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Galantamine, an Acetylcholinesterase Inhibitor and Positive Allosteric Modulator of Nicotinic Acetylcholine Receptors, Attenuates Nicotine Taking and Seeking in Rats

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Current smoking cessation pharmacotherapies have limited efficacy in preventing relapse and maintaining abstinence during withdrawal. Galantamine is an acetylcholinesterase inhibitor that also acts as a positive allosteric modulator of nicotinic acetylcholine receptors. Galantamine has recently been shown to reverse nicotine withdrawal-induced cognitive impairments in mice, which suggests that galantamine may function to prevent relapse in human smokers. However, there are no studies examining whether galantamine administration modulates nicotine self-administration and/or reinstatement of nicotine seeking in rodents. The present experiments were designed to determine the effects of galantamine administration on nicotine taking and reinstatement of nicotine-seeking behavior, an animal model of relapse. Moreover, the effects of galantamine on sucrose-maintained responding and sucrose seeking were also examined to determine whether galantamine's effects generalized to other reinforced behaviors. An inverted U-shaped dose-response curve was obtained when animals self-administered different unit doses of nicotine with the highest responding for 0.03 mg/kg per infusion of nicotine. Acute galantamine administration (5.0 mg/kg, i.p.) attenuated nicotine self-administration when animals were maintained on either a fixed-ratio 5 (FR5) or progressive ratio (PR) schedule of reinforcement. Galantamine administration also attenuated the reinstatement of nicotine-seeking behavior. No significant effects of galantamine on sucrose self-administration or sucrose reinstatement were noted. Acetylcholinesterase inhibitors have also been shown to produce nausea and vomiting in humans. However, at doses required to attenuate nicotine self-administration, no effects of galantamine on nausea/malaise as measured by pica were noted. These results indicate that increased extracellular acetylcholine levels and/or nicotinic acetylcholine receptor stimulation is sufficient to attenuate nicotine taking and seeking in rats and that these effects are reinforcer selective and not due to adverse malaise symptoms such as nausea.

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INTRODUCTION

Approximately 45 million Americans smoke tobacco products and 70–80% of smokers relapse within 6–12 months of quitting (Benowitz, 2010). This represents a national health concern as smoking-related diseases cause one in five deaths annually in the United States (Benowitz, 2010). Although tobacco smoke contains at least 4800 separate chemical compounds, the principal psychoactive chemical is nicotine, which mediates tobacco's reinforcing effects (Baker *et al*, 2004). Stimulation of nicotinic acetylcholine receptors in the central nervous system is responsible for the diverse psychoactive effects of nicotine, including mood elevation, decreased anxiety, increased arousal, improved attentiveness, decreased appetite, muscle relaxation and cognitive enhancement (Picciotto, 2003). Although there are several FDA-approved smoking cessation medications available, only about one in four smokers in the United States is able to maintain long-term (12 months) abstinence (Schnoll and Lerman, 2006). The high rate of smoking relapse highlights a critical need to develop novel, efficacious smoking cessation pharmacotherapies (Lerman *et al*, 2007).

Smoking cessation and nicotine withdrawal are associated with depressed mood, irritability, weight gain, drug craving, and cognitive deficits (Hughes and Hatsukami, 1986; Kenny and Markou, 2001). A growing literature indicates that cognitive deficits involving working memory represent a core symptom of nicotine withdrawal that predict relapse after brief periods of smoking cessation (Patterson *et al*,

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2010; Rukstalis *et al*, 2005). Consistent with these findings, nicotine re-exposure (Davis *et al*, 2005; Myers *et al*, 2008), nicotine replacement therapies (Atzori *et al*, 2008), and the $\alpha4\beta2$ nicotinic receptor partial agonist varenicline (Raybuck *et al*, 2008) reverse abstinence-induced cognitive deficits and blunt relapse in both humans and rodents. Taken together, these findings suggest that other cognitive-enhancing drugs that modulate cholinergic transmission in the brain may prevent smoking relapse.

Galantamine is an acetylcholinesterase inhibitor and positive allosteric modulator of $\alpha7$ homomeric and $\alpha4\beta2^*$ heteromeric nicotinic acetylcholine receptors (nAchRs) (Harvey, 1995; Maelicke and Albuquerque, 2000; Samochocki *et al*, 2003) that has been shown to alleviate some of the cognitive deficits associated with Alzheimer's disease (Pepeu and Giovannini, 2009; Prvulovic *et al*, 2010; Villarroya *et al*, 2007). Given that one hallmark of nicotine withdrawal is cognitive impairments, galantamine may improve nicotine withdrawal symptoms in abstinent smokers. Consistent with this hypothesis, a recent study demonstrated that galantamine administration improves cognitive performance following nicotine withdrawal in mice (Wilkinson and Gould, 2011). While these results suggest that cognitive enhancing drugs such as galantamine may prevent relapse to smoking, the role of acetylcholinesterase inhibitors in nicotine taking and seeking is not clear.

The current study employed nicotine self-administration in rats to examine the potential effects of systemic galantamine administration on nicotine taking and nicotine reinstatement, an animal model of relapse in abstinent human smokers (Mathieu-Kia *et al*, 2002; Shaham *et al*, 1997). Moreover, these experiments assessed the role of galantamine in modulating sucrose self-administration and reinstatement to examine the specificity of this drug treatment in reinforced behaviors. As with other drugs that increase cholinergic transmission, the most common adverse effects of galantamine are malaise symptoms, such as nausea and vomiting (Dunbar *et al*, 2006; Raskind *et al*, 2000; Tariot *et al*, 2000). Therefore, the effects of acute galantamine administration on pica, an animal model in which rodents consume nonnutritive substances (eg, kaolin clay) in response to nausea-inducing agents (Kanoski *et al*, 2011; Mitchell *et al*, 1976), were tested in separate cohorts of animals. Our results indicate that galantamine attenuates nicotine, but not food, taking and reinstatement in rats and that these effects are not due to galantamine-induced nausea.

MATERIALS AND METHODS

Animals and Housing

Male Sprague Dawley rats (*Rattusnorvegicus*) weighing 225–250 g were obtained from Taconic Laboratories (Germantown, NY). Initially, animals were single housed with food and water available *ad libitum*. Rats used for nicotine self-administration and reinstatement studies were mildly food restricted (approximately 15–20 g standard lab chow per day) to 85–90% of their free-feeding body weight following surgery. Mild food restriction was used to facilitate acquisition and maintenance of nicotine self-administration similar to previously published reports (Corrigall and Coen, 1989; Fowler *et al*, 2011; Yan *et al*, 2012). Separate rats used in food intake testing were maintained on *ad libitum* chow, except as noted below. All animals were housed in a colony maintained on a 12-h/12-h reverse light/dark cycle with the lights off at 0700 hours. All experimental procedures were performed during the dark phase of the light/dark cycle. All experimental protocols were in accordance with the guidelines set forth by the National Institutes of Health and were approved by the University of Pennsylvania School of Medicine Institutional Animal Care and Use Committee.

