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High-affinity α4β2 nicotinic receptors mediate the impairing effects of acute nicotine on contextual fear extinction

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ABSTRACT

Previously, studies from our lab have shown that while acute nicotine administered prior to training and testing enhances contextual fear conditioning, acute nicotine injections prior to extinction sessions impair extinction of contextual fear. Although there is also strong evidence showing that the acute nicotine's enhancing effects on contextual fear conditioning require high-affinity α4β2 nicotinic acetylcholine receptors (nAChRs), it is unknown which nAChR subtypes are involved in the acute nicotine-induced impairment of contextual fear extinction. In this study, we investigated the effects of acute nicotine administration on contextual fear extinction in knock-out (KO) mice lacking α4, β2 or α7 subtypes of nAChRs and their wild-type (WT) littermates. Both KO and WT mice were first trained and tested for contextual fear conditioning and received a daily contextual extinction session for 4 days. Subjects received intraperitoneal injections of nicotine (0.18 mg/kg) or saline 2–4 min prior to each extinction session. Our results showed that the mice that lack α4 and β2 subtypes of nAChRs showed normal contextual fear extinction but not the acute nicotine-induced impairment while the mice that lack the α7 subtype showed both normal contextual extinction and nicotine-induced impairment of contextual extinction. In addition, control experiments showed that acute nicotine-induced impairment of contextual fear extinction persisted when nicotine administration was ceased and repeated acute nicotine administrations alone did not induce freezing behavior in the absence of context-shock learning. These results clearly demonstrate that high-affinity α4β2 nAChRs are necessary for the effects of acute nicotine on contextual fear extinction.

1. Introduction

Numerous studies identified a strong bidirectional relationship between fear-related disorders such as post-traumatic stress disorder (PTSD) and smoking (Breslau, Davis, & Schultz, 2003; Breslau, Novak, & Kessler, 2004; Feldner, Babson, & Zvolensky, 2007; Koenen et al., 2005). For example, PTSD patients had higher rates of nicotine-dependence compared to healthy individuals (Lasser et al., 2000; Ziedonis et al., 2008). In addition, development of PTSD has been shown to increase smoking initiation and number of cigarettes smoked daily (Breslau et al., 2003, 2004). While nicotine dependence increases with PTSD, smoking has also been linked to increased severity of PTSD symptoms. For example, nicotine intake increased trauma-related intrusive memories (Hawkins & Cougle, 2013) as well as fear response to a trauma-related script (Calhoun et al., 2011). This suggests that PTSD may increase the severity of nicotine dependence; in return, nicotine may also worsen the fear-related symptoms. In parallel with human studies, the effects of nicotine on fear learning and memory have also been documented in laboratory animals (see Kutlu & Gould, 2015 for a review). These studies suggested that acute nicotine selectively enhances hippocampus-dependent forms of fear conditioning, such as contextual and trace fear conditioning, but not does affect hippocampus-independent cued fear conditioning (Gould & Wehner, 1999; Gould, 2003; Gould & Higgins, 2003; Gould & Lommock, 2003; Gould, Feiro, & Moore, 2004; Davis, James, Siegel, & Gould, 2005; Davis, Porter, & Gould, 2006; Davis & Gould, 2006).

Multiple studies have shown that high-affinity nicotinic acetylcholine receptors (nAChRs) are required for nicotine enhancement of hippocampus-dependent learning (Davis & Gould, 2006, 2007; Davis, Kenney, & Gould, 2007). For example, Davis and Gould (2006) showed that the high-affinity α4β2 nAChR antagonist dihydro-beta-erythroidine (DhβE) administered systemically reversed the enhancement of contextual fear conditioning by nicotine in C57BL/6J mice. However, there were no effects of the low-affinity α7 nAChR antagonist methyllycaconitine (MLA) on the acute nicotine enhancement. Furthermore, Davis et al. (2007) also suggest that the acute nicotine-induced enhancement of contextual

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fear conditioning is mediated by β2-containing receptors in the hippocampus. Davis et al.'s (2007) results demonstrated that local infusions of DhβE into the dorsal hippocampus blocked the effects of systemic injections of acute nicotine on contextual fear conditioning. In addition, Davis and Gould (2007) found that the acute nicotine-induced enhancement of contextual and trace fear conditioning was absent in the knockout mice that lack the β2 nAChR subunit whereas both α7 nAChR subunit knockout mice (KO) and respective wildtype (WT) littermates showed acute nicotine enhancement of contextual fear conditioning.

Importantly, we recently showed that acute nicotine impairs extinction of contextual fear while having no effect on generalized freezing behavior tested in a novel context (Kutlu & Gould, 2014). Together with the previous results from our lab showing that acute nicotine enhances hippocampus-dependent fear learning, the results of this study suggest that acute nicotine may have adverse effects on fear-related symptoms of PTSD as it enhances acquisition and disrupts extinction of contextual fear memories. Even though evidence from multiple studies clearly demonstrated that α4β2 nAChRs are required for the enhancing effects of acute nicotine on the acquisition of hippocampus-dependent fear memories, the neurobiological mechanisms underlying the effects of acute nicotine on extinction of contextual fear are unknown. Therefore, in the present study, we tested the involvement of specific subunits of nAChRs in the impairing effects of acute nicotine on extinction of contextual fear using mutant mice that lack β2, α4, or α7 nAChR subunits and their wildtype littermates.

