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Administration of the nicotinic acetylcholine receptor agonists ABT-089 and ABT-107 attenuates the reinstatement of nicotine-seeking behavior in rats

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Abstract

Current smoking cessation pharmacotherapies have modest efficacy, and most smokers relapse within the first few days after a quit attempt. Nicotine withdrawal-induced craving and cognitive impairments predict smoking relapse during abstinence and suggest that cognitive-enhancing drugs may prevent relapse. ABT-089 and ABT-107 are subtype-selective nAChR agonists that improve cognitive performance in laboratory animals. However, there are no studies examining the effects of ABT-089 and ABT-107 on nicotine self-administration and the reinstatement of nicotine-seeking behavior, an animal model of relapse in human smokers. The goal of the present study was to determine the effects of the $\alpha 4\beta 2^*/\alpha 6\beta 2^*$ nAChR agonist ABT-089 and the $\alpha 7$ nAChR agonist ABT-107 on nicotine taking and seeking in rats. The effects of acute ABT-089 and ABT-107 pretreatment on nicotine self-administration and reinstatement were tested in male Sprague Dawley rats. Parallel studies of ABT-089 and ABT-107 on sucrose self-administration and reinstatement were tested in separate groups of rats to determine if the effects of these drug treatments generalized to other reinforced behaviors. Nicotine and sucrose self-administration behaviors were not altered following acute administration of ABT-089 (0, 0.12, 1.2 and 12.0 mg/kg) or ABT-107 (0, 0.03 and 0.3 mg/kg). In contrast, both ABT-089 and ABT-107 pretreatment dose-dependently attenuated nicotine reinstatement. These effects were reinforcer-specific as no effects of ABT-089 or ABT-107 pretreatment on sucrose seeking were noted. Taken together, these findings suggest that ABT-089 and ABT-107 do not affect nicotine consumption, but may reduce the likelihood that a smoking lapse will lead to relapse.

Keywords

smoking; relapse; addiction; self-administration

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Conflict of Interest

The authors declare no potential conflict of interest relating to this study.

1. Introduction

Tobacco smoking remains the leading cause of preventable death worldwide. Even though smoking cessation at any age produces almost immediate health benefits that continue to improve as prolonged abstinence is maintained, only 3% of smokers are able to quit smoking successfully on their own [1]. Current FDA-approved smoking cessation medications only promote long-term smoking abstinence in 30% of patients [2]. Therefore, there is a critical need to develop novel, efficacious pharmacotherapies for smoking cessation.

Nicotine is the principal psychoactive chemical in tobacco that mediates tobacco's reinforcing effects [3]. Nicotine is a non-selective nicotinic acetylcholine receptor (nAChR) agonist that binds to and activates neuronal nAChRs. There is clear evidence that neuronal nAChRs play critical roles in nicotine reinforcement and withdrawal [4-7], which supports the development of novel smoking cessation pharmacotherapies targeting these receptors. Varenicline is currently the best in class treatment for smoking cessation and was developed as such based on its action as a partial agonist at $\alpha 4\beta 2$ nAChRs [8]. However, protracted smoking abstinence is only achieved in 23% of patients treated with varenicline [9, 10]. This limited therapeutic benefit may be due, in part, to increased expression of $\beta 2$ -containing nAChRs in the brain [11]. Similar to the effects of nicotine, chronic varenicline exposure is associated with up-regulation of neuronal $\alpha 4\beta 2$ nAChRs, which is thought to promote nicotine craving and relapse during smoking abstinence [12-15]. Based on these findings, it has been proposed that subtype-selective nAChR agonists that do not increase nAChR expression in the brain may be more effective than varenicline and nicotine replacement therapies at maintaining long-term smoking abstinence [16].

