Published in final edited form as:

Neurobiol Learn Mem. 2016 February ; 128: 17–22. doi:10.1016/j.nlm.2015.11.021.

High-affinity $\alpha 4\beta 2$ nicotinic receptors mediate the impairing effects of acute nicotine on contextual fear extinction

Munir Gunes Kutlu*, Erica Holliday, and Thomas J. Gould

Department of Psychology, Neuroscience Program, Weiss Hall, Temple University, Philadelphia, PA 19122, USA

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 $\alpha 4\beta 2$ 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 $\alpha 4$, $\beta 2$ or $\alpha 7$ 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 mins prior to each extinction session. Our results showed that the mice that lack $\alpha 4$ and $\beta 2$ subtypes of nAChRs showed normal contextual fear extinction but not the acute nicotine-induced impairment while the mice that lack the $\alpha 7$ 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 $\alpha 4\beta 2$ nAChRs are necessary for the effects of acute nicotine on contextual fear extinction.

Keywords

Nicotine; nicotinic receptors; Extinction; PTSD

Numerous studies identified a strong bidirectional relationship between fear-related disorders such as post-traumatic stress disorder (PTSD) and smoking (Breslau et al., 2003,

*Corresponding Author. Munir G. Kutlu, Ph.D., 1701 N. 13th St, Weiss Hall, Philadelphia, PA 19122, Tel: (215) 204-6554; Fax: (215) 204-5539, munir.kutlu@temple.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

We declare no potential conflict of interest.

2004; Feldner et al., 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 Kultu & 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 does not affect hippocampus-independent cued fear conditioning (Gould & Wehner 1999; Gould, 2003; Gould & Higgins; 2003; Gould & Lommock 2003; Gould et al. 2004; Davis et al. 2005, 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 et al., 2007). For example, Davis and Gould (2006) showed that the high-affinity $\alpha 4\beta 2$ nAChR antagonist dihydro-beta-erythroidine (Dh β E) administered systemically reversed the enhancement of contextual fear conditioning by nicotine in C57BL/6J mice. However, there were no effects of the low-affinity $\alpha 7$ nAChR antagonist methyllycaconitine (MLA) on the acute nicotine enhancement. Furthermore, Davis et al. (2007) also suggest that the acute nicotine-induced enhancement of contextual fear conditioning is mediated by $\beta 2$ -containing receptors in the hippocampus. Davis et al.'s (2007) results demonstrated that local infusions of Dh β E 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 $\beta 2$ nAChR subunit whereas both $\alpha 7$ 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 $\alpha 4\beta 2$ 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 $\beta 2$, $\alpha 4$, or $\alpha 7$ nAChR subunits and their wildtype littermates.

Method

Subjects

Subjects were naïve adult (8–10 weeks old), $\beta 2$, $\alpha 4$, and $\alpha 7$ global knockout mice and their wildtype littermates as well as C57BL6/J mice (Jackson Laboratory, Bar Harbor, ME). The $\beta 2$ (backcrossed to a C57BL6/J background, original breeding pairs provided by Dr. Arthur Beaudet, Baylor College of Medicine), $\alpha 4$, and $\alpha 7$ (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 $\beta 2$, $\alpha 4$, and $\alpha 7$ 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 12h 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.

Apparatus

Acquisition and extinction of contextual fear conditioning took place in 4 identical chambers ($18.8 \times 20 \times 18.3$ cm) with Plexiglass doors on the front wall of the chambers and ventilation fans mounted at the back wall of the chambers, which produce 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.

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 minutes 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 mins prior to behavioral sessions in C57BL/6J mice (Kutlu & Gould, 2014; Kutlu et al., 2014). Both saline and nicotine injection volumes were 10 ml/kg as in previous studies.

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 $\beta 2$, $\alpha 4$, and $\alpha 7$ KO and WT mice in contextual fear conditioning, in which they received two white noise-footshock (0.57 mA) pairings (see Figure 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 mins. In addition to the nicotine and saline groups, each nAChR subunit experiment ($\beta 2$, $\alpha 4$, and $\alpha 7$) 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.

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 ($\text{freezing} \times 100 / \text{initial freezing}$). This way we ensured that between-group baseline differences in contextual freezing did not affect subsequent fear extinction curves. We used separate 2 (Drug) $\times$ 2 (Genotype) $\times$ 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 $\beta 2$, 4 mice from the $\alpha 4$ experiment, and 1 mouse from the $\alpha 7$ 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 were indicated in figure captions. All statistical analyses were run using SPSS 16.0.3.