2. Method

2.1. Subjects

Subjects were naïve adult (8–10 weeks old), β2, α4, and α7 global knockout mice and their wildtype littermates as well as C57BL6/J mice (Jackson Laboratory, Bar Harbor, ME). The β2 (backcrossed to a C57BL6/J background, original breeding pairs provided by Dr. Arthur Beaudet, Baylor College of Medicine), α4, and α7 (backcrossed to a C57BL6/J background, breeding pair obtained from Jackson Laboratories) heterozygous male and female mice were bred in our animal colonies to obtain β2, α4, and α7 KO and WT mice and backcrossed to a C57BL6/J background. All mice were group housed with ad-libitum access to water and food in a colony room maintained on a 12 h light/dark cycle. All training and testing occurred between 9:00 am and 6:00 pm. Behavioral procedures used in this study were approved by the Temple University Institutional Animal Care and Use Committee.

2.2. Apparatus

Acquisition and extinction of contextual fear conditioning took place in 4 identical chambers (18.8 × 20 × 18.3 cm) with Plexiglass doors on the front wall of the chambers and ventilation fans mounted at the back wall of the chambers, which produced a background noise (65 dB). Also, another set of speakers located on the right wall of the chambers provided a white noise (85 dB) auditory conditioned stimulus (CS). The conditioning chambers were in sound attenuating boxes (MED Associates, St. Albans, VT). The chamber floors were metal grids (0.20 cm and 1.0 cm apart) connected to a shock generator, which delivered a 2 s long, 0.57 mA foot-shock unconditioned stimulus (US). The CS and the US were controlled by an IBM-PC compatible computer running MED-PC software.

2.3. Drug administration

Nicotine hydrogen tartrate salt (0.18 mg/kg freebase, Sigma, St. Louis, MO) dissolved in saline or saline alone were administered into the intraperitoneal cavity (i.p.) 2–4 min prior to each extinction session. The 0.18 mg/kg dose and the time course of the injections were chosen because we previously showed that contextual fear extinction and contextual safety discrimination were impaired at this dose administered 2–4 min prior to behavioral sessions in C57BL/6J mice (Kutlu & Gould, 2014; Kutlu, Oliver, & Gould, 2014). Both saline and nicotine injection volumes were 10 ml/kg as in previous studies.

2.4. Behavioral procedures

Freezing response to the context, which was defined as the absence of voluntary movement except respiration, was measured as the dependent variable (Davis et al., 2007; Kutlu & Gould, 2014). A time sampling method where subjects were observed every 10 s for a duration of 1 s and scored as active or freezing (Blanchard & Blanchard, 1972) was used to detect freezing response. For all 3 experiments, we trained the β2, α4, and α7 KO and WT mice in contextual fear conditioning, in which they received two white noise-footshock (0.57 mA) pairings (see Fig. 1 for the schematic experimental designs). The next day all mice were tested for initial freezing to the context. Then mice were given 4 days of contextual extinction where they received exposure to the training chamber in the absence of the footshocks in order to reduce conditioned freezing response to the context. Prior to each extinction session, mice were given i.p. nicotine (0.18 mg/kg) or saline injections 2–4 min. In addition to the nicotine and saline groups, each nAChR subunit experiment (β2, α4, and α7) also had a KO and a WT group, which produced 4 groups for each experiment: KO/Nicotine, KO/Saline, WT/Nicotine, and WT/Saline groups.

We also ran 2 additional experiments to control for the potential effects of acute nicotine on general freezing behavior. For the first experiment, C57BL6/J mice received the same training and extinction procedure described for the KO experiments. However, this group of mice received acute nicotine or saline injections only prior to the first 2 extinction sessions whereas there was no drug injection prior to the last 2 extinction sessions. For the second control experiment, C57BL6/J mice received acute nicotine or saline injections for 4 days without context-shock training or extinction. Following the last injection, all mice were placed in the conditioning chamber to test for freezing behavior.

2.5. Statistical analysis

Following Tian et al. (2008), we converted freezing response to "normalized %freezing", where freezing response measured during initial testing was normalized to 100%. Then each subsequent freezing response measured during extinction sessions was normalized to the initial freezing response (freezing × 100/initial freezing). This way we ensured that between-group baseline differences in contextual freezing did not affect subsequent fear extinction curves. We used separate 2 (Drug) × 2 (Genotype) × 5 (Trial) Repeated measures ANOVAs at $\alpha = 0.05$ for each KO group. Planned comparison *t*-tests were used for post-hoc analysis (Kutlu & Gould, 2014). Three mice from the β2, 4 mice from the α4 experiment, and 1 mouse from the α7 experiment were removed from the analysis as their freezing levels were 2 standard deviations above the mean during at least 1 extinction session. Group sizes are indicated in figure captions. All statistical analyses were run using SPSS 16.0.3.

3. Results

3.1. β2 nAChR subunit is necessary for the acute nicotine-induced impairment of contextual fear extinction

In this experiment, we measured the effects of acute nicotine on contextual extinction in β2 nAChR KO and WT mice (Fig. 2).