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ABT-089, but not ABT-107, ameliorates nicotine withdrawal-induced cognitive deficits in C57BL6/J mice

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Abstract

Nicotine withdrawal produces cognitive deficits that can predict relapse. Amelioration of these cognitive deficits emerges as a target in current smoking cessation therapies. In rodents, withdrawal from chronic nicotine disrupts contextual fear conditioning (CFC), whereas acute nicotine enhances this hippocampus-specific learning and memory. These modifications are mediated by β_2 -subunit-containing (β_2^*) nicotinic acetylcholine receptors in the hippocampus. We aimed to test ABT-089, a partial agonist of $\alpha_4\beta_2^*$, and ABT-107, an α_7 nicotinic acetylcholine receptor agonist, for amelioration of cognitive deficits induced by withdrawal from chronic nicotine in mice. Mice underwent chronic nicotine administration (12.6 mg/kg/day or saline for 12 days), followed by 24 h of withdrawal. At the end of withdrawal, mice received 0.3 or 0.6 mg/kg ABT-089 or 0.3 mg/kg ABT-107 (doses were determined through initial dose–response experiments and prior studies) and were trained and tested for CFC. Nicotine withdrawal produced deficits in CFC that were reversed by acute ABT-089, but not ABT-107. Cued conditioning was not affected. Taken together, our results suggest that modulation of hippocampal learning and memory using ABT-089 may be an effective component of novel therapeutic strategies for nicotine addiction.

Keywords

acetylcholine; addiction; cognition; hippocampus; learning; withdrawal

Introduction

Despite gains by preventive healthcare policies in the last few decades, chronic tobacco use still remains the leading preventable cause of morbidity and mortality worldwide and a major public health challenge (Agaku *et al.*, 2014). The principal addictive compound in tobacco products is nicotine, a plant alkaloid that exerts its effects on the central and peripheral nervous system by acting as an agonist at nicotinic acetylcholine receptors (nAChRs; Dani and Heinemann, 1996). nAChRs are pentameric ligand-gated ion channels

endogenously activated by acetylcholine under physiological conditions. Neuronal nAChR pentamers can be assembled from nine α (α_2 – α_{10}) and three β (β_2 – β_4) subunit monomers to form functional receptors. Among a variety of combinations, heteromeric $\alpha_4\beta_2$ and homomeric α_7 receptors are the most abundantly found nAChRs throughout the central nervous system (Changeux, 2012). Altered nAChR signaling by chronic nicotine exposure may lead to nicotine addiction (United States, Public Health Service, Office of the Surgeon General., 2010).

Nicotine withdrawal syndrome is defined by a set of somatic, affective, and cognitive symptoms that occur shortly after cessation of chronic nicotine administration (Hughes, 2007). Nicotine withdrawal-related cognitive deficits include – but are not limited to – difficulty in concentration, disruptions in working memory, and impairment of attention (Snyder *et al.*, 1989; Parrott and Craig, 1992; Blake and Smith, 1997). In the last two decades, nicotine withdrawal-related cognitive deficits have been shown to be part of a major dependence phenotype (Lerman *et al.*, 2007) and to predict relapse in addicted smokers (Rukstalis *et al.*, 2005; Patterson *et al.*, 2010). Of smokers, 50–75% relapse within the first week of a quit attempt (Garvey *et al.*, 1992; Hughes, 1992). Thus, amelioration of these cognitive deficits may aid in efforts to develop efficacious pharmacotherapy strategies.