A growing body of evidence indicates that $\alpha 4\beta 2^*$ nAChRs and $\alpha 7$ -containing nAChRs mediate the reinforcing effects of nicotine. In addition to regulating nicotine-taking and -seeking behaviors in rodents [17-23], both $\alpha 4\beta 2^*$ nAChRs and $\alpha 7$ -containing nAChRs have been shown to play important roles in cognitive function [24, 25]. Although many factors influence chronic smoking behavior, cognitive deficits are a core withdrawal phenotype that peak during the first few days of abstinence and predict smoking relapse [26, 27]. These findings indicate that cognitive-enhancing medications may prevent drug craving and relapse, in part, by reversing or normalizing nicotine withdrawal-induced cognitive impairments [28-30]. Therefore, novel subtype-selective $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR agonists that attenuate nicotine taking and seeking as well as improve nicotine withdrawal-induced cognitive deficits may represent efficacious smoking cessation pharmacotherapies [31].

ABT-089 is a partial agonist at neuronal $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs that has been shown to increase cognitive performance in non-human primates and rodents [32-35]. Consistent with these findings, recent clinical studies have shown that ABT-089 improves cognition and is well tolerated in humans [36, 37]. ABT-107 is a high affinity agonist at neuronal $\alpha 7$ nAChRs that has also been shown to increase cognitive performance in laboratory animals [38-40]. ABT-107 is safe and well tolerated in humans in a Phase 1 clinical study [41]. Moreover, repeated administration of ABT-089 or ABT-107 during nicotine withdrawal

does not increase expression of $\beta 2$ -containing nAChRs in the brain, which suggests that these compounds may promote smoking abstinence [42]. While these findings imply that ABT-089 and/or ABT-107 may reverse or normalize nicotine withdrawal-induced cognitive deficits and prevent relapse, no studies to date have examined the effects of these subtype-selective nAChR agonists on nicotine taking and seeking in rats.

Here, we examined the ability of acute systemic administration of ABT-089 and ABT-107 to attenuate nicotine self-administration and the reinstatement of nicotine seeking-behavior, an animal model of relapse in abstinent human smokers. In addition to examining the effects of ABT-089 and ABT-107 on nicotine taking and seeking, we also assessed the role of these subtype-selective nAChR agonists in modulating sucrose self-administration and reinstatement in order to examine the specificity of these drug treatments in appetitive/reinforced behaviors.

2. Materials and Methods

2.1. Animals and housing

Male Sprague Dawley rats (*Rattus norvegicus*) **approximately 3 months of age** were obtained from Taconic Laboratories (Germantown, NY, USA). All subjects were mildly food restricted (20-25 g chow daily) to 85-90% of their free-feeding body weight following recovery from surgery for the duration of these experiments. Water was continuously available in the home cage. Rats were housed in a colony maintained on a 12-h/12-h reverse light/dark cycle, with lights off at 7:00 a.m. 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.

2.2. Materials

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

2.3. Surgery

Rats were handled daily and allowed one 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 Aldrich/RBI, St. Louis, MO, USA). For rats self-administering nicotine, an indwelling silicone catheter (CamCaths; Cambridge, UK) was inserted into the right, external jugular vein and sutured securely in place. The catheter was attached to a mesh backmount that was implanted subcutaneously above the shoulder blades. To prevent infection and maintain patency, catheters were flushed daily with 0.3 ml of a solution of the antibiotic Timentin (0.93 mg/ml; Fisher, Pittsburgh, PA, USA) dissolved in heparinized 0.9% saline (Butler Schein, Dublin, OH, USA). When not in use, catheters were sealed with plastic obturators.

2.4. Nicotine self-administration and reinstatement of nicotine seeking

After surgery, rats were allowed 7 days to recover before behavioral testing commenced. Nicotine self-administration was performed as described previously [43, 44]. Initially, rats were placed in operant chambers and allowed to lever-press for intravenous infusions of nicotine (0.03 mg/kg nicotine/59 μ l saline, infused over a 5 s period) on a fixed-ratio 1 (FR1) schedule of reinforcement. Each nicotine infusion was paired with a light/tone cue. Stable responding on the FR schedules of reinforcement was defined as less than 20% variation in response rates over three consecutive self-administration days. After stable responding was achieved, the schedule of reinforcement was increased to fixed-ratio 3 (FR3) for 3-5 days and then finally increased to a fixed-ratio-5 (FR5) schedule. A 20 s time-out 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 operant sessions.