Results

$\beta 2$ 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 $\beta 2$ nAChR KO and WT mice (Figure 2). A one-way ANOVA showed no main effect of genotype on initial freezing levels ($F(1,43) = 1.368$, $p > 0.05$). However, a repeated measures ANOVA found that the Drug (Saline vs. Nicotine) $\times$ 2 Genotype (KO vs. WT) $\times$ 5 Trial interaction was significant ($F(4,164) = 2.479$; $p < 0.05$; $F(4,80) = 2.318$, $p = 0.06$), which shows that $\beta 2$ nAChR KO and WT mice were differentially affected by the acute nicotine treatment. Individual planned comparison t-tests showed that the $\beta 2$ WT/Nicotine group showed significantly higher normalized %freezing compared to the WT/Saline group in Ext-1, Ext-2, Ext-3, and Ext-4 ($ps < 0.05$) whereas the $\beta 2$ KO/Nicotine group showed no differences in normalized freezing compared to KO/Saline mice during extinction ($ps > 0.05$). This suggests that acute nicotine does not affect contextual fear extinction in mice lacking $\beta 2$ subunit of nAChR. In addition, a separate Repeated Measures ANOVA showed no significant interaction between Genotype (KO vs. WT) and Trial in the saline treated mice

($F(4,80) = 1.992$, $p > 0.05$ for normalized; $F(4,80) = 0.440$, $p > 0.05$) but a significant main effect of Trial ($F(4,80) = 40.319$, $p < 0.001$ for normalized; $F(4,164) = 34.566$, $p < 0.001$ for raw), which suggests that $\beta 2$ KO mice showed normal contextual fear extinction.

$\alpha 4$ nAChR subunit is required for the impairing effects of acute nicotine on contextual fear extinction

The effects of acute nicotine injections were measured in $\alpha 4$ nAChR subunit KO and their littermate WT mice (Figure 3). A one-way ANOVA showed no main effect of genotype on initial freezing levels ($F(1,32) = 3.642$, $p > 0.05$). However, a repeated measures ANOVA yielded a significant interaction between Drug (Saline vs. Nicotine) and Genotype (KO vs. WT) $\times$ 5 Trial ($F(4,120) = 2.902$, $p < 0.05$ for normalized; $F(4,120) = 3.425$, $p < 0.05$ for raw). This suggests that acute nicotine modulates contextual fear extinction differentially in the $\alpha 4$ nAChR subunit KO and WT mice. Furthermore, individual planned comparison t-tests indicated that $\alpha 4$ WT/Nicotine mice showed higher normalized freezing compared to the WT/Saline group at Ext-1 and Ext-2 sessions ($p < 0.05$). However, we found no differences between the KO/Nicotine and KO/Saline groups at any extinction session ($p > 0.05$). These results indicate that while $\alpha 4$ WT mice exhibited the impairing effects of acute nicotine on contextual fear extinction, $\alpha 4$ KO mice did not show this effect. Like $\beta 2$ KO mice, $\alpha 4$ nAChR subunit KO mice did not show altered baseline contextual fear extinction either as a separate Repeated Measures ANOVA showed no significant interaction between Genotype (KO vs. WT) and Trial in the saline treated mice ($F(4,60) = 1.744$, $p > 0.05$ for normalized; $F(4,60) = 0.683$, $p > 0.05$ for raw) but a significant main effect of Trial ($F(4,60) = 23.814$, $p < 0.001$ for normalized; $F(4,60) = 20.672$, $p < 0.001$ for raw).

The acute nicotine-induced impairment of contextual fear extinction does not require $\alpha 7$ nAChR subunit

In this experiment, we tested the effects of acute nicotine administration on contextual fear extinction in $\alpha 7$ nAChR subunit KO mice and their WT littermates (Figure 4). A one-way ANOVA showed no main effect of genotype on initial freezing levels ($F(1,25) = 3.251$, $p > 0.05$). Also, a repeated measures ANOVA showed that the interaction between Drug (Nicotine vs. Saline), Genotype (KO vs. WT), and Trial was not significant ($F(4,100) = 0.838$, $p > 0.05$ for normalized; $F(4,100) = 0.530$, $p > 0.05$ for raw). However, there was a significant Drug $\times$ Trial interaction ($F(4,100) = 8.484$, $p < 0.001$ for normalized; $F(4,100) = 9.324$, $p < 0.001$ for raw) suggesting that nicotine affected both genotypes in the same manner. This suggests that acute nicotine results in impaired contextual fear extinction even in the absence of the $\alpha 7$ nAChR subunit. Like $\beta 2$ and $\alpha 4$ KO mice, $\alpha 7$ KO/Saline mice did not show altered baseline contextual fear extinction either as a separate repeated measures ANOVA showed no interaction between Genotype (KO vs. WT) and Trial ($F(4,52) = 1.055$, $p > 0.05$ for normalized; $F(4,52) = 1.122$, $p > 0.05$ for raw) but a significant main effect of Trial ($F(4,52) = 51.571$, $p < 0.001$ for normalized; $F(4,52) = 21.017$, $p < 0.001$ for raw).