Materials

All self-administration experiments were conducted in ventilated, sound-attenuating operant chambers purchased from Med-Associates (East Fairfield, VT). Each operant chamber was equipped with both active and inactive response levers, a sucrose pellet dispenser, cue lights, a tone generator, as well as an automated injection pump for administering drug or vehicle solutions intravenously.

Surgery

Rats were allowed 1 week to acclimate to their home cages upon arrival. Prior to surgery, the rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine (Sigma/RBI, St. Louis, MO). An indwelling catheter (CamCaths; Cambridge, UK) was inserted into the right, external jugular vein and sutured securely in place. The catheter was connected to a mesh backmount, which was implanted subcutaneously above the shoulder blades. To prevent infection and to maintain patency, catheters were flushed daily with 0.3 ml of a solution of the antibiotic Timentin (0.93 mg/ml) dissolved in heparinized saline. When not in use, the catheters were sealed with plastic obturators.

Nicotine Self-Administration

After surgery, rats were allowed 7 days to recover before behavioral testing commenced. Initially, rats were placed in operant chambers daily and allowed to lever press for intravenous nicotine (0.03 mg/kg nicotine/59 µl saline, infused over 5 s) on a fixed-ratio 1 (FR1) schedule of reinforcement. Each nicotine infusion was paired with a light/tone cue. A cue light located above the active lever, upon which responding resulted in infusions of nicotine, was lit simultaneously with the initiation of a nicotine infusion and remained illuminated throughout the 20-s timeout period. A tone also accompanied the light cue during each nicotine infusion and subsequent timeout period. Stable responding on the FR1 schedule was defined as less than 20% variation in response rates over three consecutive self-administration days. After stable responding was achieved, animals were switched to a FR3 schedule of reinforcement for 3–5 days and then an FR5 schedule. For all FR schedules, a 20-s timeout period followed each nicotine infusion, during which time active lever responses were tabulated but had no scheduled consequences. Responses made on the inactive lever, which had no scheduled consequences, were also recorded during the FR training sessions.

Sucrose Self-Administration

Rats were trained initially to lever press for sucrose pellets (Research Diets, New Brunswick, NJ) on a FR1 schedule of sucrose reinforcement during 1-h operant sessions. Once animals achieved stable responding for sucrose (defined as $<20\%$ variation in responding over 3 consecutive days) on the FR1 schedule of reinforcement, the response requirement was increased to an FR5 schedule of reinforcement. Animals were limited to 30 sucrose pellets within a 1-h operant session and were food restricted to 15–20 g of lab chow (Harlan Teklad, Wilmington, DE) in their home cages for the duration of the experiment. Subjects were mildly food deprived to maintain consistency with the nicotine self-administration experiments (ie, ensure similar motivational states). Each successful completion of the response requirement resulted in delivery of a sucrose pellet as well as contingent presentation of a light/tone cue.

Experiment 1: Nicotine Dose-Response Curve and Mecamylamine-Induced Attenuation of Nicotine Self-Administration

Rats ($n=9$) self administered nicotine (0.03 mg/kg/infusion) on a FR5 schedule of reinforcement during daily 2-h operant sessions. Stable responding for the training dose of nicotine was defined as less than 20% deviation from the mean of the total number of infusions earned over three consecutive self-administration days per subject. After acquisition of stable responding for nicotine, self-administration of various doses of nicotine (0, 0.003, 0.01, 0.03, or 0.06 mg/kg/infusion) was tested using a between-session, within-subjects design that was based on a previously published study (Watkins *et al*, 1999). Nicotine doses were counterbalanced and rats were allowed to self-administer each dose for 3 consecutive days. After each test dose of nicotine, rats were returned to the training dose (0.03 mg/kg/infusion) for an additional 3 days to assess the stability of nicotine self-administration at this dose. Over the next subsequent 3 days, nicotine self-administration behavior was extinguished (defined as $<15\%$ of the total maximum responses for the training dose of nicotine for each rat) by replacing nicotine with saline. Each subject then reacquired stable nicotine self-administration for the training dose (0.03 mg/kg/infusion) over 3 days before testing another dose of nicotine.

The effects of the non-selective nAChR antagonist mecamylamine were tested in a separate group of rats ($n=7$) that had acquired nicotine self-administration to demonstrate pharmacological specificity of operant responding for intravenous nicotine infusions. Mecamylamine hydrochloride (0, 0.4, or 4.0 mg/kg, s.c.) was administered 20 min prior to the beginning of the self-administration session similar to previously published reports (Corrigall and Coen, 1989; Rauhut *et al*, 2002; Shoaib *et al*, 1997; Watkins *et al*, 1999). Mecamylamine doses were counterbalanced using a between-session, within-subjects design. Between doses, subjects were allowed to respond for the training dose of nicotine for at least 3 days or until they achieved baseline responding before being tested on the next dose of mecamylamine.

Experiment 2: Effects of Galantamine on Nicotine and Sucrose Self-Administration

The effects of acute galantamine were examined in animals that acquired stable nicotine or sucrose self-administration. Subjects ($n=10$) were pretreated with galantamine (0, 0.1, 1.0, or 5.0 mg/kg, i.p.) 20 min prior to the beginning of the operant session. Doses of galantamine and time course of administration were chosen based on previously published reports (Camacho *et al*, 1996; Hernandez *et al*, 2006; Sharp *et al*, 2004). A between-session, within-subjects design was used to test the effects of galantamine on nicotine- and sucrose-taking behavior. Galantamine doses were counterbalanced and tested in animals that had acquired stable nicotine or sucrose self-administration on a FR5 schedule of reinforcement. Each test day was separated by at least 2 days of nicotine self-administration to ensure that nicotine taking had stabilized between test sessions. Once all doses were tested, subjects were switched to a progressive ratio (PR) schedule of reinforcement. Under a PR schedule, the response requirement for each subsequent drug delivery increases until the subject fails to meet a requirement. In the current experiments, the response requirement for the i^{th} reinforcement was given by $R(i)=[5e^{0.2i}-5]$ and the session expired when an animal took more than 30 min to receive an injection. The effects of galantamine were studied once animals had stabilized their responding for nicotine on a PR schedule (approximately 5 days). A similar design was used to study the effects of galantamine in animals ($n=8$) trained to self administer sucrose pellets on a FR5 schedule of reinforcement.