The effects of acute ABT-089 and ABT-107 were examined in separate cohorts of rats that acquired stable nicotine self-administration on a FR5 schedule of reinforcement. A between-session, within-subjects design was used to test the effects of ABT-089 and ABT-107 on nicotine taking (Figure 1A). Each test day was separate by at least 2 days of nicotine self-administration to ensure that nicotine taking had stabilized between test sessions. Subjects were pretreated with ABT-089 (0, 0.12, 1.2 and 12.0 mg/kg, i.p.) 10 min prior to the beginning of the operant session. ABT-107 (0, 0.03 and 0.3 mg/kg, i.p.) was administered 30 min prior to self-administration sessions. Following pretreatment with ABT-089 or ABT-107, rats were placed into the operant chambers and allowed to self-administer intravenous nicotine. All doses of ABT-089 and ABT-107 were counterbalanced and tested in each rat in order to avoid potential rank order effects of drug pretreatment. Doses of ABT-089 and ABT-107 and pretreatment times were selected based on previously published reports demonstrating cognitive-enhancing properties of these compounds in rodents [33, 34, 39, 45].

The effects of ABT-089 and ABT-107 on the reinstatement of nicotine-seeking behavior were tested in separate cohorts of rats that were not previously tested with these compounds during the self-administration phase of the experiment. Following 28 daily nicotine self-administration sessions, drug-taking behavior was extinguished by replacing the nicotine with 0.9% saline such that every 5 active lever responses resulted in a saline infusion. Light/tone cues that were paired with nicotine infusions during the self-administration phase were turned off during extinction. Daily extinction sessions continued until responding on the active lever was less than 20% of the response rate maintained by nicotine self-administration under the FR5 schedule of reinforcement. Typically, it took 3-7 days for rats to meet this criterion. Once nicotine self-administration was extinguished, the ability of an acute priming injection of nicotine (0.2 mg/kg, s.c.) to reinstate nicotine seeking was assessed. During each reinstatement test session, subjects were allowed to respond for light/tone cues that were previously paired with nicotine infusions. Therefore, satisfaction of the response requirement (i.e. every 5 active lever responses) during reinstatement test sessions resulted in an infusion of saline along with contingent presentations of the light/tone cues. On subsequent reinstatement test days, ABT-089 (0, 0.12, 1.2 and 12.0 mg/kg, i.p.) and

ABT-107 (0, 0.03 and 0.3 mg/kg, i.p.) was administered 10 and 30 min, respectively, prior to a priming injection of nicotine. Animals were placed immediately into the operant chambers following administration of a priming injection of nicotine and a 2-hour reinstatement session began. Using a between-sessions reinstatement paradigm, each reinstatement test session was followed by extinction sessions until responding was again less than 20% of the response rate maintained by nicotine self-administration (Figure 1B). Generally, 1-2 days of extinction were necessary to reach extinction criterion between reinstatement test sessions. This reinstatement procedure combines nicotine priming- and cue-induced reinstatement, which has been used in several previous studies [46-48], that elicits more robust drug seeking than when only nicotine or only the cues are presented. The combination of nicotine and cues may be a more relevant model of smoking relapse in humans than cues alone or nicotine alone.

2.5. Sucrose self-administration and reinstatement of sucrose seeking

Reinforcer-specificity and potential nonspecific rate-suppressing effects of ABT-089 and ABT-107 were evaluated by assessing the influence of these compounds on sucrose taking and seeking in separate cohorts of rats. Rats were trained initially to self-administer 45 mg sucrose pellets (Research Diets, New Brunswick, NJ) on a FR1 schedule of reinforcement. Once animals achieved stable responding for sucrose (defined as less than 20% variation in responding over three 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 each 1-hour daily operant session. Each successful completion of the response requirement resulted in delivery of a sucrose pellet as well as contingent presentations of light/tone cues. Subjects were mildly food restricted in order to maintain consistency with the nicotine self-administration experiments (i.e. to ensure similar motivational states). A between-session, within-subjects design was used to test the effects of ABT-089 and ABT-107 in rats stably self-administering sucrose pellets (Figure 1A). Each test day was separate by at least 2 days of sucrose self-administration to ensure that sucrose taking had stabilized between test sessions. Subjects were pretreated with ABT-089 (0, 0.12, 1.2 and 12.0 mg/kg, i.p.) 10 min prior to the beginning of the operant session. ABT-107 (0, 0.03 and 0.3 mg/kg, i.p.) was administered 30 min prior to self-administration sessions. All doses of ABT-089 and ABT-107 were counterbalanced and tested in each rat.