Acute nicotine disrupts extinction memory consolidation rather than enhancing general freezing behavior

While our results demonstrated that WT mice that received acute nicotine showed impaired contextual fear extinction, this may be a result of an increase in general freezing behavior instead of disrupted extinction. To eliminate this possibility, we ran C57BL6/J mice in contextual fear extinction where they received acute injections of nicotine (0.18 mg/kg) or saline prior to the first 2 extinction sessions but no injections were administered before the last 2 extinction sessions. A repeated measures ANOVA showed that the interaction between Drug (saline vs. nicotine) and Trial was significant ($F(4,44) = 2.727, p < 0.05$ for normalized; $F(4,44) = 3.716, p < 0.05$ for raw). Also, individual t-tests showed that differences between groups were significant for Ext1, Ext2, Ext3, and Ext4 sessions (normalized, $ps < 0.05$). These results show that increased freezing response observed during the extinction sessions preceded by acute nicotine injections was not completely eliminated but decreased gradually in the absence of injections (Figure 5). This suggests that the acute nicotine-induced increase in freezing response during contextual fear extinction was a result of disrupted memory consolidation process rather than a direct effect of acute nicotine on general freezing behavior.

Acute nicotine injections alone do not affect general freezing behavior in the absence of context-shock training

Although our results showed that acute nicotine administration prior to contextual fear extinction sessions does not affect general freezing behavior, it is possible that repeated acute nicotine injections preceding testing may increase freezing during contextual fear extinction independent of extinction. Therefore, to control for the effects of repeated acute nicotine injections on freezing, C57BL6/J mice received 4 days of acute nicotine (0.18 mg/kg) or saline injections and were tested for freezing in conditioning chambers in the absence of context-shock presentations (Figure 6). A one-way ANOVA yielded no significant main effect of Drug ($F(1,10) = 0.172, p > 0.05$). This suggests that prior repeated administration of acute nicotine alone does not affect freezing behavior in mice. Therefore, the impairing effects of acute nicotine on contextual fear extinction in mice may be attributed to disrupted extinction rather than direct effects of nicotine on general freezing behavior.

Discussion

The results of the present study show that the impairing effects of acute nicotine were absent in the KO mice that lack $\beta 2$ and $\alpha 4$ nAChR subunits whereas $\alpha 7$ nAChR subunit KO mice exhibited the acute nicotine-induced impairment of contextual fear extinction. In line with a previous study from our lab demonstrating that acute nicotine at 0.18 mg/kg dose did not affect general freezing behavior tested in a novel context (Kutlu & Gould, 2014), our control experiments showed that acute nicotine's effects on contextual fear extinction was not completely eliminated when nicotine injections were ceased during extinction. In addition, repeated acute nicotine administration alone did not induce freezing behavior in the absence of context-shock learning. Therefore, the present results cannot be attributed to potential effects of acute nicotine on general freezing behavior. Finally, previous work from our lab

looking at the effects of acute nicotine on contextual fear conditioning showed that acute nicotine enhances acquisition but not recall of contextual fear memories (e.g. Gould & Wehner, 1999; Kenney & Gould, 2008). Specifically, acute nicotine administered during testing had no effect on contextual fear. This contrasts our data showing that acute nicotine impairs extinction of contextual fear (Kutlu & Gould, 2014). If the effects of acute nicotine on acquisition and extinction were the same, then acute nicotine administration at testing should have increased freezing, as stated this was not found in our prior work. This suggests that acquisition and extinction of contextual fear memories are mediated by different neural processes. Overall, these results suggest that the high-affinity $\alpha 4\beta 2$ nAChRs are required for the impairing effects of acute nicotine on contextual fear extinction and these effects are not due to acute nicotine-induced increase of general freezing behavior.

