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ORIGINAL INVESTIGATION

Differential effects of α4β2 nicotinic receptor antagonists and partial-agonists on contextual fear extinction in male C57BL/6 mice

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Abstract

Rationale Numerous studies have attributed the psychopathology of post-traumatic stress disorder (PTSD) to maladaptive behavioral responses such as an inability to extinguish fear. While exposure therapies are mostly effective in treating these disorders by enhancing extinction learning, relapse of PTSD symptoms is common. Although several studies indicated a role for cholinergic transmission and nicotinic acetylcholine receptors (nAChRs) in anxiety and stress disorder symptomatology, very little is known about the specific contribution of nAChRs to fear extinction

Objectives In the present study, we examined the effects of inhibition and desensitization of α4β2 nAChRs via a full antagonist (Dihydro-beta-erythroidine (DhβE)) and two α4β2 nAChR partial-agonists (varenicline and sazetidine-A) on contextual fear extinction, locomotor activity, and spontaneous recovery of contextual fear in mice.

Methods We trained and tested the subjects in a contextual fear extinction as well as an open field paradigm and spontaneous recovery following injections of DhβE, varenicline, and sazetidine-A.

Results Our results demonstrated that lower doses of DhβE (1 mg/kg) and sazetidine-A (0.01 mg/kg) enhanced contextual fear extinction whereas higher doses of varenicline (0.1 mg/kg) and sazetidine-A (0.1 mg/kg) resulted in impaired contextual fear extinction. However, the higher dose of sazetidine-A (0.1 mg/kg) decreased locomotor activity, which may contribute to increased freezing response observed during fear extinction. Finally, we found that the low dose of DhβE, but not sazetidine-A, also decreased spontaneous recovery of contextual fear following fear extinction.

Conclusions Overall, these results suggest that inhibition and desensitization of α4β2 nAChRs enhance extinction of contextual fear memories. This suggests that modulation of α4β2 nAChRs may be employed as an alternative pharmacological strategy to aid exposure therapies associated with PTSD by augmenting contextual fear extinction processes.

Keywords Nicotinic receptors · Fear extinction · Spontaneous recovery · PTSD

The lifetime prevalence of experiencing a traumatic event is nearly 90% (Breslau et al. 1998). Exposure to traumatic events can lead to negative health outcomes, including post-traumatic stress disorder (PTSD). Individuals with PTSD exhibit exaggerated fear responses to otherwise safe environments. Repeated exposure to a fear-associated stimulus or context should extinguish the fear response; however, individuals with PTSD experience difficulty in extinguishing fear (Rothbaum and Davis 2003; VanElzakker et al. 2014). PTSD is commonly treated through exposure therapy, a behavioral treatment strategy based on fear extinction. However, exposure therapy has high dropout rates due to the unpleasant experience of repeated exposure (Imel et al. 2013), and this treatment method is susceptible to relapse (Craske and Mystkowski 2006).

Individuals with PTSD exhibit high rates of comorbidity with nicotine dependence (Breslau et al. 2004). The relationship between nicotine and PTSD is bidirectional in that smoking increases the likelihood of developing PTSD, and the rate of smoking can increase after an event that triggers PTSD (Koenen et al. 2005; Breslau et al. 2003, 2004). When compared to individuals who report never experiencing a traumatic event, the prevalence of nicotine dependence is nearly 23% higher for those with PTSD (Ziedonis et al. 2008).

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Studies suggest nicotine may increase the amount of intrusive memories related to trauma (Hawkins and Cougle 2013). Similarly, in animal studies, nicotine has been shown to alter fear memories (see Kutlu and Gould 2015 for a review). Hippocampus-dependent types of fear conditioning, such as contextual and trace fear conditioning, are enhanced by acute nicotine (Gould and Wehner 1999; Gould 2003; Gould and Higgins 2003; Gould and Lommock 2003; Gould et al. 2004; Davis et al. 2005, 2006; Davis and Gould 2006). In addition to enhancing contextual fear learning, studies from our lab have shown that nicotine administration also hindered fear extinction in mice (Kutlu and Gould 2014; Kutlu et al. 2016a, 2017a, b). Specifically, acute nicotine administration impairs encoding and consolidation of contextual fear extinction memories (Kutlu and Gould 2014; Kutlu et al. 2017a) and enhances spontaneous recovery of contextual fear memories (Kutlu et al. 2016b). Combined with human studies, these results indicate a strong influence of nicotine on acquisition and extinction of fear memories.

