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β2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning

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Abstract

Acute nicotine enhances contextual fear conditioning, whereas withdrawal from chronic nicotine produces impairments. However, the nicotinic acetylcholine receptors (nAChR) that are involved in nicotine withdrawal deficits in contextual fear conditioning are unknown. The present study used genetic and pharmacological techniques to investigate the nAChR subtype(s) involved in the effects of nicotine withdrawal on contextual fear conditioning. β2 or α7 nAChR subunit knockout (KO) and corresponding wild-type (WT) mice were withdrawn from 12 days of chronic nicotine treatment (6.3 mg/kg/day), and trained with 2 conditioned stimulus (CS; 85 dB white noise)—unconditioned stimulus (US; 0.57 mA footshock) pairings on day 13. On day 14, mice were tested for contextual and cued freezing. β2 KO mice did not show nicotine withdrawal-related deficits in contextual fear conditioning, in contrast to WT mice and α7 KO mice. A follow-up study investigated if nicotine withdrawal disrupts acquisition or recall of contextual fear conditioning. The high affinity nAChR antagonist dihydro-β-erythroidine (DHβE; 3 mg/kg) was administered prior to training or testing to precipitate withdrawal in chronic nicotine-treated C57BL/6 mice. Deficits in contextual fear conditioning were observed in chronic nicotine-treated mice when DHβE was administered prior to training, but not when administered at testing. These results indicate that β2-containing nAChRs, such as the α4β2 receptor, mediate nicotine withdrawal deficits in contextual fear conditioning. In addition, nicotine withdrawal selectively affects acquisition but not recall or expression of the learned response.

Keywords: Nicotine; Withdrawal; Addiction; Learning; Acetylcholine; Dihydro-β-erythroidine

1. Introduction

Cigarette smoking is a serious health problem in the United States: over 435,000 deaths each year are attributed to smoking (Mokdad, Marks, Stroup, & Gerberding, 2004). Although 42% of smokers attempt to quit each year, less than 6% are successful, suggesting that current smoking cessation treatments are not adequate (McIlvain, Susman, Davis, & Gilbert, 1995). The addictive liability of nicotine may relate to its ability to usurp the functioning of several processes, and to produce a range of withdrawal symptoms (see Baker, Brandon, & Chassin, 2004; Mansvelder, De Rover, McGehee, & Brussaard, 2003; Nestler, 2002 for review). In order to develop more effective smoking cessation treatments, an understanding of the cellular mechanisms that underlie nicotine addiction is necessary.

In humans, disrupted cognition is frequently reported as a symptom of nicotine withdrawal (Hughes, Higgins, & Bickel, 1994; Jacobsen et al., 2005; Mendrek et al., 2006). However, few animal studies have investigated the effects of nicotine withdrawal on learning and memory. Previously, we demonstrated differential effects of acute, chronic, and withdrawal from chronic nicotine on contextual fear conditioning in mice. Acute nicotine enhanced contextual fear conditioning; however, a dose of chronic nicotine that produced similar plasma nicotine levels [and was within the range observed in smokers (Benowitz, Porchet, & Jacob, 1989; Henningfield & Keenan, 1993)] had no effect, suggesting the development of tolerance.

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Furthermore, contextual fear conditioning was disrupted in mice withdrawn from chronic nicotine treatment (Davis, James, Siegel, & Gould, 2005). These results demonstrate that nicotine withdrawal disrupts learning-related processes but because mice were withdrawn from nicotine treatment prior to conditioning, it is unknown if nicotine withdrawal disrupts learning or recall. In addition, the nicotinic acetylcholine receptors (nAChR) underlying the withdrawal-associated deficits in contextual fear conditioning are unknown.

Nicotinic acetylcholine receptors are ligand-gated cation channels consisting of 5 subunits. Twelve neuronal nAChR subunits have been identified (α2–α10 and β2–β4), with the α4β2 and α7 nAChRs being the most widely expressed nAChRs in the brain (Hogg, Raggenbass, & Bertrand, 2003; Le Novere, Corringer, & Changeux, 2002). Different nAChR subtypes have distinct functional properties that include differences in desensitization, binding affinity, and cation permeability (Cordero-Erausquin, Marubio, Klink, & Changeux, 2000; Fenster, Rains, Noerager, Quick, & Lester, 1997; Papke, Sanberg, & Shytle, 2001). For instance, α4 or β2-containing nAChR subtypes bind with high affinity to nicotine, in contrast to α7 nAChRs, which have lower affinity for nicotine (Cordero-Erausquin et al., 2000). Thus, different nAChR subtypes likely mediate the various effects of nicotine on learning and addiction.