Studies with rodents have shown that withdrawal from chronic nicotine impairs hippocampus-dependent learning (Davis *et al.*, 2005; Andre *et al.*, 2008; Portugal and Gould, 2009; Kenney *et al.*, 2011; Portugal *et al.*, 2012a, 2012b). Withdrawal from chronic nicotine selectively disrupts contextual, but not cued, fear conditioning (Davis *et al.*, 2005), a paradigm that approximates important aspects of declarative memory in humans (Kenney and Gould, 2008), whereas acute nicotine enhances this hippocampus-specific learning and memory paradigm (Gould and Wehner, 1999). Previous studies have shown that hippocampal β_2^* (where * indicates other assembled subunits) nAChRs mediate nicotine withdrawal-related deficits in contextual learning and memory (Davis and Gould, 2009; Gould *et al.*, 2012), whereas FDA-approved pharmacotherapies, such as bupropion (Portugal and Gould, 2007) and varenicline (Raybuck *et al.*, 2008), and experimental smoking cessation pharmacotherapies, such as galantamine (Wilkinson and Gould, 2011), successfully reverse these deficits.

Targeting cognitive deficits associated with abstinence from nicotine/tobacco may facilitate the treatment of nicotine addiction. Because studies have suggested that modulation of β_2^* and α_7 nAChR signaling may have cognition enhancing effects (Sarter *et al.*, 2009) and nAChRs are clearly involved in nicotine addiction, drugs that modulate signaling through these receptors may be effective in treating nicotine withdrawal-associated changes in cognition (Kenney and Gould, 2008; Portugal and Gould, 2008; Gould and Leach, 2014). ABT-089 [2-methyl-3-(2-(*S*)-pyrrolidinylmethoxy)pyridine, pozanidine] is a novel cholinergic agent that is a partial agonist at $\alpha_4\beta_2^*$ nAChRs and shows high selectivity for $\alpha_6\beta_2^*$ and $\alpha_4\alpha_5\beta_2$ nAChR subtypes (Lin *et al.*, 1997; Rueter *et al.*, 2004; Marks *et al.*, 2009). ABT-107 [5-(6-[(3*R*)-1-azabicyclo[2,2,2]oct-3-yl]oxy)pyridazin-3-yl]-1*H*-indole] is a novel nicotinic cholinergic agonist that shows selective activity at homomeric α_7 nAChRs (Bitner *et al.*, 2010). The binding affinity (K_i , nmol/l; rat) for ABT-089 to [3 H]cytosine sites was 16.7 (nicotine = 1; Lin *et al.*, 1997) and for ABT-107 to [3 H]cytosine sites was 4000 and

to [^3H]MLA was 7.2 (Malysz *et al.*, 2010). In animal studies, both ABT-089 and ABT-107 have been shown to enhance learning (Decker *et al.*, 1997; Bitner *et al.*, 2010). As amelioration of cognitive deficits associated with nicotine withdrawal has been proposed as a major target for potential smoking cessation therapies (Ashare *et al.*, 2014), we tested whether these two drugs could reverse nicotine withdrawal-related cognitive deficits in C57BL/6 mice.

Methods

Subjects

Adult male C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine, USA) at 8–12 weeks of age were used for the experiments. Mice were housed four per cage under monitored temperature and humidity, and were provided with free access to food and water. A 12-h light/dark cycle (lights on at 07:00 h and off at 19:00 h) was maintained in the housing facility throughout the study, and all experiments were carried out during the light cycle. All procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Drugs and administration

Nicotine was chronically administered through sub-cutaneous implanted osmotic mini pumps (Model 1002, 100 μl reservoir volume; Alzet, Cupertino, California, USA) that delivered 12.6 mg/kg/day nicotine hydrogen tartrate (freebase weight; Sigma Co., St Louis, Missouri, USA) in physiological saline solution (0.9% NaCl in distilled water) at a 0.21–0.25 $\mu\text{l/h}$ flow rate. Pumps were removed 12 days after the initial implant surgeries; training was conducted 24 h after pump removal and testing was performed 24 h after training. ABT-089 and ABT-107 were dissolved in saline and administered intraperitoneally 5 and 15 min before training and testing, respectively. Dose–response experiments with ABT-089 and ABT-107 were conducted to establish the doses in the withdrawal experiments that had minimal impact on baseline fear conditioning. Starting doses and pretreatment times were based on communications with Dr Lynne Rueter (AbbVie) and on prior work (Yohn *et al.*, 2014).