Nicotine is an agonist of nicotinic acetylcholine receptors (nAChRs), a class of ligand-gated ion channels located throughout the central and peripheral nervous system (Cordero-Erausquin et al. 2000). The low-affinity α7 and high-affinity α4β2 nAChRs are dominant in the brain regions critical for long-term memory formation such as hippocampus (Alkondon and Albuquerque 1993; Wada et al. 1989; Seguela et al. 1993). Previously, we have shown that α4β2, but not α7, nAChRs are necessary for the impairing effects of acute nicotine on contextual fear extinction (Kutlu et al. 2016a). α4β2 nAChRs show high-affinity nicotine binding and desensitize slowly (Fenster et al. 1997; Cordero-Erausquin et al. 2000), suggesting that activation of α4β2 nAChRs via nicotine may be responsible for the extinction deficits. Importantly, these results also indicate that inhibition of α4β2 nAChRs may lead to enhancement of contextual fear extinction. Therefore, in the present study, α4β2 nAChR partial-agonists and an antagonist (varenicline, sazetidine-A, and Dihydro-beta-erythroidine, DhβE) were tested in order to examine the effects of α4β2 nAChR inhibition on contextual fear extinction.

Methods

Subjects

Eight-week-old male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were group-housed in a colony room and maintained on a non-reversed 12 h light/dark cycle, and behavioral experiments were carried out in the light phase. Subjects had ad libitum access to food and water. Training and testing took place between 9:00 am and 6:00 pm. All behavioral procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus

Contextual fear conditioning training, testing, and spontaneous recovery took place in four identical chambers (18.8 × 20 × 18.3 cm) within sound-attenuating boxes (MED Associates, St. Albans, VT). A ventilation fan located at the back of each box produced a background noise (65 dB). A white noise conditioned stimulus (CS, 85 dB) was produced by a speaker within the right wall of the chambers. Each chamber was composed of Plexiglas walls and ceiling with metal grid floors (0.20 and 1.0 cm apart) connected to a shock generator, which produced a 2 s long, 0.57 mA footshock unconditioned stimulus (US). Both the conditioned and unconditioned stimuli were controlled by an IBM-PC compatible computer running MED-PC software. The open field testing took place within a Plexiglas arena (49.5 cm × 59.7 cm). Between subjects, the fear conditioning chambers and open field arena were cleaned with 70% ethanol.

Drugs and administration

Mice received two doses of either varenicline (0.01 or 0.1 mg/kg, freebase weight; Tocris, Minneapolis, MN, #3754), DhβE (1 or 5 mg/kg tartrate weight; Tocris, Minneapolis, MN, #2349), or sazetidine-A (0.01 or 0.1 mg/kg, freebase weight; Tocris, Minneapolis, MN, #2736) or vehicle. All compounds were prepared in saline. Varenicline doses roughly correspond to medium to high doses of oral varenicline doses tested in humans (Faessel et al. 2006). DhβE was administered subcutaneously 25 mins prior to behavioral testing whereas both varenicline and sazetidine-A were administered intraperitoneally 10 min prior to behavioral testing. All doses, routes of administration, and injection times are based on previous studies from our laboratory (Davis and Gould 2006; Turner et al. 2013). All injection volumes were 10 ml/kg.

Behavioral procedures

During fear conditioning training, mice were placed in the conditioning chambers for 120 s to assess baseline freezing. Following Kutlu and Gould (2014), subjects received two CS-US pairings in which a 30-s CS co-terminated with a 2-s 0.57 mA footshock. To measure immediate freezing to the US, freezing was assessed for 120 s after the first CS-US pairing. After the second CS-US pairing, mice remained in the chamber for 30 s before removal. For contextual testing, mice were placed in the same context they had been exposed to during training. Freezing was measured for 5 min without the auditory CS or US. For contextual fear extinction, the mice

were exposed to the same context experienced during training (Fig. 1). Re-exposure occurred each day for 5 days. Sessions were 24 h apart. Prior to each extinction session, mice received DhβE (1 mg/kg, 5 mg/kg, or saline; $n = 8$–11 per group), varenicline (0.01 mg/kg, 0.1 mg/kg, or saline; $n =$ 13–14 per group), or sazetidine-A (0.01 mg/kg, 0.1 mg/kg, or saline; $n = 7$–8 per group). Each subject was observed every 10 s for 1 s; the subject's behavior was recorded as either active of freezing. Freezing was defined as the absence of voluntary movement except respiration (Blanchard and Blanchard 1969). Scores were then converted to percent freezing. Experimenters were blinded to drug conditions when scoring.

The same group of mice was tested in an open field paradigm 1 week after the last fear extinction session to assess locomotor activity. Mice received the same doses of varenicline, DhβE, sazetidine A, or saline before open field testing. Each mouse was placed in the center quadrant of the open field arena. Locomotor activity, defined as distance traveled in inches, was recorded for 5 min using tracking software (Smart Tracking Software, Panlab).

