow-Edwards, 2005; Wooters *et al*, 2006). Since methylphenidate

is commonly administered to children and adolescents in clinical settings, an important avenue for future research will be to determine the effects of developmental exposure to low doses of oral methylphenidate on subsequent acquisition of nicotine self-administration.

Individuals diagnosed with ADHD smoke cigarettes at a higher rate than the general population (Barkley *et al*, 1990; Kollins *et al*, 2005; Rohde *et al*, 2004). Although the exact reason(s) for this relationship is yet to be determined, one potential explanation is that tobacco smoking represents a form of self-medication in the ADHD population. Nicotine has comparable therapeutic effects to methylphenidate in adolescents (Potter and Newhouse, 2004) and adults (Connors *et al*, 1996; Levin *et al*, 2001) diagnosed with ADHD, raising the possibility that tobacco smoking in the ADHD population is maintained by the positive behavioral effects of nicotine. Alternatively, given the widespread exposure to methylphenidate in ADHD-diagnosed individuals, there has been interest in determining whether methylphenidate exposure increases the risk for smoking. In one prospective longitudinal study, Lambert and Hartsough (1998) reported that 93% of adult smokers diagnosed with ADHD during childhood who were treated with stimulants were daily smokers, compared to only 80% of ADHD-diagnosed smokers who were not treated previously with stimulant drugs. Moreover, methylphenidate has been shown to increase tobacco cigarette smoking in a controlled human laboratory experiment (Rush *et al*, 2005). The present preclinical results extend this previous work by providing evidence of a direct potentiating interaction between the abuse-related behavioral effects of methylphenidate and nicotine. Taken together, these results suggest that caution may be warranted when prescribing methylphenidate to cigarette smokers with ADHD.

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DISCLOSURE/CONFLICT OF INTEREST

The authors do not have any conflict of interests (financial or otherwise) that might bias this work.

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