The effects of ABT-089 and ABT-107 on sucrose seeking were tested in separate cohorts of rats. After 14 days of sucrose-maintained responding on a FR5 schedule of reinforcement, rats underwent an extinction phase where lever pressing no longer resulted in sucrose delivery and presentation of light/tone cues. Once lever responding decreased to less than 20% of the maximum number of responses completed during sucrose self-administration, animals proceeded to reinstatement testing. During the reinstatement test sessions, rats were allowed to respond for light/tone cues that were previously paired with sucrose pellet delivery during the self-administration phase. Doses of ABT-089 and ABT-107 that attenuated nicotine priming-induced reinstatement of drug seeking were administered 10 min or 30 min, respectively, prior to the beginning of the reinstatement session. Using a within-subjects design, each animal served as its own control. The experimenter remotely administered one sucrose pellet every 2 min for the first 10 min of the reinstatement session.

Moreover, non-contingent presentation of light/tone cues was delivered for 5 seconds at the beginning of each reinstatement test session and repeated once every 2 minutes for the first 10 minutes of the reinstatement session. A between-session paradigm was used so that each daily reinstatement test session was followed by an extinction session the following day until responding was again less than 20% of the response rate maintained by sucrose (Figure 1B). Doses of ABT-089 and ABT-107 were counterbalanced across reinstatement test sessions. Rats were tested for sucrose seeking in the absence of a systemic drug infusion at the end of the experiment to ensure that reinstatement of sucrose seeking had not extinguished.

2.6. Drugs

(–)Nicotine hydrogen tartrate salt (Sigma Aldrich, St. Louis, MO, USA) was dissolved in sterile 0.9% saline (pH was adjusted to 7.4 ± 0.5 with sodium hydroxide). Nicotine doses are reported as free base concentrations. ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine dihydrochloride] and ABT-107 [5-(6-[(3R)-1-azabicyclo[2.2.2]oct-3-yl)oxy]pyridazin-3-yl)-1H-indole] were synthesized by AbbVie (North Chicago, IL, USA) and dissolved in sterile 0.9% saline [33, 39]. Doses of ABT-089 and ABT-107 used in these studies represent behaviorally relevant doses that have been shown previously to produce cognitive-enhancing effects in rodents [33, 34, 39, 45].

2.7. Statistical analyses

Data are presented as means and standard errors of the mean (S.E.M.) Total active and inactive lever responses during nicotine and sucrose self-administration and reinstatement experiments were analyzed with two-way mixed-factors analyses of variance (ANOVAs). Pairwise comparisons were made with Bonferroni *post hoc* test following two-way ANOVAs ($P < 0.05$).

3. Results

3.1. Acute ABT-089 administration has no effect on nicotine and sucrose self-administration in rats

Total lever responses for animals self-administering nicotine on a FR5 schedule of reinforcement are shown in Figure 2A ($n = 16$). Prior to the start of the operant session, rats were pretreated with 0, 0.12, 1.2 or 12 mg/kg ABT-089. These data were analyzed with a two-way ANOVA, which revealed a significant main effect of lever [$F(1,120) = 391.6$, $p < 0.0001$] but no effect of treatment [$F(3,120) = 0.48$, $p = 0.70$]. Total lever responses for animals self-administering sucrose pellets on a FR5 schedule of reinforcement are shown in Figure 2B. Prior to the start of the self-administration session, animals were pretreated with 0, 0.12, 1.2, or 12 mg/kg ABT-089 ($n = 10$). These data were analyzed with a two-way ANOVA, which revealed a significant main effect of lever [$F(1,72) = 5587$, $p < 0.0001$] but no effect of treatment [$F(3,72) = 0.51$, $p = 0.67$]. These findings indicate that acute ABT-089 administration does not influence nicotine or sucrose self-administration in rats.