Fear conditioning apparatus

Mice were trained and tested for contextual fear conditioning in four identical conditioning chambers (17.78 $\times$ 19.05 $\times$ 38.10 cm) placed in sound attenuating boxes (Med Associates, St. Albans, Vermont, USA). Each chamber was constructed of clear Plexiglas walls in the front, back, and top, and stainless steel walls on the sides. The chamber floors, which were connected to a shock generator and scrambler, were made of metal rods (0.2 cm in diameter) spaced 0.60 cm apart. Speakers for administering the conditioned stimulus (CS) were located on the right wall of each chamber. Ventilation fans, located on the right wall of each sound attenuating box, provided air exchange and background noise (69 dB). A 4 W bulb illuminated each box from above, and a computer-running MED-PC software (Med Associates, St Albans, Vermont, USA) controlled stimuli administration.

Testing for cued fear conditioning took place in a different set of four Plexiglas chambers (20.30 × 22.90 × 17.80 cm) placed in sound attenuating boxes. Similar speaker, ventilation fan, and illumination setups were used; however, these chambers were located in a different room and were visually and tactually different from those used for context training and testing. A novel olfactory cue, vanilla extract, was also applied to a paper towel and placed under each chamber to further distinguish the chambers from those used for training.

Contextual and cued fear conditioning

Mice were trained and tested in contextual fear conditioning according to the behavioral procedures described previously (Davis *et al.*, 2006). Briefly, mice were placed in conditioning chambers and training lasted for a total of 330 s. Baseline freezing was recorded during the first 2 min, followed by two co-terminating CS (30 s, 85 dB white noise)–unconditioned stimulus (2 s, 0.57 mA footshock) trials separated by a 2-min interval. Immediate freezing was recorded during the 2-min interval between the first and second trials. Freezing, defined as the absence of movement except for respiration (Blanchard and Blanchard, 1969), served as the dependent measure and was scored by the experimenter for 1 s every 10 s during each session (Gould and Higgins, 2003). Twenty-four hours after training, the mice were tested for contextual freezing to the training context for 5 min. One hour after context testing, the mice were placed in the cued fear conditioning chambers for 6 min. Freezing in the absence of the CS was scored during the first 3 min, and during the last 3 min, freezing in the presence of the CS was assessed. The chambers were cleaned with a 70% ethanol solution after all behavioral procedures.

Five-choice serial reaction time task

Mice were food-restricted to 85% of their free-feeding weight. Experiments were conducted in four identical chambers (24 × 20.3 × 12.7 cm; Med Associates Inc., St. Albans, Vermont, USA). Habituation sessions included a fixed ratio 1 schedule of reinforcement, in which a nose-poke in any of the five nose-poke holes resulted in pellet (rodent dustless precision pellets, 20 mg; Bio-Serv Inc., Frenchtown, New Jersey, USA) reinforcement. All five nose-poke holes and the food hopper were illuminated for the entire session, which ended after 30 min or after delivery of 60 pellets. Mice that fulfilled this criterion (at least 50 pellets within a session) began five-choice serial reaction time task (5-CSRTT) training. The 5-CSRTT training procedure, based on the procedure developed by Higgins and Breyse (2008), commenced with illumination of the house and food hopper lights and the delivery of a food pellet. Trials began with the illumination of one of the five nose-poke holes by pseudorandom selection, and each nose-poke hole was illuminated an equal number of times. A nose-poke response in an illuminated nose-poke hole within the limited hold (LH) time was recorded as a correct response and a pellet was delivered. Responding to a nonilluminated nose-poke hole was recorded as an incorrect response, resulting in a time-out period of 5 s, during which the house light was extinguished. A time-out also resulted from premature responding during the intertrial interval (10 s). Failure to respond resulted in an omission error.