Our results showing that high-affinity $\alpha 4\beta 2$ nAChRs governing acute nicotine's effects on contextual fear extinction are in close agreement with the previous studies showing that high-affinity $\alpha 4\beta 2$ nAChRs, but not the low-affinity $\alpha 7$ nAChRs, are required for the nicotinic modulation of contextual fear acquisition (Davis & Gould, 2007; Davis et al., 2007). This suggests that although acute nicotine has enhancing effects on the acquisition and impairing effects on the extinction of contextual fear, these phenomena may be mediated by similar nAChR mechanisms. It is also possible that the effects of acute nicotine on acquisition and extinction of contextual fear require similar nAChR subtypes located in different brain regions. Previously, studies from our laboratory identified the dorsal hippocampus but not ventral hippocampus as the critical site for the acute nicotine-induced enhancement of contextual fear conditioning (Davis et al., 2007; Kenney et al., 2008, 2012; Gould et al., 2014). These studies showed that local injections of nicotine into the dorsal hippocampus enhanced contextual fear conditioning and these effects were reversed by local injections of the high-affinity nAChR antagonist Dh β E but not the low-affinity nAChRs antagonist MLA (Davis et al., 2007). Kenney et al. (2012) also showed that nicotine infusions into the ventral hippocampus impaired contextual fear conditioning. Interestingly, in a recent study, we found that acute nicotine impaired extinction retrieval and this effect was associated with increased *c-fos* immediate early gene expression in the ventral but not dorsal hippocampus (Kutlu et al., under review). Therefore, it is possible that the high-affinity $\alpha 4\beta 2$ nAChRs in another region within the fear extinction circuitry may primarily control the impairing effects of acute-nicotine on contextual fear extinction. Future studies will clarify this possibility.

In parallel with laboratory animal studies showing the involvement of high-affinity nAChRs in the acquisition and extinction of contextual fear memories, there is also evidence from human studies showing that $\beta 2$ -containing nAChRs play an important role in symptomatology of PTSD. Importantly, using the radiotracer [123 I]5-IA-85380 and single-photon emission computed tomography (SPECT), Czermak et al. (2008) demonstrated that non-smoker PTSD patients showed a significantly higher density of $\beta 2$ nAChRs in the mesiotemporal cortex, including the amygdala and hippocampus, compared to non-smoker healthy individuals. These brain regions are widely related to pathogenesis of PTSD (Martin et al., 2009). Also, Czermak et al. (2008) found a significant positive correlation between $\beta 2$ nAChR levels in the mesiotemporal cortex and PTSD symptoms such as re-experiencing.

These results indicate that $\beta 2$ -containing nAChRs are critically involved in the PTSD symptomatology. It is possible that a higher density of $\beta 2$ -containing nAChRs leads to greater activation of the cholinergic system and that acute nicotine produces a similar increased activation of cholinergic processes mediated by $\alpha 4\beta 2$ nAChRs resulting in deficits in contextual fear extinction, but this possibility needs further examination. Nevertheless, Czermak et al.'s (2008) results are important as they draw a parallel between human and animal studies in terms of the involvement of high-affinity $\beta 2$ -containing nAChRs in the fear-related symptoms of PTSD.

Overall, our results show that $\alpha 4$ and $\beta 2$ but not $\alpha 7$ nAChR subunits are necessary for impairment of contextual fear extinction by acute nicotine. This suggests that these nAChR subunits may also be critically involved in fear-related symptoms of PTSD. Given that PTSD patients show impaired extinction learning our results suggest areas of research that may potentially lead to the advancement of the pharmacological interventions to counteract the negative effects of nicotine on fear-related symptoms of PTSD.

Acknowledgements

The authors would like to thank Chicora Oliver for her assistance with breeding the $\beta 2$ knockout and wildtype animals. This work was funded with grant support from the National Institute on Drug Abuse (T.J.G., DA017949).