The present study examined nAChR involvement in the effects of nicotine withdrawal on learning and memory by comparing withdrawal effects between α7 knockout (KO), β2 KO, and wild-type (WT) mice, and through the use of dihydro-β-erythroidine (DHβE; a high affinity nAChR antagonist) precipitated withdrawal. The question of whether nicotine withdrawal disrupts learning or recall was addressed by comparing DHβE-precipitated withdrawal at training versus testing. Establishing whether nicotine withdrawal disrupts acquisition or recall of memories will advance the understanding of learning processes and factors that influence nicotine addiction. Furthermore, identifying the nAChRs involved in withdrawal effects on cognitive processes will help determine if different nicotine withdrawal symptoms involve common or divergent nAChRs, and may aid in development of more efficacious therapeutics for assisting in nicotine abstinence.

2. Materials and methods

2.1. Subjects

For genetic knockout experiments, male and female α7 and β2 nAChR subunit KO and WT mice (ages 8–12 weeks at training) were bred from mice heterozygous for the null mutation for the α7 or β2 nAChR subunit. In the laboratory of Dr. Beaudet, mutant mouse lines were created in 129/SvEv cells, and were backcrossed to C57BL/6 mice for more than 7 generations (see Orr-Urtreger et al., 1997 and Xu et al., 1999 for a more detailed explanation). For pharmacological experiments, the subjects were male C57BL/6 mice (ages 8–12 weeks). Mice were maintained on a 12 h light–dark cycle (lights on at 7:00 am) and housed in groups of two with continuous access to food and water. All procedures occurred during the light phase and were approved by the Temple University Institutional Animal Care and Use Committee.

2.2. Apparatus

The training and testing of contextual fear conditioning occurred in four identical chambers (17.78 cm × 19.05 cm × 38.10 cm) housed in sound attenuating boxes (Med-Associates, St. Albans, VT). The front, back, and top chamber walls were Plexiglas, and the sidewalls were stainless steel. The floors of the chambers were composed of metal rods that were connected to a shock generator and scrambler. Ventilation fans provided air exchange and background noise (69 dB) in each sound attenuating box. Speakers attached to the right wall of each chamber were used to administer the white noise CS (conditioned stimulus). Med-PC software controlled stimulus administration. Testing for freezing to the CS occurred in four altered chambers (20.32 × 22.86 × 17.78 cm) housed in sound attenuating boxes in a different room. The side chamber walls were made of aluminum, and all other walls were composed of Plexiglas. The chamber floors were covered in white plastic. Speakers for delivering the CS were mounted on the left wall of each chamber. A vanilla extract olfactory cue was added to further distinguish these chambers from the training chambers.

2.3. Behavioral procedures: contextual and cued fear conditioning

Freezing, used as the behavioral measure of learning, was assessed with a time-sampling procedure described in detail elsewhere (Gould & Wehner, 1999). Briefly, mice were observed for one second every 10 s and were scored as freezing or active. During training, baseline activity was scored for 120 s followed by two co-terminating CS (30 s 85 dB white noise)—US (unconditioned stimulus; 2 s 0.57 mA footshock) pairings separated by a 120 second inter-trial interval (ITI). Immediate freezing was scored during the 120 s ITI. The training session ended with a 30 s period during which freezing behavior was not recorded. Twenty-four hours later, mice were tested for freezing to the context by returning them to the training chambers; freezing was scored for 5 min. One-hour later, freezing to the CS was assessed. During the first 3 min, generalized freezing was assessed in the absence of the CS and then freezing to the CS was measured for the next 3 min; the auditory cue was present for the entire 3 min.

2.4. Drug administration and experimental design

For the KO experiments, nicotine hydrogen tartrate salt (6.3 mg/kg/day nicotine reported in freebase nicotine weight; Sigma, St. Louis, MO) was dissolved in saline and administered via mini-osmotic pumps (model 1002; Alzet, Cupertino, CA). The selection of this dose of nicotine was based on previous research demonstrating that withdrawal from chronic nicotine administration of this dose will produce impairments in contextual fear conditioning in C57BL/6 mice, and this dose produces plasma nicotine levels comparable to what is observed in smokers (Benowitz et al., 1989; Davis et al., 2005; Henningfield & Keenan, 1993). Pumps were removed 12 days after pump implantation. Training and testing took place on days 13 and 14, respectively (n's = 8).