Experiment 3: Effects of Galantamine on Ad Libitum Food Intake and Pica

The effects of acute galantamine on *ad libitum* food intake and pica were studied in a separate cohort of rats ($n=20$). All animals were single housed on a 12-h/12-h reverse light/dark cycle with the lights off at 0900 hours. Consistent with the aforementioned studies of galantamine on nicotine self-administration, all experimental procedures were performed during the dark phase of the light/dark cycle. Initially, rats were habituated to *ad libitum* kaolin pellet (Research Diets; K50001) access for 1 week while maintained on standard rodent chow. Baseline kaolin intake during the habituation phase was negligible (mean kaolin intake <0.5 g for 24 h; data not shown). Before experimental testing, rats were mildly food restricted from 1600 hours to 0900 hours, with the majority of food restriction occurring during the subjects' light cycle to ensure an acute, energy-depleted state similar to the chronic deprivation state of rats used in the nicotine self-administration studies. Subjects were pretreated with galantamine (0, 0.1, 1.0, and 5.0 mg/kg, i.p.) 20 min prior to testing. Pre-weighed standard rodent chow (Purina 5001) and kaolin pellets were placed in each cage at the onset of the dark cycle. Cumulative chow and kaolin intake (± 0.1 g) were recorded 2 h and 24 h following onset of the dark cycle. Changes in body weight were also recorded over the 24-h testing session. A within-subject, Latin-square design was used to test the potential effects of galantamine on *ad libitum* food consumption and kaolin intake. Testing days were separated by a minimum of 48 h

to ensure that animals maintained stable feeding behavior and body weight gain between treatments.

Cisplatin, a widely studied chemotherapy drug known to induce nausea in humans, was administered to a separate cohort of rats as a positive control for pica. All animals were single housed on a 12-h/12-h reverse light/dark cycle with the lights off at 0900 hours. Briefly, rats were habituated with *ad libitum* access to kaolin for 1 week prior to experimental testing. Prior to experimental testing, rats were mildly food restricted similar to the aforementioned experiment examining the effects of galantamine on *ad libitum* food intake and pica. Acute cisplatin (6.0 mg/kg; i.p., $n=7$) or saline ($n=5$) was administered approximately 20 min prior to testing. Rodent chow and kaolin pellets were placed in each cage at the onset of the dark cycle. Cumulative chow and kaolin consumption as well as changes in bodyweight were recorded 24 h following drug treatment.

Experiment 4: Effects of Galantamine on Nicotine and Sucrose Reinstatement

The effects of acute galantamine on nicotine or sucrose reinstatement were studied in separate cohorts of animals. Following approximately 21 days of daily nicotine self-administration sessions, drug-seeking behavior was extinguished by replacing the nicotine with 0.9% saline. Light/tone cues that were previously paired with nicotine infusions during the self-administration phase were turned off. Daily 2-h extinction sessions continued until responding on the active lever was $<15\%$ of the response rate maintained by nicotine self-administration under the FR5 schedule of reinforcement. Typically, it took approximately 7 days for rats to meet this criterion. Once nicotine self-administration behavior was extinguished, the ability of a priming injection of nicotine (0.2 mg/kg, s.c.) plus light/tone cues to reinstate nicotine seeking was assessed. During the reinstatement test session, subjects ($n=9$) were allowed to respond for light/tone cues that were previously paired with nicotine infusions. When an animal completed each response requirement (ie, five presses on the active lever), an intravenous infusion of saline was administered along with contingent presentations of the light/tone cues. Additionally, non-contingent presentation of the light/tone cue was delivered for 20 s at the beginning of each reinstatement test session. On subsequent test days, galantamine (0, 0.5 or 5.0 mg/kg, i.p.) was administered 20 min prior to a priming injection of nicotine. Animals were placed immediately into the operant chambers following administration of a priming injection of nicotine and the 2-h reinstatement session began. Using a between-session paradigm, each daily reinstatement session was followed by extinction days until responding was less than 15% of the maximum number of responses maintained by nicotine self-administration. In general, it took 2–3 days of extinction for each animal to reach criterion between reinstatement sessions.

The effect of galantamine on the reinstatement of sucrose-seeking behavior was tested in a separate cohort of animals ($n=8$). After 2 weeks of sucrose-maintained responding on the FR5 schedule of reinforcement, responding was extinguished by inactivating the food dispenser so that every five lever presses had no scheduled consequences. Light/tone cues that were previously paired with delivery of sucrose pellets during the self-administration phase were turned off. Once lever responding decreased to $<15\%$ of the maximum number of responses completed during sucrose self-administration, animals proceeded to reinstatement testing. During the reinstatement test session, subjects were allowed to respond for light/tone cues that were previously paired with sucrose pellet delivery during the self-administration phase. Acute injections of galantamine (0, 0.5, and 5.0 mg/kg, i.p.) were administered 20 min prior to the beginning of the reinstatement session. The experimenter remotely administered one sucrose pellet every 2 min for the first 10 min of the reinstatement session along with a 20-s non-contingent presentation of the light/tone cue at the beginning of the reinstatement test session. A between-session paradigm was used so that each daily 1-h reinstatement session was followed by an extinction session the following day until responding was again $<15\%$ of the response rate maintained by sucrose. A within-subjects design was used for the sucrose studies and doses of galantamine were counterbalanced across self-administration and reinstatement sessions.

Statistical Analyses

Total active and inactive lever responses for the nicotine dose-response curve, mecamylamine, and galantamine experiments were analyzed with one-way mixed-factors analyses of variance (ANOVAs). One-way ANOVAs were also used to analyze total nicotine infusions and total nicotine administered as well as the effects of galantamine on total *ad libitum* chow consumed, total kaolin intake, sucrose self-administration and sucrose reinstatement. Pairwise comparisons for all one-way ANOVAs were made with Tukey's HSD ($p<0.05$). Paired *t*-tests were used to analyze PR data and unpaired *t*-tests were used to analyze the effects of cisplatin on total chow and kaolin consumed.

Drugs

Nicotine hydrogen tartrate salt (Sigma) was dissolved in sterile 0.9% saline (pH adjusted to 7.4 ± 0.5 with sodium hydroxide). Mecamylamine hydrochloride and galantamine hydrobromide were purchased from Tocris (Ellisville, MS) and dissolved in sterile 0.9% saline. Cisplatin (*cis*-diammineplatinum(II) dichloride) was purchased from Sigma-Aldrich and dissolved in sterile 0.9% saline. Nicotine doses are reported as freebase concentrations, whereas mecamylamine, galantamine, and cisplatin doses are reported as their salt concentrations.