3.2. Acute ABT-107 administration has no effect on nicotine and sucrose self-administration in rats

Total active and inactive lever responses in animals pretreated with 0, 0.03 and 0.3 mg/kg ABT-107 are shown in Figure 3A ($n = 7$). These data were analyzed with a two-way ANOVA, which revealed a significant main effect of lever [$F(1,36)=91.44, p<0.0001$] but no effect of treatment [$F(2,36)=0.05, p=0.95$]. The effects of ABT-107 pretreatment on sucrose self-administration are shown in Figure 3B ($n = 9$). There was no effect of drug pretreatment on lever responding [$F(2,48)=0.07, p=0.93$] suggesting that acute ABT-107 administration has no effect on nicotine or sucrose taking in rats.

3.3. Acute ABT-089 administration attenuated nicotine, but not sucrose, reinstatement in rats

Total active and inactive lever responses for rats pretreated with 0, 0.12, 1.2 and 12.0 mg/kg ABT-089 prior to a systemic priming injection of nicotine are shown in Figure 4A ($n = 11$). Total lever responses were analyzed with a two-way ANOVA, which revealed significant main effects of treatment [$F(3,80)=4.00, p<0.05$] and lever [$F(1,80)=121.5, p<0.0001$] as well as a significant treatment $\times$ lever interaction [$F(3,80)=3.83, p<0.05$]. Subsequent pairwise analyses showed that the total active lever responses were significantly different between rats pretreated with vehicle and 12.0 mg/kg ABT-089 (Bonferroni, $p<0.05$). Total lever responses for animals pretreated with 0 ($n = 10$), 0.12, 1.2 and 12.0 mg/kg ABT-089 ($n = 9$) prior to sucrose reinstatement tests are shown in Figure 4B. These data were analyzed with a two-way ANOVA, revealed a significant main effect of lever [$F(1,66)=152.7, p<0.0001$] but no effect of treatment [$F(3,66)=0.37, p=0.81$]. Taken together, these data indicate that ABT-089 administration attenuates the reinstatement of nicotine-seeking behavior and that these effects are reinforcer-specific.

3.4. Acute ABT-107 administration attenuated nicotine, but not sucrose, reinstatement in rats

Total lever responses in rats pretreated with 0, 0.03 and 0.3 mg/kg ABT-107 ($n = 8$) prior to a systemic priming injection of nicotine are shown in Figure 5A. These data were analyzed with a two-way ANOVA, which revealed significant main effects of treatment [$F(2,42)=8.07, p<0.01$] and lever [$F(1,42)=124.2, p<0.0001$] as well as a significant treatment $\times$ lever interaction [$F(2,42)=6.90, p<0.01$]. Subsequent pairwise analyses showed that the total active lever responses were significantly different between rats pretreated with vehicle and 0.3 mg/kg ABT-107 (Bonferroni, $p<0.05$). The effects of ABT-107 pretreatment on sucrose seeking were tested in a separate cohort of rats ($n = 8$). Total lever responses during the sucrose reinstatement test sessions are shown in Figure 5B and were analyzed with a two-way ANOVA. No effects of ABT-107 pretreatment on sucrose seeking were observed [$F(2,42)=2.24, p=0.12$]. These results indicate that ABT-107 administration selectively attenuates nicotine-seeking behavior in rats.

4. Discussion

This is the first study to examine the effects of the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR partial agonist ABT-089 and the $\alpha 7$ nAChR agonist ABT-107 on nicotine self-administration and the

reinstatement of nicotine-seeking behavior. Acute administration of ABT-089 and ABT-107 did not influence nicotine or sucrose self-administration in rats. In contrast, acute administration of ABT-089 and ABT-107 dose-dependently attenuated the reinstatement of nicotine-seeking behavior in rats. Moreover, ABT-089 and ABT-107 administration did not influence sucrose seeking indicating that these effects are reinforcer specific and not due to general motor-suppressant effects of drug treatment. Taken together, these results indicate that $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$ and $\alpha 7$ nAChRs play a critical role in the reinstatement of nicotine-seeking behavior and that subtype-selective agonists targeting these nAChRs may have efficacy in preventing smoking relapse during abstinence.