Finally, a separate group of mice were trained and tested in fear conditioning and received 5 days of contextual fear extinction as described above. To assess spontaneous recovery (SR), mice were returned to the conditioning chambers 7 days after the final extinction session. Subjects were administered lower doses of DhβE ($n = 7$–8 per group) or sazetidine-A ($n =$ 8 per group) before the SR session as described for the contextual fear extinction experiment.

Statistical analysis

For statistical analysis, a two-way mixed-design ANOVA examined three levels of drug across six trials (one testing and five extinction trials) for each compound. Open field data were examined via three separate 2-way ANOVAs with three drug levels. Differences between groups for each extinction session were analyzed using Bonferroni corrected *t* tests. For the open field data, we used one-way ANOVAs followed by Bonferroni corrected *t* tests for group comparisons. For spontaneous recovery experiments, only two drug levels were examined using separate two-way ANOVAs for each compound. In order to eliminate potential between-group baseline differences in contextual freezing, which may affect subsequent fear extinction curves, the dependent variable was percent freezing to the context normalized to the individual freezing levels at the initial testing session (freezing × 100/initial freezing; Tian et al. 2008; Kutlu et al. 2016a). We also depicted raw percent freezing scores. For the spontaneous recovery experiment, both raw %freezing scores during re-testing and %Rebound (freezing during re-testing × 100/initial freezing) scores were used. Following our previous studies (Kutlu et al. 2016b, 2017a), we employed an extinction criterion for the spontaneous recovery experiments where four mice that did not show freezing levels below 30% of their initial freezing levels at the end of the extinction phase were removed from analysis as freezing levels above this level may indicate incomplete extinction.

Results

Antagonism of α4β2 nAChR with DhβE dose-dependently enhances contextual fear extinction

First, we administered DhβE prior to each extinction session to examine the effects of α4β2 antagonism on contextual fear extinction. A repeated measures ANOVA demonstrated that both the drug × trial interaction ($F(10,130) = 1.917$, $p < 0.05$) and drug main effect ($F(2,26) = 5.130$, $p < 0.05$) were significant. Furthermore, the simple main effect of drug was significant for the low dose of DhβE (1 mg/kg; $F(1,19) = 9.726$, $p < 0.01$) but not for the high dose of DhβE (5 mg/kg; $F(1,17) = 0.123$, $p > 0.05$), which suggests that DhβE dose-dependently affects contextual fear extinction. That is, while the low dose of DhβE augments contextual fear extinction, the high-dose DhβE has no effect (Fig. 2a–b). These results suggest that partial inhibition of α4β2 nAChRs may augment contextual fear extinction.

α4β2 nAChR partial-agonist varenicline impairs contextual fear extinction

In addition to α4β2 nAChR antagonist DhβE, we also investigate whether varenicline, a partial agonist of α4β2 nAChRs that keeps the receptor activation at a sub-maximal level, has any effect on contextual fear extinction. A repeated measures ANOVA showed that both the drug × trial interaction ($F(10,185) = 2.285$, $p < 0.05$) and drug main effect ($F(2,37) = 11.821$, $p < 0.01$) were significant. Furthermore, the simple main effect of drug was significant for Saline/Var 0.1 mg/kg comparison ($F(1,25) = 16.482$, $p < 0.01$) but not for Saline/Var 0.01 mg/kg comparison ($F(1,24) = 0.087$, $p > 0.05$), suggesting that the effect is mainly driven by the high dose. Therefore, our results show that the high dose of varenicline impairs contextual fear extinction whereas the low dose of varenicline did not have an effect (Fig. 2c–d).

α4β2 nAChR partial-agonist sazetidine-A has bi-directional effects on contextual fear extinction

Given that sazetidine-A uniquely desensitizes α4β2 nAChRs without activating these receptors, we also examine the effects of sazetidine-A on contextual fear extinction. A repeated measures ANOVA revealed that for sazetidine-A both the drug ×