RESULTS

Nicotine Dose-Response Curve

Subjects ($n=9$) acquired stable nicotine self-administration at the training dose (0. 03 mg/kg/infusion) within approximately 16 days. The mean of the second and third day of self-administration at each unit dose of nicotine was used for the analysis. Total lever responses (mean ± SEM) during the nicotine self-administration sessions are presented in Figure 1a. Total active lever responses were analyzed with a one-way ANOVA, which revealed a significant main effect

of treatment ($F(4,40) = 6.581$, $p < 0.001$). Subsequent pairwise analyses showed that total active lever responses were significantly different between the saline and the 0.01, 0.03 and 0.06 mg/kg/infusion nicotine treatments (Tukey's HSD, $p < 0.05$). Inactive lever responses for the nicotine dose-response curve were analyzed with a one-way ANOVA. No significant differences in responses made on the inactive lever were revealed in subjects responding for different unit doses of nicotine ($F(4,40) = 2.246$, $p < 0.081$). Total infusions of nicotine (mean ± SEM.) earned for each unit dose are plotted in Figure 2b. These data were analyzed with a one-way ANOVA, which revealed a significant main effect of treatment ($F(4,40) = 6.696$, $p < 0.001$). Further pairwise analyses revealed a significant difference in total infusions of nicotine between the saline and the 0.01, 0.03, and 0.06 mg/kg/infusion nicotine treatments (Tukey's HSD, $p < 0.05$). Total nicotine infused (mean ± SEM) is plotted in Figure 2c. These data were analyzed with a one-way ANOVA, which revealed a significant main effect of treatment ($F(4,40) = 27.61$, $p < 0.0001$). Subsequent pairwise analyses showed that total nicotine infused was significantly different between the saline and the 0.01, 0.03, and 0.06 mg/kg/infusion nicotine treatments (Tukey's HSD, $p < 0.05$). Total lever responses (mean ± SEM) for animals pretreated with saline, 0.4 or 4.0 mg/kg mecamylamine ($n = 7$) prior to the nicotine self-administration session are plotted in Figure 1d. Active lever responses were analyzed with a one-way ANOVA, which revealed a significant main effect of treatment ($F(2,20) = 5.676$, $p < 0.05$). Subsequent pairwise analyses revealed a significant difference in total active lever responses between the saline or 0.4 mg/kg mecamylamine and 4.0 mg/kg mecamylamine treatments (Tukey's HSD, $p < 0.05$). No significant differences on inactive lever responding were found between treatments ($F(2,20) = 0.09257$, $p < 0.91$).

Systemic Galantamine Administration Dose-dependently Attenuated Nicotine, but not Sucrose, Self-Administration in Rats

Saline, 0.1, 1.0, or 5.0 mg/kg galantamine ($n = 10$) was administered 20 min prior to the start of a self-administration session. Total lever responses (mean ± SEM) for animals self-administering nicotine on a FR5 schedule are shown in Figure 2a. Total active lever data were analyzed with a one-way ANOVA, which revealed a significant main effect of treatment ($F(3,36) = 8.009$, $p < 0.001$). Subsequent pairwise analyses showed that total active lever responses were significantly different between the saline and 5.0 mg/kg galantamine treatments (Tukey's HSD, $p < 0.05$). No significant differences were found on inactive lever responding between treatments ($F(3,36) = 1.272$, $p < 0.30$). Total lever responses (mean ± SEM) for animals self administering nicotine on a PR schedule of reinforcement are plotted in Figure 2b. Total active lever responses were significantly different following administration of 5.0 mg/kg galantamine

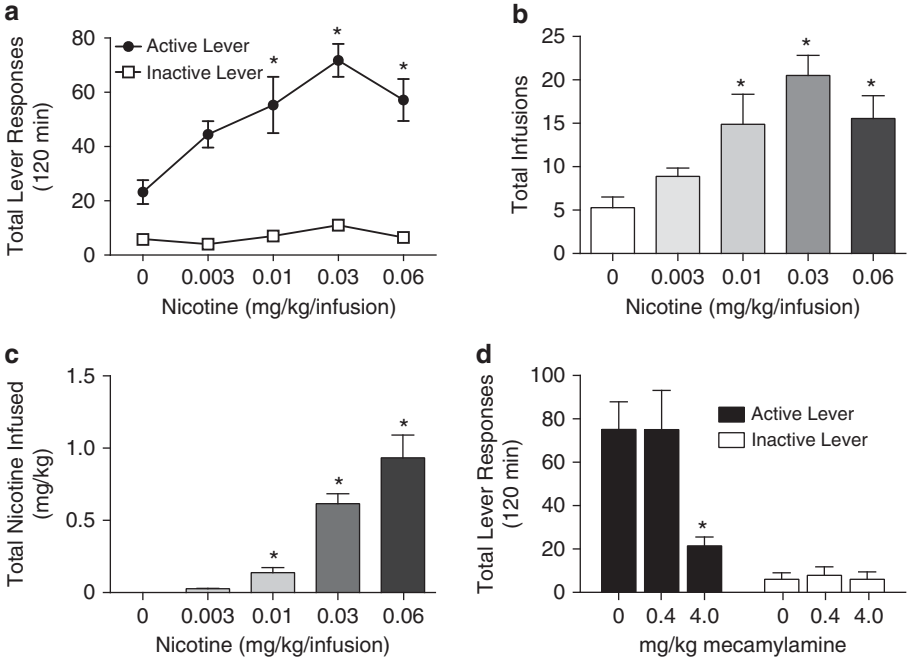

Figure 1 Nicotine dose-response curve and attenuation of nicotine self-administration following systemic administration of the nicotinic acetylcholine receptor antagonist mecamylamine. Data in panel (a) depict the total number of responses (mean ± SEM) on the active and inactive levers for each unit dose of nicotine self-administered ($n = 9$ per treatment). The asterisks denotes a significant difference in active lever responding between animals self-administering 0.01, 0.03, or 0.06 mg/kg/infusion of nicotine when compared with saline (Tukey's HSD, $p < 0.05$). Dose-response curves for total infusions (b) and total nicotine infused (c) are also displayed for increasing unit doses of nicotine. Data are presented as mean ± SEM. Asterisks denote significant differences in total infusions and total nicotine infused during the daily 2-h operant session between animals self-administering 0.01, 0.03, or 0.06 mg/kg/infusion of nicotine *vs* saline ($n = 9$ per treatment). (d) Total number of responses (mean ± SEM) on the active and inactive levers in animals self-administering nicotine following pretreatment with saline, 0.4, and 4.0 mg/kg mecamylamine ($n = 7$ per treatment). The asterisk denotes a significant difference on active lever responding between 4.0 mg/kg mecamylamine and saline and 0.4 mg/kg mecamylamine treatments (Tukey's HSD, $p < 0.05$). No significant differences in responding on the inactive lever (mean ± SEM) were found between treatments.