At the beginning of training, the signal duration (SD) was 30 s, with a 30 s LH, but over the course of training the SD and LH were decreased to 1 and 5 s, respectively. The

performance criterion for progressing through the stages of training was 80% accuracy [correct response/(correct + incorrect responses) $\times$ 100] and 20% omissions (omissions/number of trials $\times$ 100). After the mice fulfilled the performance criterion for at least 3 days on the final training protocol (SD 1 s), an osmotic mini pump containing saline or nicotine (18 mg/kg/day) was implanted. The dose of nicotine was chosen on the basis of prior work that demonstrated that this dose produced withdrawal deficits in contextual fear conditioning (Wilkinson, Turner *et al.*, 2013). A higher dose was not used because higher doses can produce somatic withdrawal signs (Damaj *et al.*, 2003; Jackson *et al.*, 2008), which could confound interpretation of the results. The intention was to then find the minimum dose that produced withdrawal changes in 5-CSRTT, but as no withdrawal deficits were detected in 5-CSRTT with this dose, lower doses were not examined. Mice were assigned to drug groups on the basis of baseline performance over the prior 3 days such that both groups performed similarly before pump implantation. Starting 24 h after implantation of the mini pumps, mice were trained for 12 days during chronic nicotine administration. The two groups had near-identical mean (SEM) percent correct scores on the last day of training before withdrawal: chronic saline = 83.8 ± 2.6 , chronic nicotine = 83.9 ± 2.3 . After training on day 12, mini pumps were removed; 24 h later, mice were tested using the final training protocol (SD 1 s, LH 5 s) for 4 days.

Statistical analyses

Fear conditioning data were analyzed with one-way analyses of variance (ANOVAs; SPSS Version 20.0; SPSS Inc., Chicago, Illinois, USA) and followed up with post-hoc Least Significant Difference tests. Student's *t*-test was also used when appropriate. 5-CSRTT was analyzed with a repeated-measures ANOVA. Results were considered significant at *P* 0.05. All data are expressed as means $\pm$ SEM.

Results

Dose determination for ABT-089 effects on contextual fear conditioning

To determine the doses of ABT-089 that had minimal effect on baseline fear conditioning, we conducted an initial dose–response experiment. ABT-089 (0.6, 1.2, 6, 12 mg/kg) or vehicle was administered to C57BL/6 mice 5 min before training and testing of contextual fear conditioning (Fig. 1). A one-way ANOVA revealed a significant effect when mice were tested for contextual fear conditioning [$F_{(4,55)} = 3.31$, $P < 0.05$] and no difference when they were tested for cued fear conditioning [$F_{(4,55)} = 0.02$, NS]. Post-hoc least significant difference tests revealed that there was a significant enhancement in freezing to context with all tested doses of ABT-089 as compared with vehicle ($P < 0.05$). No significant effects of ABT-089 were observed for baseline freezing, immediate freezing, or pre-CS freezing. A separate follow-up experiment was conducted to test whether a lower dose of ABT-089, 0.3 mg/kg, would alter fear conditioning. Student's *t*-test revealed no significant effect of 0.3 mg/kg ABT-089 on any measure ($P > 0.05$; contextual fear conditioning: saline = 56.7 ± 5.4 , ABT-089 = 61.7 ± 7.3 ; cued fear conditioning: saline = 80.2 ± 5.8 , ABT-089 = 72.9 ± 4.7 ; percent total time freezing mean $\pm$ SEM).