In dihydro-β-erythroidine (DHβE; Sigma Co., St. Louis, MO, dissolved in saline (0.09%)) precipitated withdrawal experiments, mini-osmotic pumps filled with 6.3 mg/kg/day nicotine or saline were subcutaneously implanted in C57BL/6 mice. Training and testing occurred on days 13 and 14. In the DHβE administration on training day experiment, naïve mice received a subcutaneous injection of 3.0 mg/kg DHβE ($n = 29$; 15 chronic nicotine, 14 chronic saline) or saline ($n = 30$; 17 chronic nicotine, 13 chronic saline) 25 min before training on day 13. The dose of DHβE and method of drug administration was based on prior research, which demonstrated that DHβE can block the enhancing effect of acute nicotine

(Davis & Gould, 2006). Twenty-four hours later (day 14), mice in all groups were given a subcutaneous injection of saline 25 min before testing for contextual and cued conditioning.

The DHβE administered on testing day experiment followed a similar design, except that naïve mice were all treated with saline 25 min before training on day 13. On the testing day (day 14), mice were given 3.0 mg/kg DHβE ($n = 20$; 10 chronic nicotine, 10 chronic saline) or saline ($n = 20$; 10 chronic nicotine, 10 chronic saline) subcutaneously 25 min prior to testing. An additional subcutaneous injection of DHβE or saline was delivered 25 min before testing freezing to the CS to test if precipitated withdrawal disrupted recall of cued conditioning even though prior results with spontaneous withdrawal showed that nicotine withdrawal did not disrupt cued fear conditioning (Davis et al., 2005). In the final experiment (DHβE-precipitated withdrawal on training and testing), naïve mice were treated with subcutaneous injections of 3.0 mg/kg DHβE ($n = 20$; 10 chronic nicotine, 10 chronic saline) or saline ($n = 17$; 9 chronic nicotine, 8 chronic saline) 25 min before training and testing of contextual and cued fear conditioning; thus mice received a total 3 injections of either DHβE or saline.

2.5. Statistical analyses

Data from experiments with knockout mice were analyzed using 2 (gender: male, female) × 2 (genotype: wild-type, knockout) × 2 (drug treatment: nicotine, saline) ANOVAs. Given that no significant main effects or interactions were found with gender, the data from male and female subjects were combined. Freezing scores from all periods (baseline, immediate, contextual freezing, pre-CS, and cued freezing) were analyzed using 2 (genotype) × 2 (drug treatment) ANOVAs. Data from experiments with C57BL/6 mice were evaluated with 2 (chronic drug treatment: nicotine, saline) × 2 (acute drug treatment: DHβE, saline) ANOVAs. For all ANOVAs, a Games-Howell post hoc test was used in instances where the assumption of homogeneity of variance (determined by the Levene statistic) was not met; otherwise a Tukey post hoc test was used to test pair-wise comparisons.

3. Results

3.1. β2 nAChR subunit knockout mice do not exhibit nicotine withdrawal deficits in contextual fear conditioning

The effects of nicotine withdrawal on conditioning in β2 KO and WT mice were measured (Fig. 1). A 2 (genotype) × 2 (drug treatment) ANOVA revealed significant main effects for drug treatment [$F(1,28) = 8.40$, $p < 0.05$] and genotype [$F(1,28) = 16.21$, $p < 0.05$] when mice were tested for contextual fear conditioning. Furthermore, a significant interaction between drug treatment and genotype was found [$F(3,28) = 18.81$, $p < 0.05$]. Tukey post hoc comparisons revealed that β2 WT mice withdrawn from chronic nicotine exhibited significantly lower levels of freezing to the context compared to all other groups ($p < 0.05$); the β2 KO group withdrawn from nicotine did not differ from saline-treated groups. No significant differences were observed in baseline or immediate freezing measured on training day, suggesting that all groups were similar in locomotor activity. Furthermore, there were no differences between groups in pre-CS freezing or in cued fear conditioning ($p > 0.05$). Overall, the results from this experiment demonstrate that the β2 nAChR subunit is critically involved in nicotine withdrawal-related deficits in contextual fear conditioning.