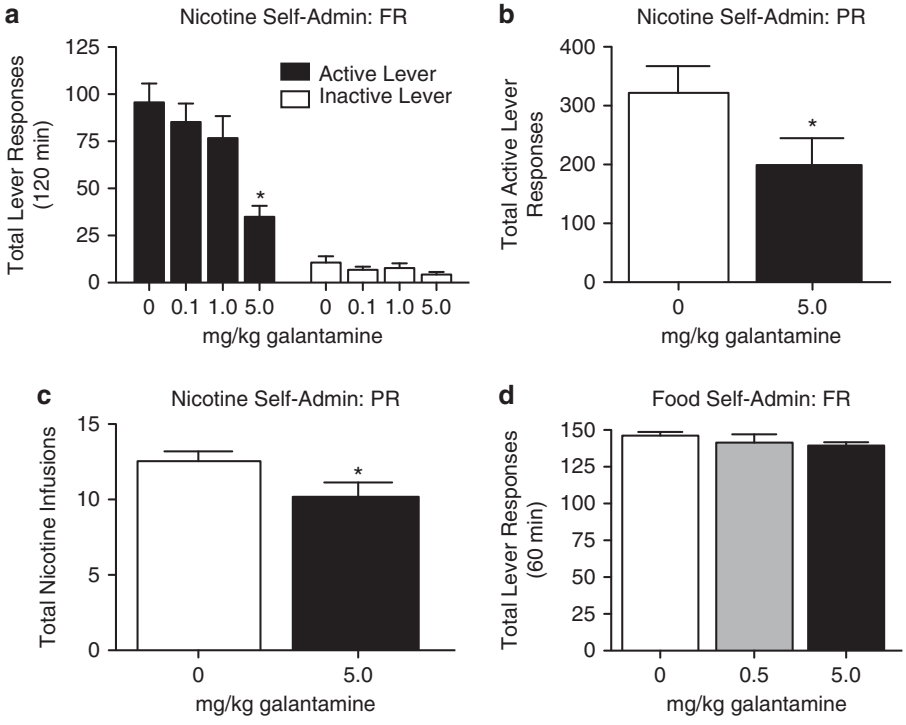

Figure 2 Systemic administration of the acetylcholinesterase inhibitor galantamine dose-dependently attenuated nicotine, but not food, self-administration. (a) Total number of responses (mean ± SEM) on the active and inactive levers following systemic administration of 0, 0.1, 1.0, or 5.0 mg/kg galantamine in rats self administering nicotine on a FR5 schedule of reinforcement ($n = 10$ per treatment). The asterisk represents a significant difference from vehicle, 0.1, and 1.0 mg/kg galantamine (Tukey's HSD, $p < 0.05$). No significant differences in responding on the inactive lever (mean ± SEM) were found between treatments. Total active lever responses (b) and total nicotine infused (c) are plotted for animals pretreated with 5.0 mg/kg galantamine prior to nicotine self-administration on a PR schedule of reinforcement ($n = 11$ per treatment). The asterisks represent a significant difference from vehicle (Paired t-test, $p < 0.001$). (d) Total number of lever responses (mean ± SEM) in subjects ($n = 8$ per treatment) self-administering food pellets on a FR5 schedule of reinforcement. No significant differences in responding were noted between subjects pretreated with vehicle, 0.5, or 5.0 mg/kg galantamine. FR, fixed ratio; PR, progressive ratio; Self-admin, self-administration.

when compared with saline ($t(10) = 8.464$, $p < 0.001$). Total nicotine infusions (mean ± SEM) for animals self administering nicotine on a PR schedule are displayed in Figure 2c. Galantamine administration significantly decreased total active lever responses on a PR schedule ($t(10) = 6.094$, $p < 0.001$). No significant differences in inactive lever responding were noted between treatments ($t(10) = 0.45$, $p < 0.67$) in animals responding for nicotine on a PR schedule of reinforcement (data not shown).

The behaviorally relevant dose of galantamine that attenuated nicotine taking had no effect on sucrose self-administration (see Figure 2d). Saline, 0.5 or 5.0 mg/kg galantamine ($n = 8$) was administered 20 min prior to the beginning of a sucrose self-administration session. Total active lever responses (mean ± SEM) were analyzed with a one-way ANOVA. No significant differences in active lever responding were found between treatments ($F(2,21) = 0.821$, $p < 0.50$).

Systemic Galantamine Administration Does Not Alter Food Intake or Pica in Rats

To determine whether the effects of galantamine on nicotine self-administration were due to drug-induced nausea/malaise, total chow intake, and kaolin consumption were measured in a separate cohort of animals. Total chow consumed (mean ± SEM) following systemic administration of galantamineis plotted in Figure 3a. No significant differences were found between treatments at 2 h ($F(3,76) = 0.7680$, $p < 0.52$) or 24 h ($F(3,76) = 0.241$, $p < 0.87$) following injection. Figure 3b depicts total kaolin consumed (mean ± SEM) following systemic administration of galantamine. No significant differences were found between treatments on kaolin intake at 2 h ($F(3,76) = 0.466$, $p < 0.71$) or 24 h ($F(3,76) = 0.959$, $p < 0.42$) following injection. Galantamine administration also did not affect 24 h bodyweight (data not shown). To serve as a positive control for the pica model, the chemotherapeutic drug cisplatin was administered in a separate cohort of animals. Total chow consumed and total kaolin intake (mean ± SEM) are plotted in Figures 3c and d, respectively. Cisplatin administration significantly decreased total 24 h chow intake ($t(10) = 2.716$, $p < 0.05$) and increased 24 h total kaolin intake ($t(10) = 2.414$, $p < 0.05$) when compared with saline controls. Total body weight gained was also significantly less in rats pretreated with cisplatin (14.06 ± 2.11 g) when compared with vehicle (2.9 ± 2.53 g) ($t(10) = 2.990$, $p < 0.05$).