Acute ABT-089 reverses nicotine withdrawal-induced deficits in contextual fear conditioning

In two separate experiments, we tested whether the doses of ABT-089 that were noneffective and minimally effective in enhancing baseline contextual fear conditioning would ameliorate nicotine withdrawal-induced deficits in contextual fear conditioning. Following withdrawal from chronic nicotine or vehicle administration (24 h after removal of osmotic mini pumps), mice were treated with 0.3 mg/kg ABT-089 or vehicle 5 min before training and testing for contextual fear conditioning (Fig. 2a). A one-way ANOVA revealed a significant effect of drug treatment on contextual fear conditioning [$F_{(3,50)} = 9.30$, $P < 0.001$] and no significant effect on cued fear conditioning [$F_{(3,40)} = 0.135$, NS]. Post-hoc tests revealed that mice withdrawn from chronic nicotine and treated acutely with saline showed significantly less freezing to context ($P < 0.05$) – that is, a deficit in contextual fear conditioning. In contrast, mice withdrawn from chronic nicotine that received acute administration of 0.3 mg/kg ABT-089 showed no deficit in contextual fear conditioning and actually had enhanced contextual fear conditioning relative to saline controls ($P < 0.05$). No significant effects of ABT-089 or withdrawal from chronic nicotine were observed for baseline freezing, immediate freezing, or pre-CS freezing.

In the study with the 0.6 mg/kg dose of ABT-089 (Fig. 2b), a one-way ANOVA revealed a significant effect of drug treatment on contextual fear conditioning [$F_{(3,40)} = 11.35$, $P < 0.001$] and no significant effect on cued fear conditioning [$F_{(3,40)} = 0.04$, NS]. Post-hoc tests revealed that mice withdrawn from chronic nicotine and treated with saline had a deficit in contextual fear conditioning ($P < 0.05$), whereas mice withdrawn from chronic nicotine that received acute administration of 0.6 mg/kg ABT-089 had no withdrawal deficit in contextual fear conditioning and showed significantly enhanced freezing to context as compared with saline controls ($P < 0.05$). No significant effects of ABT-089 or withdrawal from chronic nicotine were observed for baseline freezing, immediate freezing, or pre-CS freezing. Taken together, these results suggest that ABT-089 ameliorates nicotine withdrawal-related cognitive deficits in contextual fear conditioning.

Dose determination for ABT-107 effects on contextual fear conditioning

To determine the doses of ABT-107 that had minimal effect on baseline fear conditioning, we conducted an initial dose–response experiment. ABT-107 (0.003, 0.03, 0.3 mg/kg) or vehicle was administered to C57BL/6 mice 15 min before training and testing for contextual fear conditioning (Fig. 3). A one-way ANOVA revealed a significant effect of drug treatment on contextual fear conditioning [$F_{(3,27)} = 3.899$, $P < 0.05$]; however, this was driven by a significant difference in freezing to the context between the 0.03 and the 0.3 mg/kg groups ($P < 0.05$); there were no significant differences in any of the treatment groups as compared with the vehicle. There were no significant differences in cued fear conditioning [$F_{(3,27)} = 2.02$, NS], baseline freezing, immediate freezing, or pre-CS freezing.

Acute ABT-107 does not reverse nicotine withdrawal-induced deficits in contextual fear conditioning

In this experiment, we examined whether 0.3 mg/kg ABT-107 would ameliorate nicotine withdrawal-induced cognitive deficits in contextual fear conditioning. A oneway ANOVA

revealed a significant effect of drug treatment on contextual fear conditioning [$F_{(3,28)} = 7.71$, $P < 0.005$] and no significant effect on cued fear conditioning [$F_{(3,28)} = 0.15$, NS; Fig. 4]. Post-hoc tests revealed that mice withdrawn from chronic nicotine showed significantly less freezing to context regardless of ABT-107 treatment ($P < 0.05$).

5-CSRTT and nicotine withdrawal

A repeated-measures ANOVA showed no effect of nicotine withdrawal on percent correct performance [$F_{(1,23)} = 0.07$, NS; Fig. 5], accuracy [$F_{(1,23)} = 0.17$, NS], percent incorrect performance [$F_{(1,23)} = 0.11$, NS], or percent omission errors [$F_{(1,23)} = 0.06$, NS]. These data, combined with the present results for contextual fear conditioning and prior studies (Wilkinson *et al.*, 2013), suggest that the effects of nicotine withdrawal on contextual fear conditioning and attention, as assessed by the 5-CSRTT, may be mediated by different substrates at the doses tested.