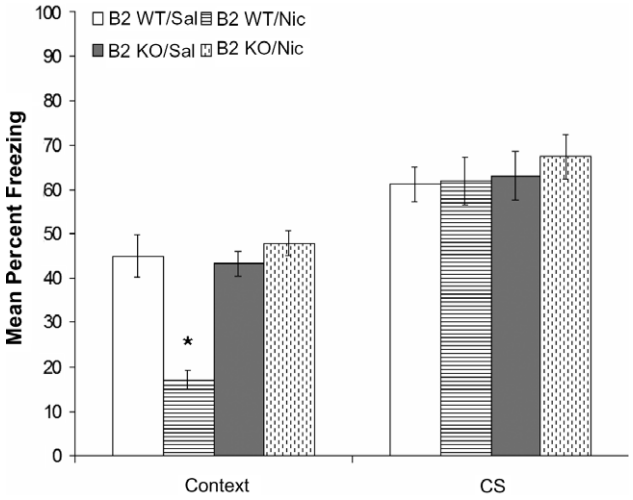

Fig. 1. The effects of withdrawal from chronic nicotine on fear conditioning in β2 KO and WT mice. β2 WT but not KO mice exhibited significant nicotine withdrawal deficits in contextual fear conditioning, suggesting that β2-containing nAChRs mediate nicotine withdrawal-related deficits in contextual fear conditioning. No differences in cued fear conditioning were observed between groups. Error bars indicate SEM, and * indicates $p < 0.05$ compared to all other groups.

3.2. The α7 nAChR subunit is not critically involved in nicotine withdrawal-associated deficits in contextual fear conditioning

α7 KO and WT mice were tested for deficits in conditioning following withdrawal from chronic nicotine (Fig. 2). A 2 (genotype) × 2 (drug treatment) ANOVA

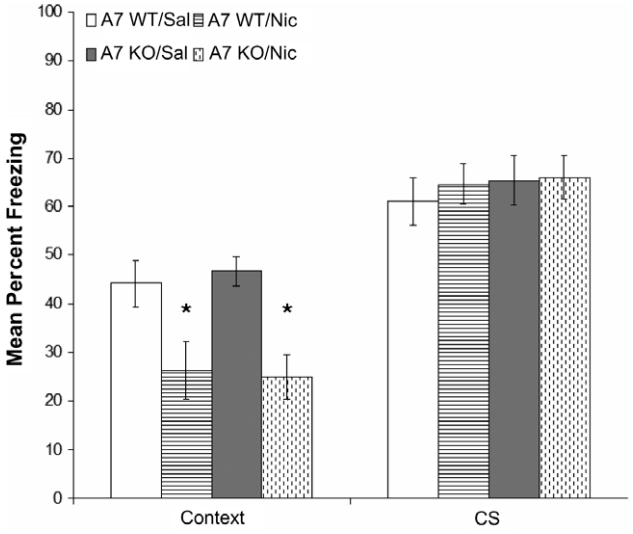

Fig. 2. The effects of withdrawal from chronic nicotine on fear conditioning in α7 KO and WT mice. Both α7 WT and KO mice demonstrated withdrawal deficits in contextual fear conditioning. These data indicate that the α7 nAChR subunit does not play a critical role in the effects of nicotine withdrawal on contextual fear conditioning. No differences in cued fear conditioning were observed between groups. Error bars indicate SEM, and * indicates $p < 0.05$ compared to both chronic saline-treated groups.

revealed a significant main effect for drug treatment [$F(1,28) = 20.71$, $p < 0.05$] when mice were tested for contextual fear conditioning. However, no main effect for genotype or drug by genotype interaction was observed ($p > 0.05$). Tukey post hoc comparisons determined that α7 KO and WT mice withdrawn from chronic nicotine treatment exhibited significantly lower levels of freezing to the context compared to both saline groups ($p < 0.05$). In addition, α7 KO and WT mice in the same drug treatment condition did not differ ($p > 0.05$). No significant differences were observed during baseline freezing, immediate freezing, pre-CS freezing or cued fear conditioning ($p > 0.05$). Taken together, these data suggest that the α7 nAChR subunit does not critically mediate the nicotine withdrawal deficits in contextual fear conditioning.