The lack of effect of acute ABT-089 administration on nicotine self-administration was surprising given the role for $\alpha 4$ -, $\alpha 6$ - and $\beta 2$ -containing nAChR subtypes in mediating the reinforcing effects of nicotine. Partial agonists of $\alpha 4\beta 2^*$ nAChRs and antagonists of $\alpha 4\beta 2^*$ nAChRs decrease nicotine self-administration in rats [17-20, 49]. Consistent with these findings, mutant mice lacking $\beta 2^*$ nAChRs self-administer less nicotine than wildtype controls, deficits that are reversed following viral-mediated re-expression of $\beta 2$ nAChR subunits in the ventral tegmental area [50-52]. Moreover, the reinforcing properties of nicotine are also mediated by $\alpha 4^*$ nAChRs. Nicotine self-administration is reduced in mutant mice lacking $\alpha 4^*$ nAChRs compared to wildtype controls [52] [however see, 53]. Transgenic mice expressing hypersensitive $\alpha 4^*$ nAChRs have increased sensitivity to nicotine reward providing further support for the role of $\alpha 4^*$ nAChRs in nicotine reinforcement [53, 54]. $\alpha 6\beta 2^*$ nAChRs also play an important role in nicotine self-administration. Pharmacological inhibition of $\alpha 6\beta 2^*$ nAChRs decrease nicotine taking in rats [55, 56] and mutant mice lacking $\alpha 6^*$ nAChRs do not acquire nicotine self-administration [52]. Taken together, these findings support a key role for $\alpha 4$ -, $\alpha 6$ - and $\beta 2$ -containing nAChR subtypes in regulating nicotine self-administration. However, despite considerable evidence from behavioral pharmacology and genetic studies that decreased $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR function reduces nicotine taking in rodents [6], we found no effect of the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR partial agonist ABT-089 on nicotine self-administration. The doses of ABT-089 used in this study were based on doses that improve cognitive performance in rats [33, 34]. It is possible that higher acute doses of ABT-089 may be required to attenuate nicotine self-administration in rats. Moreover, chronic dosing of smoking cessation medications is required to prevent nicotine craving and relapse in human smokers. Therefore, it is possible that sustained elevated plasma levels of ABT-089, not achieved through our acute dosing regimen, are necessary to attenuate nicotine self-administration in rats. While future studies are needed to examine the effects of repeated ABT-089 on nicotine self-administration, the present findings indicate that acute ABT-089 does not affect nicotine consumption at the doses tested.

The present findings also demonstrate that acute administration of the $\alpha 7$ nAChR agonist ABT-107 has no effect on stable nicotine self-administration in rats. These results are not surprising given the mixed evidence supporting a role for $\alpha 7$ nAChRs in mediating the reinforcing properties of nicotine. Pharmacological inhibition of $\alpha 7$ nAChRs and decreased expression of $\alpha 7$ nAChR subunits does not alter nicotine taking in rodents [52, 57]. In contrast, one study demonstrated reduced nicotine taking in rats pretreated with an $\alpha 7$ nAChR antagonist [21]. While the precise contribution of $\alpha 7$ nAChRs to nicotine

reinforcement remains to be determined, our findings suggest that $\alpha 7$ nAChRs do not play a role in nicotine taking. However, there is some evidence that $\alpha 7$ nAChRs regulate nicotine consumption in a temporally dependent manner. Mice with constitutive knockdown of neuronal $\alpha 7$ nAChR expression display reduced consumption of nicotine compared to controls after prolonged, but not acute, nicotine exposure [58]. Therefore, the present study examining the effects of ABT-107 on nicotine taking may be limited by the short duration (i.e. 28 days) of nicotine self-administration. It is possible that the effects of ABT-107 on drug taking may not be realized until rats have self-administered nicotine for a minimum of 60 days.