Systemic Administration of Galantamine Dose-dependently Attenuates the Reinstatement of Nicotine, but not Sucrose, Seeking in Rats

Total active lever responses (mean ± SEM) following systemic administration of galantamine prior to the nicotine

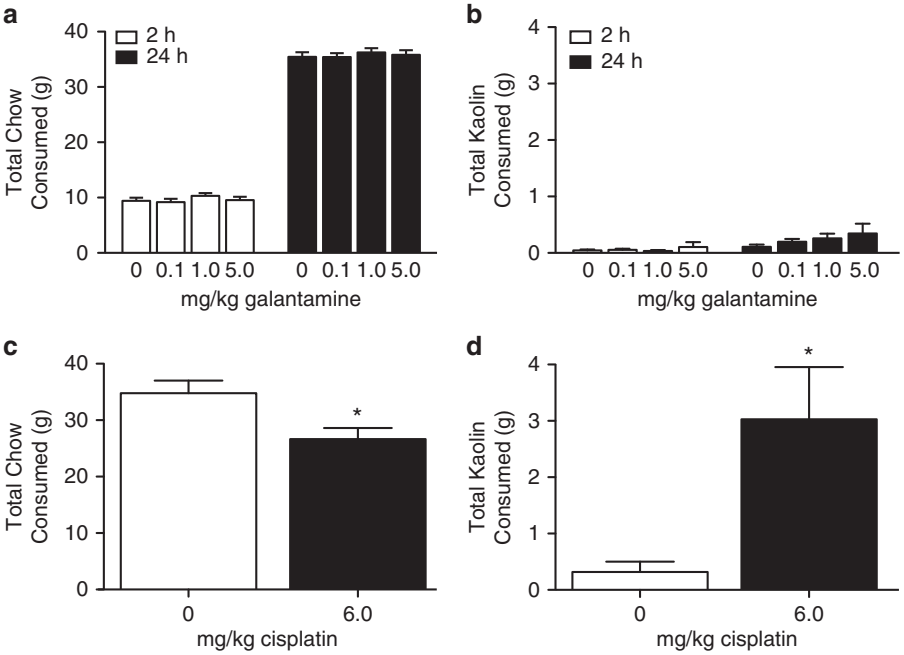

Figure 3 Systemic galantamine administration did not alter feeding behavior or pica in rats. Total chow (a) and kaolin (b) consumed (mean ± SEM) 2 and 24 h after systemic administration of 0, 0.1, 1.0, and 5.0 mg/kg galantamine are plotted ($n = 20$ per treatment). No significant differences in total chow or kaolin consumed were noted between treatments. In contrast, systemic administration of the alkylating agent cisplatin, a chemotherapeutic drug, significantly decreased total chow (c) and increased kaolin (d) consumed (mean ± SEM) over a 24-h test session. The asterisks represent a significant decrease between animals pretreated with saline ($n = 5$) and 6.0 mg/kg cisplatin ($n = 7$)(un-paired *t*-test, $p < 0.05$).

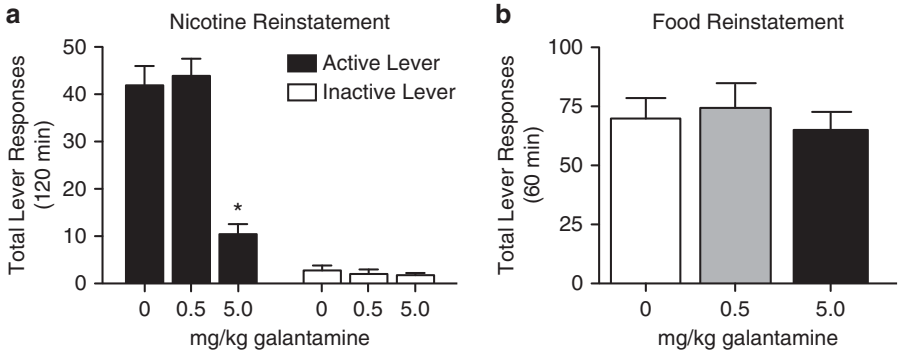

Figure 4 Systemic galantamine administration dose dependently attenuated the reinstatement of nicotine-, but not food-, seeking behavior in rats. (a) Total number of lever responses (mean ± SEM) on the active and inactive levers following systemic administration of 0, 0.5 or 5.0 mg/kg galantamine ($n = 9$ per treatment). Galantamine dose dependently attenuated drug seeking induced by a priming injection of nicotine and cues previously associated with nicotine self-administration. The asterisk represents a significant decrease in responding on the active lever compared with vehicle and 0.5 mg/kg galantamine (Tukey's HSD, $p < 0.001$). No significant differences in responding on the active lever (mean ± SEM) were noted between treatments. (b) Total number of lever responses (mean ± SEM) were not significantly different between treatments ($n = 8$ per treatment) during tests of food seeking.

reinstatement test session are plotted in Figure 4a ($n = 9$). Total active lever responses were analyzed using a one-way ANOVA, which revealed a significant main effect of treatment ($F(2,24) = 30.65$, $p < 0.0001$). Pairwise analyses revealed a significant difference in responding on the active lever between the saline or 0.5 mg/kg and 5.0 mg/kg galantamine treatments (Tukey's HSD, $p < 0.05$). No significant differences on inactive lever responding were found between treatments ($F(2,24) = 0.3697$, $p < 0.69$). The effects of galantamine administration ($n = 8$) on sucrose reinstatement are shown in Figure 4b. Total active lever responses (mean ± SEM) were analyzed with a one-way ANOVA. No significant differences in active lever responding were found between treatments ($F(2,21) = 0.2958$, $p < 0.75$).

DISCUSSION

In the current study, nicotine maintained robust self-administration in rats and this behavioral response was attenuated by the nonselective nAChR antagonist mecamylamine, similar to previously published reports (Corrigall and Coen, 1989; Watkins *et al*, 1999). However, this is the first study to demonstrate that systemic administration of

galantamine, a pharmacological inhibitor of acetylcholinesterase and positive allosteric modulator of $\alpha7$ and $\alpha4\beta2^*$ nAChRs attenuates nicotine self-administration and the reinstatement of nicotine-seeking behavior. The present results include four novel findings: i) acute administration of galantamine dose-dependently attenuated nicotine self-administration in animals maintained on FR and PR schedules of reinforcement; ii) acute galantamine administration did not affect *ad libitum* feeding behavior or sucrose self-administration; iii) acute galantamine administration dose-dependently attenuated the reinstatement of nicotine, but not sucrose, seeking; and iv) acute galantamine administration did not produce nausea/malaise (as measured by kaolin intake) in rats. Collectively, these results indicate that increased cholinergic transmission in the brain is sufficient to attenuate nicotine, but not food, reinforcement and reinstatement and that these effects are not due to adverse effects such as nausea/malaise.