3.3. The administration of DHβE prior to training precipitates withdrawal deficits in contextual fear conditioning

DHβE (a high affinity nAChR antagonist) was administered on training day to determine if precipitated nicotine withdrawal disrupts the learning of contextual fear conditioning (Fig. 3). A 2 (chronic drug treatment – nicotine or saline) × 2 (acute drug treatment – DHβE or saline) ANOVA revealed a significant main effect for chronic drug treatment [$F(1,55) = 14.74$, $p < 0.05$] in contextual fear conditioning. Furthermore, a significant interaction between acute drug treatment and chronic drug treatment was found [$F(3,55) = 5.08$, $p < 0.05$]. Subsequent Games-Howell post hoc comparisons revealed that chronic nicotine-treated mice that were given DHβE on training day exhibited significantly lower levels of contextual fear conditioning when compared to all other groups ($p < 0.05$). No significant differences were observed for baseline freezing, immediate freezing, pre-CS freezing or cued fear conditioning ($p > 0.05$).

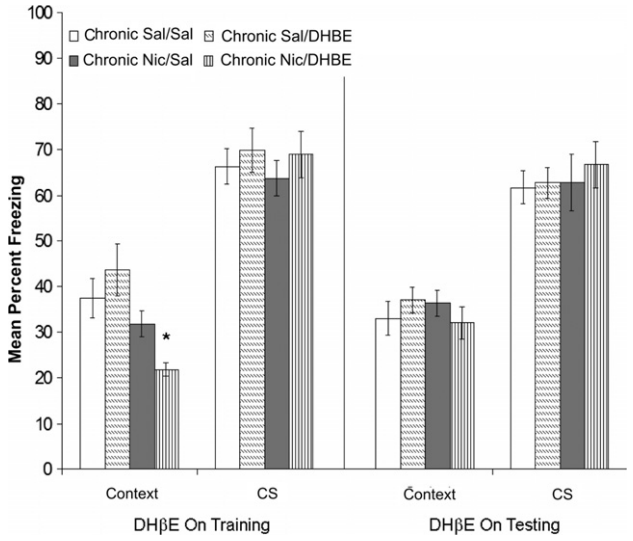

Fig. 3. The effects of DHβE administration at training day or testing day on fear conditioning in chronic nicotine or saline-treated mice. DHβE administered on training day to chronic nicotine-treated mice produced deficits in contextual fear conditioning. However, DHβE administration on testing day produced no deficits. No significant differences in cued fear conditioning were found. These results suggest that nicotine withdrawal selectively affects learning but not recall. Error bars indicate SEM, and * indicates $p < 0.05$ compared to all other groups.

3.4. DHβE administered prior to testing has no effect on contextual fear conditioning in chronic nicotine or saline-treated mice

DHβE was administered on testing day to determine if precipitated nicotine withdrawal disrupts the recall or expression of contextual fear conditioning (Fig. 3). A 2 (chronic drug treatment – nicotine or saline) × 2 (acute drug treatment – DHβE or saline) ANOVA revealed no significant main effects or interactions in contextual fear conditioning ($p > 0.05$). No significant differences were observed in baseline freezing, immediate freezing, pre-CS freezing or cued fear conditioning ($p > 0.05$). Taken together, the results from the two DHβE-precipitated withdrawal experiments suggest that nicotine withdrawal disrupts learning but not recall.

3.5. The effects of DHβE-precipitated withdrawal are not due to state dependent effects

Although DHβE-precipitated withdrawal appears to affect the learning (but not the recall) of contextual fear conditioning, these results could also potentially be explained as state-dependent learning. State-dependent learning theory proposes that impairments can occur if animals are trained and tested in different drug states (Overton, 1991). In order to rule out this explanation, mice chronically treated with nicotine or saline were administered DHβE or saline on both training and testing days (Fig. 4). A 2 (chronic drug treatment – nicotine or saline) × 2 (acute drug treatment – DHβE or saline) ANOVA revealed a significant main effect for acute drug treatment [$F(1,33) = 8.69$, $p < 0.05$] when mice were tested for contextual fear conditioning. In addition, a significant interaction between acute drug treatment and chronic drug treatment was found [$F(3,33) = 8.05$, $p < 0.05$]. Tukey post hoc comparisons revealed that chronic nicotine-treated mice that received DHβE on training and testing day exhibited significantly lower levels of contextual conditioning when compared to all other groups ($p < 0.05$). No significant differences were observed during the baseline or immediate phases of training, or during the pre-CS or cued fear conditioning phases ($p > 0.05$). Given that chronic nicotine-treated mice displayed impairments in contextual fear conditioning when given DHβE on both training and testing days, it is unlikely that the results seen when DHβE was administered on training day only were due to state dependent effects.