References

- Blanchard DC, Blanchard RJ. Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology*. 1972; 81(2):281. [PubMed: 5084445]
- Breslau N, Davis GC, Schultz LR. Posttraumatic stress disorder and the incidence of nicotine, alcohol, and other drug disorders in persons who have experienced trauma. *Archives of General Psychiatry*. 2003; 60(3):289–294. [PubMed: 12622662]
- Breslau N, Novak SP, Kessler RC. Psychiatric disorders and stages of smoking. *Biological Psychiatry*. 2004; 55(1):69–76. [PubMed: 14706427]
- Calhoun PS, Wagner HR, McClernon FJ, Lee S, Dennis MF, Vrana SR, Beckham JC. The effect of nicotine and trauma context on acoustic startle in smokers with and without posttraumatic stress disorder. *Psychopharmacology*. 2011; 215(2):379–389. [PubMed: 21188354]
- Czermak C, Staley JK, Kasserman S, Bois F, Young T, Henry S, Neumeister A. $\beta 2$ Nicotinic acetylcholine receptor availability in post-traumatic stress disorder. *International Journal of Neuropsychopharmacology*. 2008; 11(3):419–424. [PubMed: 18190729]
- Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology*. 2006; 184(3–4):345–352. [PubMed: 15988571]
- Davis JA, Gould TJ. $\beta 2$ subunit-containing nicotinic receptors mediate the enhancing effect of nicotine on trace cued fear conditioning in C57BL/6 mice. *Psychopharmacology*. 2007; 190(3):343–352. [PubMed: 17136517]
- Davis JA, James JR, Siegel SJ, Gould TJ. Withdrawal from chronic nicotine administration impairs contextual fear conditioning in C57BL/6 mice. *The Journal of Neuroscience*. 2005; 25(38):8708–8713. [PubMed: 16177040]
- Davis JA, Porter J, Gould TJ. Nicotine enhances both foreground and background contextual fear conditioning. *Neuroscience Letters*. 2006; 394(3):202–205. [PubMed: 16260086]
- Davis JA, Kenney JW, Gould TJ. Hippocampal $\alpha 4\beta 2$ nicotinic acetylcholine receptor involvement in the enhancing effect of acute nicotine on contextual fear conditioning. *The Journal of Neuroscience*. 2007; 27(40):10870–10877. [PubMed: 17913920]

- Feldner MT, Babson KA, Zvolensky MJ. Smoking, traumatic event exposure, and post-traumatic stress: A critical review of the empirical literature. *Clinical Psychology Review*. 2007; 27(1):14–45. [PubMed: 17034916]
- Gould TJ. Nicotine produces a within-subject enhancement of contextual fear conditioning in C57BL/6 mice independent of sex. *Integrative Physiological & Behavioral Science*. 2003; 38(2): 124–132. [PubMed: 14527181]
- Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. *Behavioural Brain Research*. 1999; 102(1):31–39. [PubMed: 10403013]
- Gould TJ, Higgins JS. Nicotine enhances contextual fear conditioning in C57BL/6J mice at 1 and 7 days post-training. *Neurobiology of Learning and Memory*. 2003; 80(2):147–157. [PubMed: 12932430]
- Gould TJ, Lommock JA. Nicotine enhances contextual fear conditioning and ameliorates ethanol-induced deficits in contextual fear conditioning. *Behavioral Neuroscience*. 2003; 117(6):1276–1282. [PubMed: 14674846]
- Gould TJ, Feiro O, Moore D. Nicotine enhances trace cued fear conditioning but not delay cued fear conditioning in C57BL/6 mice. *Behavioural Brain Research*. 2004; 155(1):167–173. [PubMed: 15325790]
- Gould TJ, Wilkinson DS, Yildirim E, Poole RL, Leach PT, Simmons SJ. Nicotine shifts the temporal activation of hippocampal protein kinase A and extracellular signal-regulated kinase 1/2 to enhance long-term, but not short-term, hippocampus-dependent memory. *Neurobiology of Learning and Memory*. 2014; 109:151–159. [PubMed: 24457151]
- Hawkins KA, Coughle JR. The effects of nicotine on intrusive memories in nonsmokers. *Experimental and Clinical Psychopharmacology*. 2013; 21(6):434–442. [PubMed: 24099352]
- Kenney JW, Gould TJ. Nicotine enhances context learning but not context-shock associative learning. *Behavioral Neuroscience*. 2008; 122(5):1158. [PubMed: 18823171]
- Kenney JW, Raybuck JD, Gould TJ. Nicotinic receptors in the dorsal and ventral hippocampus differentially modulate contextual fear conditioning. *Hippocampus*. 2012; 22(8):1681–1690. [PubMed: 22271264]
- Koenen KC, Hitsman B, Lyons MJ, Niaura R, McCaffery J, Goldberg J, Tsuang M. A twin registry study of the relationship between posttraumatic stress disorder and nicotine dependence in men. *Archives of general psychiatry*. 2005; 62(11):1258–1265. [PubMed: 16275813]
- Kutlu MG, Gould TJ. Acute nicotine delays extinction of contextual fear in mice. *Behavioural Brain Research*. 2014; 263:133–137. [PubMed: 24487010]
- Kutlu MG, Gould TJ. Nicotine modulation of fear memories and anxiety: Implications for learning and anxiety disorders. *Biochemical pharmacology*. 2015
- Kutlu MG, Oliver C, Gould TJ. The effects of acute nicotine on contextual safety discrimination. *Journal of Psychopharmacology*. 2014; 28(11):1064–1070. [PubMed: 25271215]
- Kutlu MG, Holliday E, Gould TJ. Acute nicotine enhances spontaneous recovery of contextual fear and changes c-fos early gene expression in infralimbic cortex, hippocampus, and amygdala. *Learning & Memory*. (under review).
- Lasser K, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: a population-based prevalence study. *Jama*. 2000; 284(20):2606–2610. [PubMed: 11086367]
- Martin EI, Ressler KJ, Binder E, Nemeroff CB. The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. *Psychiatric Clinics of North America*. 2009; 32(3):549–575. [PubMed: 19716990]
- Tian S, Gao J, Han L, Fu J, Li C, Li Z. Prior chronic nicotine impairs cued fear extinction but enhances contextual fear conditioning in rats. *Neuroscience*. 2008; 153(4):935–943. [PubMed: 18440720]
- Ziedonis D, Hitsman B, Beckham JC, Zvolensky M, Adler LE, Audrain-McGovern J, Riley WT. Tobacco use and cessation in psychiatric disorders: National Institute of Mental Health report. *Nicotine & Tobacco Research*. 2008; 10(12):1691–1715. [PubMed: 19023823]