Nicotine Reinforcement and Galantamine

Data from both human and rodent nicotine self-administration studies depict an inverted U-shaped dose-response curve, with maximal rates of responding occurring at intermediate doses of nicotine (Fowler *et al*, 2011; Rose and Corrigall, 1997; Watkins *et al*, 1999). Consistent with these findings, the present experiments demonstrated that, when given access to a range of nicotine doses, rats self-administered intravenous infusions of nicotine according to an inverted U-shaped dose-response curve. Increased responding on the ascending limb of the dose-response curve likely reflects the increasing reinforcing efficacy of nicotine as the unit dose increases (Lynch and Carroll, 2001). In contrast, decreased responding for increasing unit doses of nicotine on the descending limb likely reflects increasing aversive properties of these doses and/or the development of drug satiation (Lynch and Carroll, 1999). The total daily nicotine infused for the training (0.03 mg/kg) and high (0.06 mg/kg) doses of nicotine were consistent with previous studies utilizing short access (1–2 h/day) nicotine self-administration (Matta *et al*, 2007). Thus, the total number of infusions per session, dose range, shape of the dose-response curve, and total daily nicotine infused confirm and extend previously published reports of nicotine self-administration in rodents (Corrigall, 1999; Fowler *et al*, 2011; Matta *et al*, 2007; Watkins *et al*, 1999). Similar to previous studies, nicotine self-administration was attenuated in rats pretreated with the nonselective nAChR antagonist mecamylamine (Spealman and Goldberg, 1982; Watkins *et al*, 1999). Therefore, the behavioral pattern and pharmacological specificity of nicotine self-administration in the present experiments is consistent with the published literature and supports a model in which to study the reinforcing effects of nicotine.

With regard to the self-administration paradigm, recent evidence suggests that the primary reinforcing and reinforcement-enhancing effects (ie, responding for visual stimuli) of nicotine are behaviorally dissociable (Chaudhri *et al*, 2006; Palmatier *et al*, 2006; Palmatier *et al*, 2007). One limitation of the current study is that it does not differentiate between the potential effects of galantamine on these two distinct properties of nicotine self-administration. Future studies are required to delineate the precise role of galantamine in regulating these two aspects of nicotine taking.

In the present experiments, two measures were used to evaluate potential nonspecific rate suppressing effects of systemic injections of galantamine. First, each operant chamber was equipped with an inactive lever, responses on which are often used as a measure of nonspecific alterations in lever responding. Whereas systemic administration of galantamine had no significant effect on inactive lever responding, one could argue that responses were uniformly too low to meaningfully assess potential rate-suppressant effects of drug treatment. Therefore, we also assessed the ability of systemic galantamine infusions to alter sucrose self-administration. Galantamine pretreatment did not significantly affect sucrose self-administration and these results suggest that the effects of galantamine are not generalized to other reinforced behaviors.

Although a growing body of evidence indicates that enhanced cholinergic signaling is critically involved in nicotine addiction (Changeux, 2010; De Biasi and Dani, 2011; Tuesta *et al*, 2011), a potential role for acetylcholinesterase in the reinforcing effects of nicotine and the reinstatement of nicotine seeking is unclear. Galantamine increases cholinergic transmission in the brain by inhibiting acetylcholinesterase, thereby increasing extracellular acetylcholine levels (Harvey, 1995). Extracellular acetylcholine levels in the brain are increased 20 min following systemic administration of galantamine and remain elevated for 60 min (Giorgetti *et al*, 2010). The net effect of galantamine pretreatment in animals self-administering nicotine is an overall decrease in responding, which may reflect an increase in the reinforcing efficacy of nicotine and/or increased drug satiation analogous to higher unit doses of nicotine. Future studies are required to determine exactly how galantamine shifts the dose-response curve for nicotine self-administration. It is unlikely that galantamine-induced attenuation of nicotine taking is due to increased aversive effects (ie, motor suppressant effects, sickness, etc.) because galantamine administration had no effect on total chow intake or sucrose self-administration. Therefore, the effects of galantamine are reinforcer-specific and decreased nicotine self-administration on a FR schedule of reinforcement may reflect a leftward or downward shift in the dose-response curve. Consistent with these findings, galantamine decreased the breakpoint for nicotine self-administration on a PR schedule of reinforcement, which suggests that animals are less motivated to self-administer nicotine following galantamine pretreatment. Decreased responding on a PR schedule may also reflect drug satiation, as increasing the reinforcing efficacy of nicotine with higher unit doses engenders higher rates of responding on a PR schedule (Donny *et al*, 1999). Taken together, these data suggest that galantamine reduces the reinforcing efficacy of nicotine, in part, by increasing nicotinic neurotransmission in the brain.

Galantamine also functions as a positive allosteric modulator at $\alpha7$ homomeric and $\alpha4\beta2^*$ heteromeric nAChRs (Maelicke and Albuquerque, 2000; Samochocki *et al*, 2003). Galantamine binds to distinct sites on α subunits of nAChRs and potentiates the frequency of channel opening in response to acetylcholine and nicotine agonist stimulation

without interfering with binding of these ligands to the receptors (Schrattenholz *et al*, 1996; Woodruff-Pak *et al*, 2002). Recent evidence indicates that systemic galantamine administration increases dopaminergic cell firing in the ventral tegmental area (VTA), which promotes dopamine release in the medial prefrontal cortex (Schilstrom *et al*, 2007). Galantamine-induced enhancement of dopaminergic cell firing is regulated, in part, by allosteric potentiation of α7-containing nAChRs located on glutamate terminals in the VTA (Schilstrom *et al*, 2007). Nicotine also stimulates presynaptic α7-containing nAChRs and promotes glutamate release in the VTA (Mansvelder and McGehee, 2000; Schilstrom *et al*, 2000, 2003), effects that are associated with increased VTA dopamine cell firing and dopamine release in terminal regions (Grenhoff *et al*, 1986; Schilstrom *et al*, 1998; Woodruff-Pak *et al*, 2002). Moreover, nicotine stimulates heteromeric β2-containing nAChRs on dopamine cell bodies and γ-amino-byturic acid (GABA)-ergic interneurons, which promote dopaminergic cell firing in the VTA (Mansvelder *et al*, 2002; Picciotto *et al*, 1998; Schilstrom *et al*, 2003). Galantamine also binds to and potentiates α4β2* nAChRs (Samochocki *et al*, 2003; Smulders *et al*, 2005). α4β2* nAChRs have a critical role in nicotine reinforcement (Epping-Jordan *et al*, 1999; Maskos *et al*, 2005; Picciotto *et al*, 1998) and drugs that target α4β2 nicotinic acetylcholine receptors, such as varenicline, decrease nicotine self-administration in rats (George *et al*, 2011). These findings highlight similar effects of galanatamine and nicotine at α7 homomeric and α4β2* heteromeric nAChRs and suggest that galantamine pretreatment in conjunction with nicotine self-administration enhances extracellular dopamine to levels obtained following self-administration of higher unit doses of nicotine alone, possibly resulting in a leftward shift in the dose-response curve. Taken together, these findings suggest that galantamine modulates nicotine taking through diverse effects on different neurotransmitter systems that are mediated, in part, by endogenous acetylcholine levels as well as α7 and α4β2* nAChRs.