For all experiments, we observed no abnormal behavior at training or testing that would be suggestive of somatic

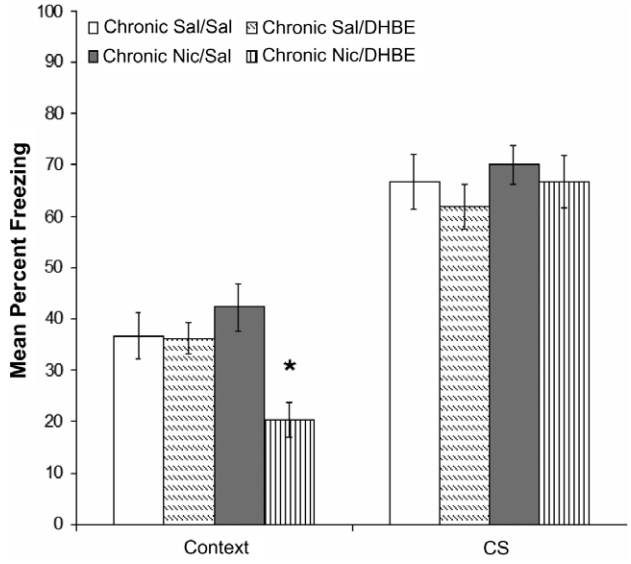

Fig. 4. The effects of DHβE administration at training and testing day on fear conditioning in chronic nicotine or saline-treated mice. DHβE administered on both days produced deficits in contextual fear conditioning in chronic nicotine-treated mice ($p < 0.05$) but had no effect on cued fear conditioning. These data suggest that the effects of DHβE administration on training day (see Fig. 3) were not due to a state dependent effect. Error bars indicate SEM, and * indicates $p < 0.05$ compared to all other groups.

nicotine withdrawal symptoms such as head shakes, tremors, backing, and scratching. This result supports previous research that has shown that somatic nicotine withdrawal symptoms are not present in mice that are withdrawn from 6 mg/kg/day nicotine (Damaj, Kao, & Martin, 2003).

4. Discussion

Through the use of pharmacological and genetic knockout approaches, the results of the present study demonstrate that β2-containing nAChRs are critically involved in the impairment of contextual fear conditioning following withdrawal from chronic nicotine administration. β2 KO mice did not show nicotine withdrawal-related deficits in contextual fear conditioning in contrast to their WT littermates. However, both α7 KO and WT mice exhibited impairments in contextual fear conditioning when withdrawn from chronic nicotine. Previous studies report that DHβE-precipitated withdrawal disrupted sustained attention (Shoaib & Bizarro, 2005) and decreased brain reward function (Epping-Jordan, Watkins, Koob, & Markou, 1998). However, given that DHβE binds to several high affinity nAChRs such as the α4β2, α4β4, and α2β4 nAChR subtypes (Harvey, Maddox, & Luetje, 1996; Williams & Robinson, 1984), the specific nAChR subunit(s) that mediate these effects are unclear. By using genetic knockout mice, the present results are the first to conclusively demonstrate the involvement of β2-containing nAChRs in nicotine withdrawal deficits, and suggest that α7 nAChRs are not critically involved in nicotine withdrawal deficits in contextual fear conditioning.