Discussion

Withdrawal symptoms during periods of abstinence are one of the features that drive nicotine addiction. There is not one universal withdrawal symptom, instead a constellation of symptoms exists, and expression of these symptoms varies across individuals (for review see Gould, 2006). As discussed, changes in cognition during abstinence are a major withdrawal symptom that is associated with relapse (Rukstalis *et al.*, 2005; Patterson *et al.*, 2010). This study is the first to demonstrate that ABT-089, a partial $\alpha_4\beta_2^*$ agonist, reverses nicotine withdrawal-induced cognitive deficits. Specifically, our results showed that acute administration of two doses of ABT-089 reversed nicotine withdrawal-associated deficits in contextual fear in C57BL/6 mice. In contrast, the α_7 agonist ABT-107 was ineffective at ameliorating nicotine withdrawal-induced deficits in contextual fear conditioning. In addition, consistent with previous research (Davis *et al.*, 2005; Andre *et al.*, 2008; Gould *et al.*, 2012), we found that hippocampus-dependent learning (e.g. contextual fear conditioning) was more sensitive to the effects of nicotine withdrawal than hippocampus-independent learning (e.g. cued fear conditioning; Davis *et al.*, 2005; Andre *et al.*, 2008; Portugal and Gould, 2009; Kenney *et al.*, 2011; Portugal *et al.*, 2012a, 2012b). These results suggest that nicotine withdrawal may alter hippocampal $\alpha_4\beta_2^*$ nAChR function and that a drug that targets high-affinity nAChRs, such as ABT-089, may be effective in treating cognitive withdrawal symptoms in smokers.

The influence of hippocampal β_2^* nAChR signaling on contextual fear conditioning has been extensively studied. Nicotine enhances contextual fear conditioning (Gould and Wehner, 1999; Gould and Higgins, 2003; Davis *et al.*, 2006) and β_2^* nAChRs mediate this effect, as demonstrated by antagonist (Davis *et al.*, 2007) and knock-out (KO) studies (Wehner *et al.*, 2004; Davis and Gould, 2007). Abrupt discontinuation of chronic nicotine administration produces deficits in contextual fear conditioning (Davis *et al.*, 2005; Andre *et al.*, 2008; Portugal and Gould, 2009), and this effect is also mediated by hippocampal β_2^* nAChRs; β_2 KO mice do not exhibit nicotine withdrawal-related deficits in contextual fear conditioning (Portugal *et al.*, 2008), and either systemic or intrahippocampal administration of high-affinity $\alpha_4\beta_2^*$ nAChR antagonist dihydro- β -erythroidine impairs learning in mice

treated with chronic nicotine (Davis and Gould, 2009). Furthermore, time-limited changes in nicotine withdrawal-related deficits in contextual learning are associated with temporal changes in upregulation of high-affinity hippocampal β_2^* nAChR binding (Gould *et al.*, 2012). From this perspective, the selectivity of ABT-089 toward high-affinity $\alpha_4\beta_2^*$ nAChRs might underlie the amelioration of nicotine withdrawal-related deficits in contextual fear conditioning on acute administration of the drug.

The changes responsible for withdrawal symptoms are unknown. However, it has been hypothesized that during chronic nicotine treatment, nAChRs are desensitized and upregulated, but during withdrawal, desensitized nAChRs resensitize and thus, because of the increased nAChR concentration due to upregulation, the nAChR system becomes hypersensitive (Dani and Heinemann, 1996; Gould *et al.*, 2012, 2014). In support of this, acute nicotine was observed to produce a greater net change in learning in mice withdrawn from chronic nicotine compared with mice withdrawn from chronic saline treatment (Wilkinson and Gould, 2013). The current results similarly show that ABT-089 produced a significant enhancement of contextual fear conditioning in mice withdrawn from chronic nicotine treatment compared with saline-treated mice, but the same dose administered to mice withdrawn from chronic saline treatment did not significantly enhance learning. If the nAChR system is hypersensitized during withdrawal, partial agonists such as varenicline and ABT-089 may be effective in ameliorating nicotine withdrawal deficits in learning by dampening nAChR activity (Raybuck *et al.*, 2008). In support of this, varenicline was seen to produce weak tonic activation of nAChRs and decreased activity of full agonists (Papke *et al.*, 2011).