Both the locomotor-stimulating effects and the reinforcing properties of nicotine are mediated, in part, by increased dopamine signaling in the ventral striatum [59-61]. When compared to nicotine, ABT-089 is 30% less efficacious and 25-fold less potent in stimulating dopamine release from rat striatal slices [62]. While these findings suggest that the lack of effect of ABT-089 on nicotine self-administration may be due, in part, to its reduced potency to stimulate dopamine release, future studies are required to determine the exact neurochemical effects of ABT-089 in rat self-administering nicotine. With regard to ABT-107, a recent study demonstrated that ABT-107 administration does not produce locomotor sensitization or cross-sensitize to nicotine when administered to nicotine-experienced rats [39]. In contrast to the behavioral-activating effects of nicotine, these results suggest that ABT-107 does not stimulate striatal dopamine release to promote hyperlocomotion. Thus, it is possible that rats continue to self-administer nicotine at baseline levels following pretreatment with ABT-089 and ABT-107 due to the relatively weak influence of these agonists on nucleus accumbens dopamine transmission.

In contrast to a growing literature examining the role of nAChR subtypes in nicotine reinforcement, there is a paucity of studies investigating nAChR subtypes in the reinstatement of nicotine-seeking behavior. Administration of varenicline, an $\alpha 4\beta 2$ nAChR partial agonist and $\alpha 7$ nAChR agonist, attenuates nicotine seeking in rats [17, 23] [however see, 63]. Furthermore, chronic nicotine exposure increases $\alpha 7$ nAChR/NMDA receptor complex formation in the brain and disrupting this interaction attenuates cue-induced nicotine seeking [22]. Our results extend these findings and indicate that administration of the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR partial agonist ABT-089 and the $\alpha 7$ nAChR agonist ABT-107 is sufficient to attenuate the reinstatement of nicotine seeking elicited by a priming injection of nicotine and cues previously paired with nicotine taking. A recent study, however, found that administration of the $\alpha 7$ nAChR antagonist MLA attenuated cue-induced nicotine seeking in rats [64]. The reason for these discrepant findings is not clear but may involve altered brain permeability of MLA following chronic nicotine exposure and/or non-specific effects of MLA at other nAChR subtypes [65, 66]. The ability of ABT-089 and ABT-107 to attenuate nicotine, but not sucrose, seeking indicates that these effects do not generalize to other reinforced behaviors or are due to general motor impairments following drug administration. Collectively, these data highlight the need to further identify the role of nAChRs in the reinstatement of nicotine-seeking behavior and investigate the efficacy of subtype-selective nAChR agonists to attenuate nicotine seeking. One limitation of the current study is that it does not examine the effects of ABT-089 and ABT-107 on drug priming-versus cue-induced reinstatement of nicotine seeking. Future studies are required to

identify the effects of these nAChR agonists on regulating these two aspects of nicotine seeking.

Chronic dosing of smoking cessation medications is required to prevent nicotine craving and relapse in human smokers. Therefore, it will be necessary to study the effects of repeated dosing of ABT-089 and ABT-107 during nicotine withdrawal on subsequent drug seeking. It is possible that ABT-089 and ABT-107 treatment alone may have limited efficacy in human smokers. Whether ABT-089 and/or ABT-107 represent first-line pharmacotherapies or adjunctive therapies for smoking cessation remains to be determined. Interestingly, a recent study found that acute administration of ABT-089 or ABT-107 reversed/normalized anxiety-like behaviors during nicotine withdrawal in mice [42]. Together with the present findings, these results indicate that the nAChR partial agonists ABT-089 and ABT-107 may alleviate nicotine withdrawal-induced anxiety and attenuate nicotine relapse in human smokers.

The present results indicate that acute administration of ABT-089 and ABT-107 is sufficient to attenuate the reinstatement of nicotine-seeking behavior in rats. These results suggest that subtype-selective nAChR agonists that target neuronal $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$ and $\alpha 7$ nAChRs may prevent nicotine craving and relapse in abstinent human smokers. Future clinical trials are required to determine the efficacy of ABT-089 and ABT-107 as smoking cessation pharmacotherapies in treatment-seeking smokers.