Nicotine Reinstatement and Galantamine

Similar to relapse in humans, reinstatement of drug seeking in animals can be elicited by stress, drug-associated stimuli and/or re-exposure to the drug itself (Schmidt *et al*, 2005; Shalev *et al*, 2002). For example, following extinction of nicotine self-administration, a subcutaneous injection of nicotine or re-exposure to cues previously paired with nicotine infusions reinstates operant responding in the absence of drug reinforcement in rodents (Andreoli *et al*, 2003; Bespalov *et al*, 2005; Fowler and Kenny, 2011; O'Connor *et al* 2010; Shaham *et al*, 1997; Yan *et al*, 2012). A combination of a priming injection of nicotine and cues elicits more robust nicotine seeking than a drug prime or cues alone (Feltenstein *et al*, 2011; O'Connor *et al* 2010). The present experiments also demonstrated that galantamine administration is sufficient to attenuate the reinstatement of nicotine-seeking behavior precipitated by a priming injection of nicotine and re-exposure to cues previously paired with nicotine infusions. These findings suggest that galantamine may prevent or attenuate nicotine craving and smoking relapse in abstinent human smokers.

Adverse Effects of Galantamine Administration

A common limitation to prescribing galantamine to enhance cognition in patients with Alzheimer's disease is its propensity to elicit adverse symptoms such as nausea and vomiting (Dunbar *et al*, 2006; Raskind *et al*, 2000; Tariot *et al*, 2000). However, studies of emesis in rodents are limited due to the lack of a vomiting reflex in rats and mice (Andrews and Horn, 2006). Despite the lack of a vomiting reflex, rodents show pica, or the consumption of nonnutritive substances such as kaolin clay, following administration of emetic agents (De Jonghe *et al*, 2009; Kanoski *et al*, 2011; Liu *et al*, 2005; Mitchell *et al*, 1976; Yamamoto *et al*, 2002). Administration of the chemotherapeutic agent cisplatin induces severe nausea and vomiting in humans (Evans *et al*, 1981). Consistent with these findings, administration of cisplatin significantly increases kaolin intake in rats (present findings). In contrast, systemic galantamine administration did not alter short (2 h)- or long (24 h)-term kaolin intake, which indicates that galantamine-induced attenuation of nicotine self-administration and reinstatement is not due to drug-induced nausea or malaise. Thus, galantamine may prevent smoking relapse within a dose range that does not induce nausea and vomiting in humans.

Galantamine and Smoking Cessation

To date, there are relatively few clinical studies of acetylcholinesterase inhibitors including galantamine on smoking behavior and smoking relapse. Administration of the acetylcholinesterase inhibitors rivistagmine and galantamine effectively reduce craving and smoking in alcohol-dependent patients (Diehl *et al*, 2006; Diehl *et al*, 2009). However, galantamine does not reduce cigarette smoking in patients with schizophrenia (Kelly *et al*, 2008). These conflicting clinical results are likely due to different patient populations (ie, genetic variability, neuropsychiatric disorders, etc.), comorbid drug use, and small sample sizes. Therefore, studies are needed to further define the efficacy of galantamine in treating smoking relapse in different populations of smokers. Together with the present findings that galantamine attenuates the reinstatement of nicotine seeking in rats, these results suggest that galantamine may be an efficacious smoking cessation pharmacotherapy.

Smoking cessation and nicotine abstinence are associated with cognitive deficits and recent evidence indicates that impaired cognitive function is a core symptom of nicotine withdrawal that predicts relapse after periods of smoking cessation (Hughes and Hatsukami, 1986; Kenny and Markou, 2001; Patterson *et al*, 2010; Rukstalis *et al*, 2005). Galantamine is a cognitive enhancer and therefore may attenuate drug seeking by improving cognitive deficits during periods of drug abstinence (Sofuoglu *et al*, 2011). This hypothesis is supported by a recent study that demonstrated that galantamine administration reversed nicotine withdrawal-induced cognitive deficits in mice (Wilkinson and Gould, 2011). While galantamine improves cognitive performance in aged rats (Barnes *et al*, 2000; Gould and Feiro, 2005) and mice with cholinergic deficits (Sweeney *et al*, 1990; Sweeney *et al*, 1988), galantamine administration in naïve rodents produces cognitive impairments or no change in cognition (Barnes *et al*, 2000; Gould and Feiro,

2005; Sweeney *et al*, 1990; Sweeney *et al*, 1988). Therefore, galantamine may selectively restore cognitive function to normal levels in animals experiencing nicotine withdrawal/abstinence-induced cognitive impairments without altering normal cognitive function. Future studies are required to determine if galantamine normalizes cognitive impairments during nicotine abstinence in rats with a history of nicotine self-administration.

Summary and Conclusions

The present findings indicate that galantamine attenuates nicotine taking and seeking in rats and that these effects are not due to adverse side effects such as galantamine-induced nausea/malaise. These results suggest that pharmacological treatments that modulate extracellular acetylcholine levels and/or potentiate signaling of $\alpha7$ and $\alpha4\beta2$ nAChRs may prevent nicotine craving and smoking relapse in abstinent human smokers. Furthermore, increased signaling at $\alpha7$ and $\alpha4\beta2$ nAChRs may attenuate nicotine craving and relapse by regulating other neurotransmitter systems, including dopamine and glutamate, known to mediate drug-seeking behavior (Schmidt *et al*, 2005; Schmidt and Pierce, 2010). These preclinical studies also provide rationale for clinical studies of galantamine on smoking behavior. The behaviorally relevant dose of galantamine that attenuated nicotine reinforcement and reinstatement has been shown to reduce acetylcholinesterase activity approximately 50% in rats (Marcos *et al*, 2008), which is comparable to levels achieved in Alzheimer's patients treated with galantamine (Bickel *et al*, 1991; Jackisch *et al*, 2009). Therefore, the dose of galantamine that attenuates nicotine reinforcement and reinstatement is likely to represent a clinically relevant dose in humans. Future clinical studies are required to determine the efficacy of cognitive-enhancing drugs such as galantamine and allosteric modulators of nAChRs as smoking-cessation pharmacotherapies.

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DISCLOSURE

The authors declare no conflict of interest.

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