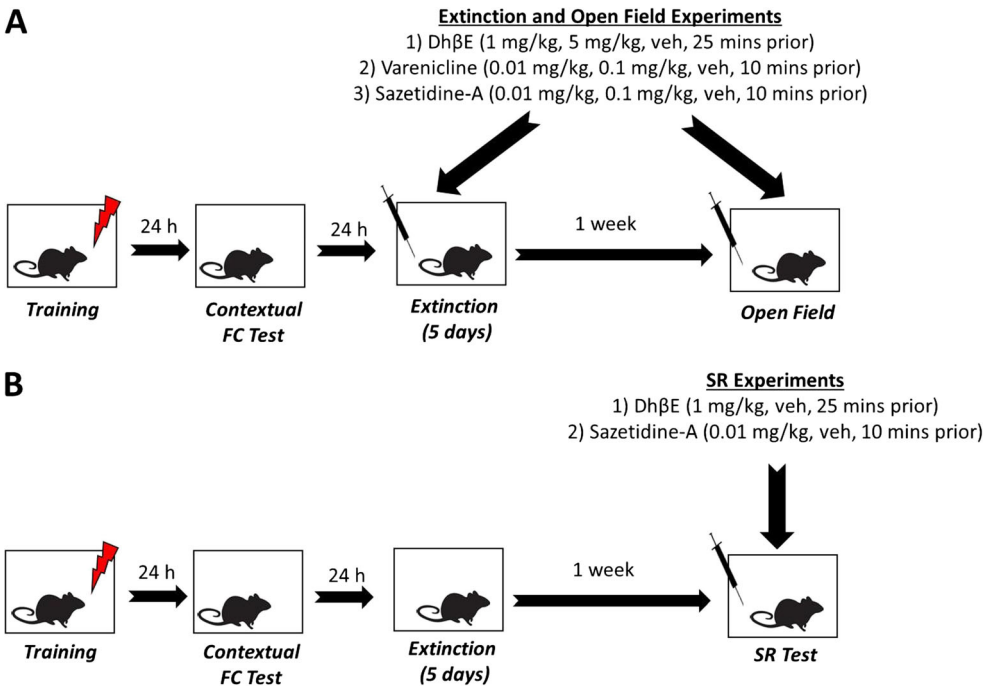

Fig. 1 The schematic of experimental designs. While each box represents a phase of the experiment, the syringe represents DhβE, varenicline, sazetidine-A, or saline injections and the lightning bolt symbol indicates the presentations of the footshocks

trial interaction ($F(10,95) = 7.111, p < 0.01$) and the drug main effect ($F(2,19) = 28.749$, $p < 0.01$) were significant. Furthermore, the simple main effect of drug was significant both for Saline/Saz-A 0.1 mg/kg ($F(1,13) = 23.528, p < 0.01$) and for Saline/Saz-A 0.01 mg/kg ($F(1,12) = 9.577, p < 0.01$). This suggests that sazetidine-A has both impairing and enhancing effects on contextual fear extinction depending on the administered dose (Fig. 2e–f). That is, the low sazetidine-A dose enhanced whereas the high dose impaired contextual fear extinction.

A high dose of sazetidine-A decreases locomotor activity

While our results showed that nAChR partial agonists varenicline and sazetidine-A as well as antagonist DhβE affected freezing behavior during contextual fear extinction, it is possible that altered locomotor activity may contribute to these effects. Therefore, we ran the same group of mice in an open field paradigm following varenicline (0.1 mg/kg, 0.01 mg/kg, or saline), sazetidine-A (0.1 mg/kg, 0.01 mg/kg, or saline), and DhβE (1 mg/kg, 5 mg/kg, or saline) injections. Our results showed that the drug main effect was significant for varenicline ($F(2,37) = 3.886$, $p < 0.05$) and sazetidine-A ($F(2,19) = 47.874$, $p < 0.01$) but not for DhβE ($F(2,26) = 1.917$, $p > 0.05$). Tukey post hoc tests revealed that the differences between the high dose (0.1 mg/kg) and low dose (0.01 mg/kg) of sazetidine-A groups as well as the difference between the high dose of sazetidine-A group and vehicle controls were significant ($ps < 0.05$). The high-dose varenicline group also showed significantly reduced levels of locomotor activity compared to the group that received the low dose of varenicline ($p < 0.05$). However, the high- and low-dose varenicline groups' locomotor activity failed to differ from saline controls ($ps > 0.05$). These results show that the full antagonist of α4β2 nAChRs DhβE does not affect locomotor activity (Fig. 3a) suggesting the enhancement of contextual fear extinction observed following DhβE administration is specific to extinction learning. In contrast, the high dose of the α4β2 nAChR partial agonist sazetidine-A and the high dose of varenicline altered locomotor function (Fig. 3b, c), which may contribute to the increased freezing response observed during contextual fear extinction.

DhβE reduces spontaneous recovery of extinguished contextual fear

Finally, we examined whether the doses of DhβE (1 mg/kg) and sazetidine-A (0.01 mg/kg) that were effective in enhancing contextual fear extinction enhanced retrieval of extinction memories and consequently reduced spontaneous recovery. We only focused on DhβE and sazetidine-A, but not varenicline, due to lack of effect of varenicline on fear extinction. Separate one-way ANOVAs conducted using %freezing during re-testing showed that the main effect of drug was significant for DhβE (5.442, $p < 0.05$), but not for sazetidine-A ($F(1,14) = 0.242$, $p > 0.05$), which demonstrates that DhβE reduces spontaneous recovery (Fig. 4a) whereas sazetidine-A has no effect (Fig. 4c). However, the drug main effect was not significant for %Rebound scores for either DhβE ($F(1,13) = 2.461$, $p > 0.05$; Fig. 4b) or sazetidine-A ($F(1,14) = 0.240$, $p > 0.05$; Fig. 4d) suggesting a transient