ABT-089 has affinity for nAChRs that include $\alpha_4\beta_2^*$, $\alpha_6\beta_2^*$, and $\alpha_4\alpha_5\beta_2$ nAChR subtypes (Lin *et al.*, 1997; Rueter *et al.*, 2004; Marks *et al.*, 2009). It is unclear which of these nAChR subtypes is involved in ameliorating the cognitive withdrawal deficit; however, if upregulated nAChRs are the therapeutic target of ABT-089, $\alpha_6\beta_2\alpha_4$ nAChRs may not be involved. Specifically, whereas chronic nicotine treatment upregulated $\alpha_4\beta_2$ nAChRs, $\alpha_6\beta_2\alpha_4$ nAChRs were downregulated (Perez, *et al.*, 2008), and another study found no change in α_6^* nAChRs after chronic nicotine treatment in 12 of 14 areas examined, whereas decreased binding was seen in the striatum and lateral geniculate nucleus (Doura *et al.*, 2008). Thus, because $\alpha_6\beta_2\alpha_4$ nAChRs are not upregulated, the effects of ABT-089 on nicotine withdrawal-related deficits in learning may involve other nAChRs; however, more work is needed to identify the subunits involved and to determine whether nAChR upregulation contributes to the withdrawal phenotype.

The involvement of homomeric α_7 nAChRs in higher cognitive functions, such as learning, memory, and attention, has been extensively investigated [for reviews, see Levin (2012); Lendvai *et al.* (2013)]. In animal studies, α_7 KO mice have been shown to exhibit deficits in radial arm maze learning (Levin *et al.*, 2009) and delayed procedural learning (Young *et al.*, 2011). In addition, overexpression of hippocampal α_7 nAChRs improves spatial memory performance (Ren *et al.*, 2007), and α_7 agonist drugs have procognitive properties in various preclinical models (Leiser *et al.*, 2009). However, previous studies have shown that intrahippocampal administration of the α_7 nAChR antagonist methyllycaconitine has no significant effect on the enhancement of contextual fear conditioning by nicotine (Davis *et*

et al., 2007), acute nicotine enhances contextual fear conditioning in both wild-type and α_7 KO mice (Wehner *et al.*, 2004; Davis and Gould, 2007), α_7 KO mice show similar deficits in context freezing following withdrawal from chronic nicotine as wild-type mice (Portugal *et al.*, 2008), and no changes are observed in hippocampal cytosine-resistant ^{125}I -epibatidine binding after withdrawal (Gould *et al.* 2012). Consistent with these previous findings, the present study showed no significant enhancement of contextual fear conditioning by the α_7 agonist ABT-107, and ABT-107 did not reverse nicotine withdrawal-induced deficits in contextual fear conditioning. However, it is possible that doses of ABT-107 not tested in the present experiments could be efficacious. However, higher doses were not tested because the drug solution became saturated at higher doses.