Extending previous research (Davis & Gould, 2006; Davis & Gould, 2007a, 2007b; Davis et al., 2005; Gould & Higgins, 2003; Gould & Wehner, 1999; Wehner et al., 2004), this study demonstrates that the effects of nicotine are specific to contextual fear conditioning. Throughout all experiments, cued fear conditioning was not affected by withdrawal from chronic nicotine or by DHβE-precipitated withdrawal. This suggests that the deficits observed in contextual fear conditioning were not due to generalized changes in processes such as motor activity or anxiety because such changes would be expected to alter both contextual and cued fear conditioning. In addition, because contextual fear conditioning involves the hippocampus, but cued fear conditioning does not (Kim & Fanselow, 1992; Logue, Paylor, & Wehner, 1997; Phillips & LeDoux, 1992), our results suggest that nicotine withdrawal may disrupt hippocampal function or function of areas projecting to the hippocampus. Thus, learning-related withdrawal deficits in mice may model cognitive deficits and abnormal hippocampal functioning reported in abstaining smokers (Due, Huettel, Hall, & Rubin, 2002; Jacobsen, Slotkin, Westerveld, Mencl, & Pugh, 2006; Zubieta et al., 2006).

Withdrawal from chronic nicotine could produce deficits by altering the acquisition or recall of contextual fear conditioning. In order to determine which process was affected by nicotine withdrawal, DHβE was used to precipitate withdrawal on training day, testing day, or on both days. When DHβE was administered on training day to chronic nicotine-treated mice, impairments in contextual fear conditioning were observed, whereas DHβE had no effect when given on testing day. This effect cannot be accounted for by state dependent learning theory given that deficits in contextual fear conditioning were also observed when DHβE was administered on both days to mice treated chronically with nicotine. It should be noted that these experiments used precipitated nicotine withdrawal to investigate if withdrawal disrupted learning or recall and precipitated withdrawal may not be the same as spontaneous withdrawal, especially since nicotine is present through precipitated withdrawal experiments. Nonetheless, these data suggest that nicotine withdrawal may selectively affect learning but not recall.

In addition to their role in the effects of nicotine on learning and memory, high-affinity nAChRs mediate the effects of nicotine on reward and reinforcement as well. Nicotine conditioned place preference (CPP), nicotine self-administration, and intracranial self-stimulation (ICSS) have been used to investigate the rewarding and reinforcing effects of nicotine. DHβE blocked nicotine CPP, nicotine self-administration, and nicotine enhancement of ICSS (Corrigall, Coen, & Adamson, 1994; Grottick et al., 2000; Harrison, Gasparini, & Markou, 2002; Kenny & Markou, 2006; Walters, Brown, Changeux, Martin, & Damaj, 2006). In contrast, the α7 nAChR antagonist MLA had no effect on nicotine CPP or nicotine

self-administration (Grottick et al., 2000; Walters et al., 2006 but see Markou & Paterson, 2001). Furthermore, β2 KO mice did not self-administer nicotine or develop nicotine CPP, whereas α7 KO mice did develop nicotine CPP (Besson et al., 2006; Picciotto et al., 1998; Walters et al., 2006). Thus, β2-containing nAChRs are involved in many of the effects of nicotine that may contribute to nicotine addiction. This may explain why the partial α4β2 agonist varenicline is one of the most effective therapeutics for treating nicotine addiction (Gonzales et al., 2006; Jorenby et al., 2006; Rollema et al., 2007).

Prior to the present study, however, evidence for β2-containing nAChR involvement in nicotine withdrawal symptoms was sparse. For instance, DHβE-precipitated withdrawal produced limited somatic signs of nicotine withdrawal with higher doses of nicotine (Damaj et al., 2003). However, DHβE antagonizes both β2 and β4-containing nAChRs (Harvey et al., 1996; Williams & Robinson, 1984) so it is unclear if the DHβE-precipitated somatic withdrawal symptoms were associated with β2 or β4-containing nAChRs. Genetic knockout studies have resolved this issue. β2 KO mice showed somatic symptoms, such as excessive rearing, body shakes or over-grooming, during withdrawal precipitated by the broad-spectrum nAChR antagonist mecamylamine (Besson et al., 2006; Salas, Pieri, & De Biasi, 2004). In contrast, β4 KO mice exhibited significantly reduced somatic symptoms during mecamylamine-precipitated withdrawal (Salas et al., 2004), suggesting that somatic symptoms of nicotine withdrawal involve β4-containing but not β2-containing nAChRs. Overall, these investigations together with the current findings demonstrate that β2-containing nAChRs are involved in the effects of nicotine on learning, reward/reinforcement, and cognitive withdrawal symptoms, but not somatic nicotine withdrawal symptoms. Thus, different nAChR subtypes mediate different processes that contribute to nicotine addiction and nicotine withdrawal symptoms.