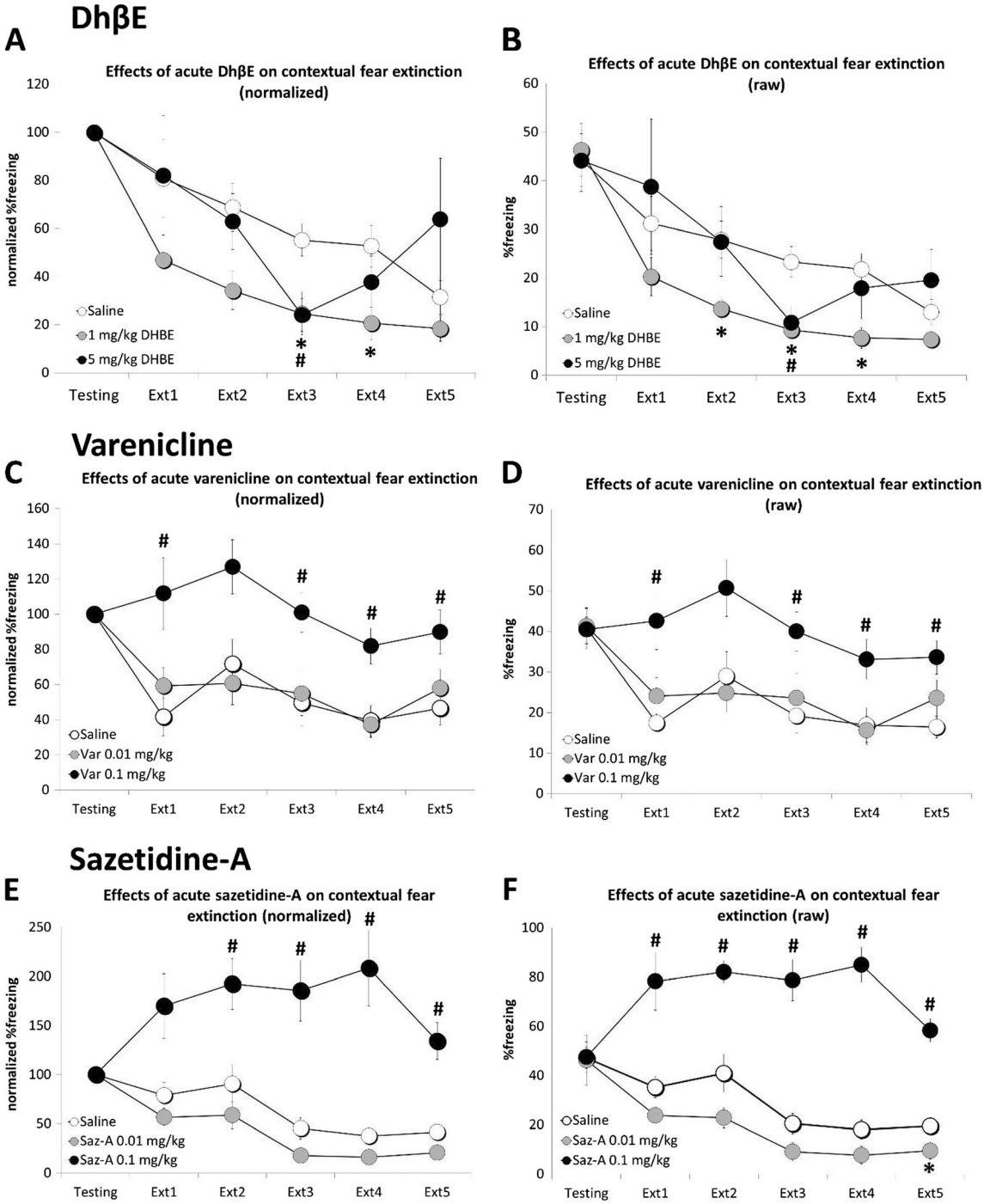

Fig. 2 Effects of DhβE, varenicline, and sazetidine-A on contextual fear extinction. Normalized and raw %freezing scores across initial testing and five extinctions sessions. **a**–**b** Effects of acute injections of DhβE (DhβE 1 and DhβE 5 mg/kg) and saline on contextual fear extinction. **c**–**d** Effects of acute varenicline (Var 0.01 mg/kg and Var 0.1 mg/kg) and saline injections on contextual fear extinction. **e**–**f**: Effects of acute sazetidine-A (Saz-A 0.01 mg/kg and Saz-A 0.1 mg/kg) and saline injections on contextual fear extinction. Error bars show standard error of the mean. Asterisk denotes significant differences between lower dose drug groups and saline controls at p value < 0.05. Number sign denotes significant differences between higher dose drug groups and saline controls at p value < 0.05

effect of DhβE on spontaneous recovery. Overall, in addition to their modulatory role in encoding of contextual fear extinction, our results indicate a potential role of α4β2 nAChRs in the retrieval of extinction memories.