Figure 1.
The schematic of experimental designs. While each box represents a phase of the experiment, the syringes represent nicotine or saline injections and the lightning bolt symbol indicates the presentations of the footshocks.

Figure 2. $\beta 2$ nAChR subunit is necessary for the acute nicotine-induced impairment of contextual fear extinction

Acute-nicotine (0.18 mg/kg) administration prior to the extinction sessions impaired contextual fear extinction in the WT/Nicotine group compared to the WT/Saline group but had no effect in the $\beta 2$ nAChR subunit KO mice that received acute nicotine (KO/Nicotine group; $n=10-12$ per group). Left panel: Normalized results. Right Panel: Raw data. Error bars indicate Standard Error of the Mean (SEM) and asterisks represent significant differences from the WT/Saline group at the $p<0.05$ level.

Figure 3. $\alpha 4$ nAChR subunit is required for the impairing effects of acute nicotine on contextual fear extinction

Acute-nicotine (0.18 mg/kg) administration prior to the extinction sessions impaired contextual fear extinction in the WT/Nicotine group compared to the WT/Saline group but the $\alpha 4$ nAChR subunit KO mice (KO/Nicotine group) showed no effects of acute nicotine on contextual fear extinction compared to saline controls (KO/Saline; $n=7-10$ per group). Left panel: Normalized results. Right Panel: Raw data. Error bars indicate Standard Error of the Mean (SEM) and asterisks represent significant differences from the WT/Saline group at the $p<0.05$ level.

Figure 4. The acute nicotine-induced impairment of contextual fear extinction does not require $\alpha 7$ nAChR subunit

Acute-nicotine (0.18 mg/kg) administration prior to the extinction sessions impaired contextual fear extinction both in the WT/Nicotine and KO/Nicotine groups compared to the WT/Saline and KO/Saline groups, respectively (n=7–8 per group). Left panel: Normalized results. Right Panel: Raw data. Error bars indicate Standard Error of the Mean (SEM) and asterisks represent significant differences from the WT/Saline group at the $p < 0.05$ level.

Figure 5. Acute nicotine disrupts extinction memory consolidation rather than enhancing general freezing behavior

Acute-nicotine-induced impairment of contextual fear extinction persists following cessation of nicotine treatment in C57BL6/J mice (Ext 3 & 4; $n=6-7$ per group). Left panel: Normalized results. Right Panel: Raw data. Error bars indicate Standard Error of the Mean (SEM) and asterisks represent significant differences from the WT/Saline group at the $p < 0.05$ level.

Figure 6. Acute nicotine injections alone do not affect general freezing behavior in the absence of context-shock training

Acute-nicotine (0.18 mg/kg) administration prior to testing had no effect on subsequent general freezing behavior in C57BL6/J mice (n=6 per group). Error bars indicate Standard Error of the Mean (SEM).