Changes to learning processes during nicotine withdrawal may facilitate and/or maintain nicotine addiction (Gould, 2006). In humans, nicotine withdrawal is associated with a variety of maladaptive behavioral changes including disrupted cognition (Baker et al., 2004; Hughes, Gust, Skoog, Keenan, & Fenwick, 1991; Jacobsen et al., 2005; Mendrek et al., 2006). Therefore, it is possible that in some individuals, relapse may occur in an attempt to ameliorate such deficits. In support, a previous study demonstrated that nicotine withdrawal deficits in contextual fear conditioning could be ameliorated by acute nicotine (Davis et al., 2005). Furthermore, the current data from experiments with DHβE revealed that precipitated nicotine withdrawal selectively impairs acquisition, but not the recall of contextual learning. This finding suggests that nicotine withdrawal impairs the acquisition of new memories, but old memories, including drug-stimuli associations, may remain intact. Therefore, prior maladaptive drug-stimuli associations may contribute to cravings for nicotine during withdrawal; these cravings, combined with disrupted learning, may facilitate relapse.

The present study demonstrates that β2-containing nAChRs (most likely α4β2 nAChRs) are critically involved in the cognitive deficits observed during nicotine withdrawal. However, the question remains as to whether cognitive deficits are a universal symptom or a symptom that has variable expression based on factors such as genetics. Resolving this issue could greatly impact treatment strategies for nicotine addiction. In both humans and mice, polymorphisms in the α4 nAChR subunit gene (*Chrna4*) yield receptors that vary in their response to nicotine. In mice, a single nucleotide polymorphism in *Chrna4* produces either an alanine (A) or threonine (T) residue at amino acid position 529 of the α4 nAChR subunit (Stitzel, Dobelis, Jimenez, & Collins, 2001). Mice with the A529 variant exhibited enhanced sensitivity to the effects of nicotine and decreased consumption of nicotine, compared to mice with the T529 variant of the α4 nAChR subunit (Butt, King, Hutton, Collins, & Stitzel, 2005; Butt et al., 2003; Dobelis et al., 2002). In human research, polymorphisms in *Chrna4* have been identified that may be protective against nicotine dependence (Feng et al., 2004; Li et al., 2005), and some evidence suggests that polymorphisms in the β2 nAChR subunit gene (*Chrnb2*) may be associated with smoking initiation (Greenbaum et al., 2006 but see Lueders et al., 2002; Silverman et al., 2000). Although polymorphisms in *Chrna4* and *Chrnb2* have been implicated in nicotine addiction, the effects of polymorphisms of *Chrna4* or *Chrnb2* on nicotine withdrawal remain unknown. An important next step in determining the role of α4 and β2-containing nAChRs in nicotine withdrawal will be to determine whether polymorphisms in *Chrna4* or *Chrnb2* produce variability in the cognitive deficits that result from nicotine withdrawal.

Finally, whereas this study found that β2-containing nAChRs are critically involved in nicotine withdrawal-related disruption of contextual fear conditioning, the data also suggests that β2-containing nAChRs are not critically involved in acquisition of contextual fear conditioning in mice treated chronically with saline. The β2 KO mice treated chronically with saline had levels of conditioning similar to WT mice, and C57BL/6 mice treated chronically with saline and administered DHβE also showed levels of conditioning similar to controls. These results are in good agreement with past studies that reported that the nAChR antagonists mecamylamine (Gould & Wehner, 1999) and DHβE (Davis & Gould, 2006) did not disrupt conditioning. In addition, other studies have found that young β2 KO mice have normal levels of conditioning (Caldarone, Duman, & Picciotto, 2000; Davis & Gould, 2007a, 2007b). However, Wehner et al. (2004) found a deficit in contextual fear conditioning in β2 KO mice that they described as small but significant. In addition, whereas Caldarone et al. (2000) found normal contextual fear conditioning in young β2 KO mice, aged male β2 KO mice had reduced levels of conditioning. These results suggest that

under certain conditions, β2-containing nAChR may have a more significant role in contextual fear conditioning. In a previous study (Gould & Lewis, 2005), we proposed that nAChRs and glutamate receptors may interact to support conditioning; thus under normal circumstances disruption of nAChR function may not alter conditioning because glutamate receptors may compensate. However, under circumstances where glutamate receptor function is altered, disruption of nAChR function may have a greater impact on conditioning.

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