Discussion

The results of the present study demonstrate that the nAChR antagonist DhβE and partial-agonists varenicline and sazetidine-A alter contextual fear extinction and spontaneous

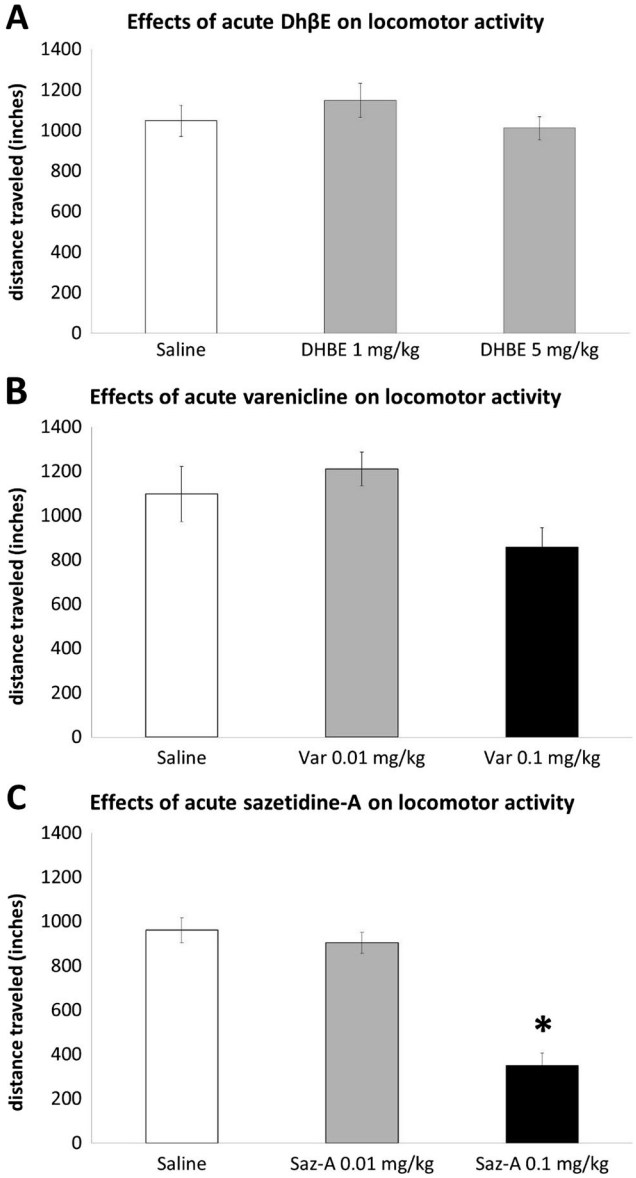

Fig. 3 Locomotor activity following DhβE, varenicline, sazetidine-A. Distance traveled (inches) in an open field paradigm. **a** Effects of acute injections of DhβE (DhβE 1 and DhβE 5 mg/kg) and saline on locomotor activity. **b** Effects of acute varenicline (Var 0.01 mg/kg and Var 0.1 mg/kg) and saline injections on locomotor activity. **c** Effects of acute sazetidine-A (Saz-A 0.01 mg/kg and Saz-A 0.1 mg/kg) and saline injections on locomotor activity. Error bars show standard error of the mean. Asterisk denotes Tukey post hoc results showing significant differences between drug groups and saline controls with p value < 0.05

recovery of contextual fear. Whereas DhβE and sazetadine-A dose dependently enhanced contextual fear extinction, varenicline administration resulted in impaired contextual fear extinction. In addition, only DhβE, at the dose that was effective in enhancing contextual fear extinction, decreased spontaneous recovery of contextual fear whereas sazetidine-A did not affect spontaneous recovery. These results indicate that inactivation of α4β2 nAChRs results in enhancement of contextual fear extinction. This hypothesis is also supported by previous work from our lab. For example, we found that acute administration of nicotine, an agonist of high-affinity α4β2 nAChRs, impaired contextual fear extinction but did not affect general freezing behavior (Kutlu and Gould 2014; Kutlu et al. 2016a, 2017a, b). In addition, acute nicotine also augmented spontaneous recovery of extinguished contextual fear while not altering recall of unextinguished fear memories (Kutlu et al. 2016b, 2017b). We also showed that knockout mice that lacked functional α4 or β2 nAChR subunits, but not α7 subunits, did not exhibit acute nicotine-induced impairment of contextual fear extinction (Kutlu et al. 2016a). In the Kutlu et al. (2016a) study, we observed a modest enhancement of contextual fear extinction in β2 nAChR knockout mice (Kutu et al. 2016a; Fig. 2). However, this effect was not as robust as we achieved with α4β2 nAChR antagonism, which suggests that deletion of both α4 and β2 nAChR subunits may be required to achieve this effect in nAChR knockout mice. Together with our previous research, these results strongly suggest that α4β2 nAChRs modulate contextual fear extinction processes.