Finally, an important issue for future studies is determining which cognitive domains are most sensitive to nicotine withdrawal. The answer is most likely complex, with factors such as age and genotype contributing to individual differences (Portugal *et al.*, 2012a, 2012b). Although the current studies were not specifically designed to address this issue, prior work and the present results suggest that the effects of nicotine withdrawal on hippocampus-dependent learning, such as contextual fear conditioning, are separate from the effects on attention. Multiple studies have found that nicotine withdrawal disrupts hippocampus-dependent learning, whereas modified versions of the same tasks that are hippocampus-independent were not altered (Davis *et al.*, 2005; Andre *et al.*, 2008; Portugal and Gould, 2009; Kenney *et al.*, 2011; Portugal *et al.*, 2012a, 2012b). If the nicotine withdrawal-induced deficits in contextual fear conditioning were related to changes in attention, one might expect both hippocampus-dependent and hippocampus-independent learning to be altered. In addition, the anterior cingulate cortex is believed to be involved in attention (Devinsky *et al.*, 1993), but acute nicotine infusion into the cingulate cortex did not alter fear conditioning, whereas infusion into the hippocampus selectively enhanced contextual fear conditioning (Gulick and Gould, 2009). Interestingly, the same study found that acute nicotine infusion into the cingulate cortex reversed ethanol-associated deficits in both cued and contextual fear conditioning, but infusion into the hippocampus had no effect on ethanol-associated deficits; this suggests that ethanol could be altering attention. Furthermore, the present study found that a dose of nicotine that produced withdrawal-induced deficits in contextual fear conditioning (Wilkinson *et al.*, 2013) did not produce changes in attention. This does not suggest that nicotine withdrawal does not alter attention, as deficits in 5-CSRTT have been seen in rats (Shoaib and Bizarro, 2005; Semenova *et al.*, 2007), but instead raises the possibility that different cognitive domains can be differentially affected by nicotine withdrawal and these effects can vary independently.

In summary, from the perspective of translating basic science to the clinic, the effects of nicotine withdrawal on contextual fear conditioning appear to be consistent with the cognitive deficits reported by addicted smokers (Hughes *et al.*, 1991; Jacobsen *et al.*, 2005; Mendrek *et al.*, 2006). Moreover, amelioration of cognitive deficits in this behavioral paradigm by current FDA-approved smoking cessation therapies (nicotine replacement therapy, varenicline and bupropion) suggests that contextual fear conditioning might be a strong screening tool for potential pharmacotherapeutic agents for smoking cessation (Davis *et al.*, 2005; Portugal and Gould, 2007; Raybuck *et al.*, 2008). Taken together, the results of

the present study suggest that modulation of hippocampal learning and memory by ABT-089 may be an effective component of novel therapeutic strategies for nicotine addiction.

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Fig. 1.

Dose–response of acute ABT-089 in contextual fear conditioning. ABT-089 enhances freezing to context at all doses tested. *Significant difference from vehicle group ($P < 0.05$). Error bars indicate SEM. For all fear conditioning data, the vertical axis is the percent freezing for each period. CS, conditioned stimulus.

Fig. 2.

A dose of (a) 0.3 mg/kg ABT-089 and (b) 0.6 mg/kg ABT-089 ameliorates nicotine withdrawal-induced cognitive deficits in contextual fear conditioning. *Significant difference from the withdrawal from the saline + vehicle group ($P < 0.05$); **significant difference from the withdrawal from the nicotine + vehicle group and from the saline + vehicle group ($P < 0.05$). Error bars indicate SEM. CS, conditioned stimulus; W/D, withdrawal.

Fig. 3.

Dose-response of acute ABT-107 in contextual fear conditioning. ABT-107 does not enhance freezing to context at any of the doses tested compared with vehicle. *Significant between the 0.03 and the 0.3 mg/kg ABT-107 groups ($P < 0.05$). Error bars indicate SEM. CS, conditioned stimulus.

Fig. 4.

A dose of 0.3 mg/kg ABT-107 does not ameliorate nicotine withdrawal-induced cognitive deficits in contextual fear conditioning. *Significant difference as compared with withdrawal from chronic nicotine ($P < 0.05$). Error bars indicate SEM. CS, conditioned stimulus; W/D, withdrawal.

Fig. 5.

Withdrawal did not alter performance in the 5-CSRTT. No difference in the percentage of correct responses was found across the 4 days of testing after cessation of chronic nicotine treatment ($P > 0.05$). Error bars indicate SEM. CS, conditioned stimulus; 5-CSRTT, five-choice serial reaction time task; W, withdrawal.