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Highlights

- Acute administration of ABT-089 or ABT-107 attenuated the reinstatement of nicotine seeking.
- ABT-089 or ABT-107 administration did not affect sucrose-seeking behavior.
- Nicotine and sucrose self-administration were not affected by ABT-089 or ABT-107 pretreatment.

A.

B.

Figure 1. Timeline for the nicotine and sucrose self-administration and reinstatement studies

A between-sessions and within-subjects design was used to study the effects of ABT-089 (vehicle, 0.12, 1.2 and 12.0 mg/kg) and ABT-107 (vehicle, 0.03 and 0.3 mg/kg) pretreatments on nicotine and sucrose self-administration behaviors (A) as well as the reinstatement of nicotine- and sucrose-seeking behaviors (B) in separate cohorts of rats. See Methods for more detail.

A. Nicotine Self-Administration

B. Sucrose Self-Administration

Figure 2. Acute, systemic administration of ABT-089 did not affect nicotine and sucrose self-administration

(A) ABT-089 pretreatment did not alter nicotine-taking behavior in rats self-administering nicotine on a FR5 schedule of reinforcement. No significant differences in responding (mean $\pm$ SEM) on the active and inactive levers were found in rats pretreated with vehicle, 0.12, 1.2 and 12.0 mg/kg ABT-089 ($n = 16/\text{treatment}$) prior to nicotine self-administration test sessions ($p > 0.05$). **(B)** Total number of active lever responses (mean $\pm$ SEM) in subjects self-administering sucrose pellets on a FR5 schedule of reinforcement ($n = 10/\text{treatment}$). No significant differences in responding were noted between subjects pretreated with vehicle, 0.12, 1.2 and 12.0 mg/kg ABT-089 ($p > 0.05$).

A. Nicotine Self-Administration

B. Sucrose Self-Administration

Figure 3. Nicotine and sucrose self-administration were not altered in rats pretreated with ABT-107

(A) No significant differences in responding (mean±SEM) on the active and inactive levers were found in rats pretreated with vehicle, 0.03 and 0.3 mg/kg ABT-107 ($n = 7/\text{treatment}$) prior to nicotine self-administration test sessions ($p > 0.05$). (B) Acute, systemic ABT-107 administration did not alter sucrose-taking behavior in rats ($n = 9/\text{treatment}$). No significant differences in responding were noted between subjects pretreated with vehicle, 0.03 and 0.3 mg/kg ABT-107 ($p > 0.05$).

A. Nicotine Reinstatement

B. Sucrose Reinstatement

Figure 4. Acute, systemic administration of ABT-089 attenuated nicotine, but not sucrose, seeking

(A) Total number of responses (mean±SEM) on the active and inactive levers during the reinstatement test session following a priming injection of nicotine (0.2 mg/kg, s.c.) in rats pretreated with vehicle, 0.12, 1.2 and 12.0 mg/kg ABT-089 ($n=11$ /treatment). ABT-089 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 in animals treated with 12.0 mg/kg ABT-089 when compared with saline-treated controls (Bonferroni, $p<0.05$). **(B)** No differences in total active lever responses (mean±SEM) during sucrose reinstatement test sessions were noted in animals pretreated with vehicle ($n=10$), 0.12 ($n=9$), 1.2 ($n=9$) or 12.0 ($n=9$) mg/kg ABT-089 ($p>0.05$).

A. Nicotine Reinstatement

B. Sucrose Reinstatement

Figure 5. Acute, systemic administration of ABT-107 dose-dependently attenuated nicotine, but not sucrose, seeking

(A) Total number of responses (mean±SEM) on the active and inactive levers during the reinstatement test session following a priming injection of nicotine (0.2 mg/kg, s.c.) in rats pretreated with vehicle, 0.03 or 0.3 ABT-107 ($n = 8/\text{treatment}$). Total active lever responses were significantly decreased in rats pretreated with 0.3 mg/kg ABT-107 when compared to saline-treated controls (Bonferroni, $*p < 0.05$). **(B)** No differences in total active lever responses (mean±SEM) during sucrose reinstatement test sessions were observed in rats pretreated with vehicle, 0.03 and 0.3 mg/kg ABT-107 ($n = 8/\text{treatment}$) ($p > 0.05$).