It is important to note that partial agonists of α4β2 nAChRs may have potential side-effects. For example, varenicline has been shown to cause several side effects in humans including fatigue, abnormal dreams, and dry mouth (Patterson et al. 2009). In parallel, we showed that the higher dose of sazetidine-A reduced locomotor activity in an open field paradigm. We also showed a trending effect of the higher varenicline dose on locomotor activity, though a previous study from our laboratory showed that the same doses of varenicline did not induce freezing behavior in the absence of prior footshock presentations (Raybuck et al. 2008). The doses of varenicline and sazetidine-A that altered or trended to alter locomotion also impaired fear extinction suggesting that effects on locomotor activity could contribute to increased freezing response observed in these groups. Previous studies using 129SvJ-C57BL/6J hybrid mice did not report locomotor effects of sazetidine-A at 0.1 mg/kg dose (Turner et al. 2010, 2013). This suggests a potential susceptibility of C57BL/6J mice for the locomotor effects of α4β2 nAChR partial-agonists. Nevertheless, our data showed that DhβE enhanced contextual fear extinction and reduced spontaneous recovery without affecting locomotor activity, which indicates that full antagonism of α4β2 nAChRs may be required for successful augmentation of fear extinction memories.

In this study, we selected three compounds due to their effects on α4β2 nAChRs. Varenicline, a partial agonist of α4β2 nAChRs, limits nicotinic receptor activation at a submaximal level (Coe et al. 2005; Rollema et al. 2007). Sazetidine A is also a partial agonist of α4β2 nAChRs; however, it desensitizes α4β2 nAChRs without activating them (Xiao et al. 2006; Zwart et al. 2008). Finally, DhβE is a direct antagonist that deactivates receptors by blocking the binding of acetylcholine to the receptors (Luetje et al. 1990). Our data

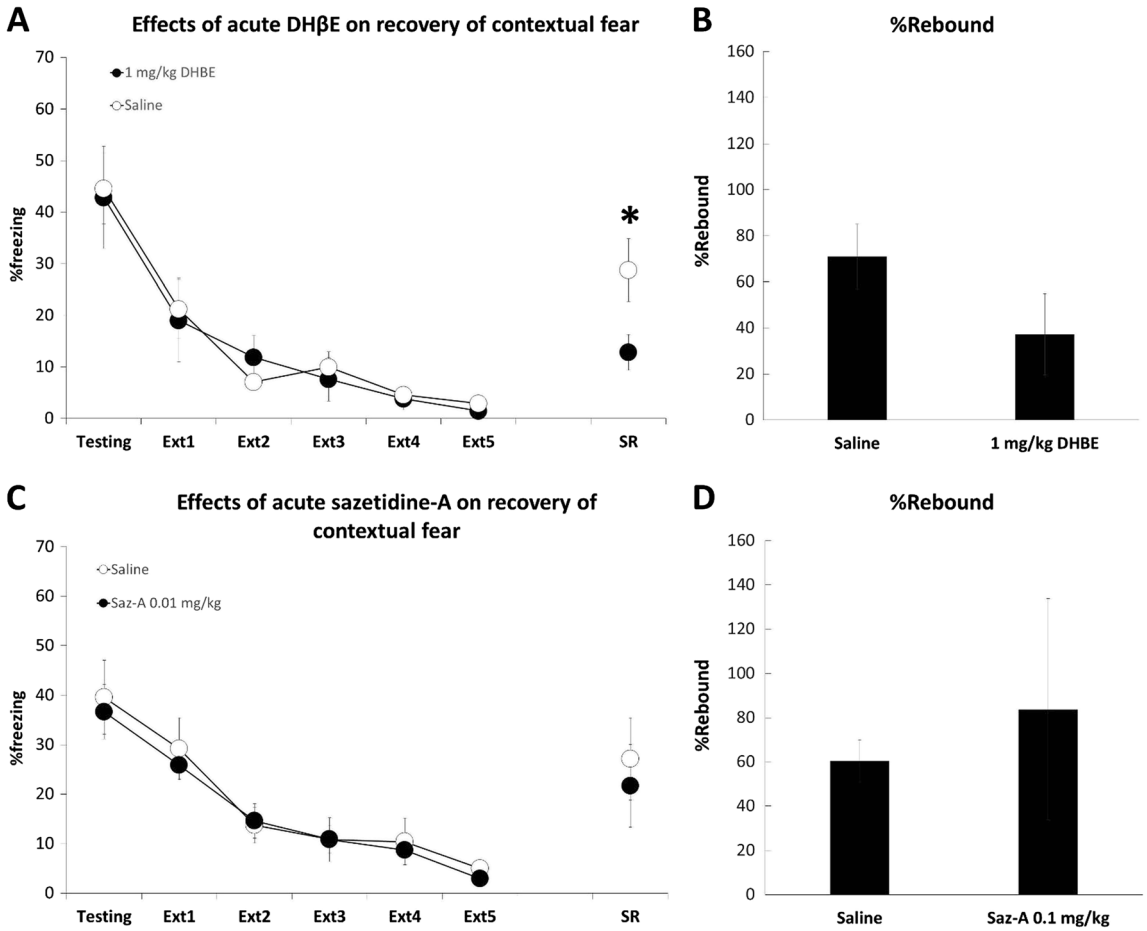

Fig. 4 DhβE decreases spontaneous recovery of contextual fear following extinction. %freezing and %Rebound scores during re-testing 7 days following the last fear extinction session. **a** Effects of low dose DhβE (DhβE 1 mg/kg) on spontaneous recovery of contextual fear when administered prior to re-testing. **b** %Rebound values for the DhβE 1 mg/kg and saline controls. **c** Effects of low dose sazetidine-A (Saz-A 0.01 mg/kg) on spontaneous recovery of contextual fear. **d** %Rebound values for the Saz-A 0.01 mg/kg and saline controls. Error bars show standard error of the mean. Asterisk denotes significant main effect of drug with p value < 0.05

indicate that DhβE and sazetidine-A enhanced fear extinction whereas varenicline resulted in impaired fear extinction. This suggests that even limited activation of α4β2 nAChRs, as could occur with varenicline may contribute to deficits in contextual fear extinction. Although the present study did not aim to investigate the involvement of downstream targets, we recently showed that during contextual fear extinction, acute nicotine disrupted phosphorylation of ventral hippocampal cell signaling kinases associated with memory consolidation such as ERK1/2 and JNK1 (Kutlu et al. 2017a). In addition, we showed that acute nicotine augments spontaneous recovery of contextual fear by enhancing neuronal activity in the ventral hippocampus and basolateral amygdala and reducing neuronal activity in the infralimbic cortex (Kutlu et al. 2016b). Given that nicotine, an agonist of α4β2 nAChRs, impairs contextual fear extinction and the opposite effect is achieved with inhibition of α4β2 nAChRs, it is possible that alterations in ventral hippocampal cell signaling cascades may also underlie the enhancing effects of DhβE and sazetidine-A on contextual fear extinction and recovery. However, future studies are necessary to validate these hypotheses.

It is also important to note that all three compounds tested in the present study also act on other nAChR receptor subtypes. For example, varenicline is also a full agonist of α7 nAChRs and shows high efficacy at α3β4 and α3β2 receptors, though at lower potency than for α4β2 nAChRs (Mihalak et al. 2006). Similarly, sazetidine-A exhibits differential effects on α4β2 nAChR subtypes; acting as an agonist of (α4)2(β2)3 pentamers, but as an antagonist of (α4)3(β2)2 pentamers (Zwart et al. 2008). These unique characteristics may shed light on the mechanisms underlying the differential effects of varenicline, sazetidine-A, and DhβE on contextual fear extinction. For instance, it is possible that varenicline's action on α7 nAChRs counteracts the potential enhancing effects of its α4β2 nAChR partial-agonism on contextual fear extinction. Therefore, future studies that will test these compounds in combination with agonists and antagonists of other nAChR subtypes are needed to determine the main site of action critical for mediating fear extinction memories.

The clinical use of nicotinic agents such as varenicline (Chantix®, Pfizer, Mission, KS) is generally limited to smoking cessation (Gibbons and Mann 2013). However, there are reports showing that these compounds have cognitive and emotional effects in humans (Patterson et al. 2009; Loughead et al. 2010). For example, a recent report showed that varenicline had destabilizing effects on mental health of veterans with PTSD (Campbell and Anderson 2010), suggesting potential adverse effects of nicotinic agents on mental health. Nevertheless, our results suggest that inhibition and desensitization of α4β2 nAChRs may be helpful for PTSD treatment as they potentially enhance fear extinction and reduce recovery of fear memories. Therefore, α4β2 nAChR compounds that decrease nAChR activation may provide a new line of pharmacotherapies to aid exposure therapy for PTSD. Investigating α4β2 nAChR antagonists and partial-agonists may be especially important given the inadequate development of new medications of for PTSD (PTSD Psychopharmacology Working Group; Krystal et al. 2017).

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