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The effects of acute nicotine on contextual safety discrimination

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Abstract

Anxiety disorders, such as post-traumatic stress disorder (PTSD), may be related to an inability to distinguish safe versus threatening environments and to extinguish fear memories. Given the high rate of cigarette smoking in patients with PTSD, as well as the recent finding that an acute dose of nicotine impairs extinction of contextual fear memory, we conducted a series of experiments to investigate the effect of acute nicotine in an animal model of contextual safety discrimination. Following saline or nicotine (at 0.0275, 0.045, 0.09 and 0.18 mg/kg) administration, C57BL/6J mice were trained in a contextual discrimination paradigm, in which the subjects received presentations of conditioned stimuli (CS) that co-terminated with a foot-shock in one context (context A (CXA)) and only CS presentations without foot-shock in a different context (context B (CXB)). Therefore, CXA was designated as the 'dangerous context', whereas CXB was designated as the 'safe context'. Our results suggested that saline-treated animals showed a strong discrimination between dangerous and safe contexts, while acute nicotine dose-dependently impaired contextual safety discrimination (Experiment 1). Furthermore, our results demonstrate that nicotine-induced impairment of contextual safety discrimination learning was not a result of increased generalized freezing (Experiment 2) or contingent on the common CS presentations in both contexts (Experiment 3). Finally, our results show that increasing the temporal gap between CXA and CXB during training abolished the impairing effects of nicotine (Experiment 4). The findings of this study may help link nicotine exposure to the safety learning deficits seen in anxiety disorder and PTSD patients.

Keywords

Animal model; anxiety; conditioned stimuli; contextual discrimination; danger; fear conditioning; memories; mouse study; nicotine; post-traumatic stress disorder; safety learning

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Conflict of interest

The authors declare that there are no conflicts of interest.

Introduction

Individuals who suffer from post-traumatic stress disorder (PTSD) show negative emotional responses such as re-experiencing the trauma events, avoidance of trauma-related memories and hyperarousal, which persist in novel contexts that were previously perceived as safe (Rauch et al., 2006; Rothbaum and Davis, 2003). There are a number of studies showing that extinction learning, in which a fear response to a stimulus diminishes with exposure, is delayed in individuals with anxiety disorders (Blecher et al., 2007; Michael, et al., 2007; Milad et al., 2009). In addition to the delayed extinction, patients with anxiety disorders also show impaired safety discrimination learning, a form of inhibitory learning where a stimulus or a context is trained to signal safety versus danger and impaired ability to transfer learned inhibition to a fear-eliciting stimulus (Jovanovic et al., 2009, 2010, 2012; Lissek et al., 2009). For a review, see Christianson et al. (2012).

Therefore, it was suggested that pathological anxiety can be attributed to deficits in fear response inhibition (Davis et al., 2000; Lissek et al., 2005). In support of a role of impaired safety learning in anxiety disorders, several studies found that following a Stimulus A-Shock/Stimulus B-No Shock differential in safety training, war veterans (Jovanovic et al., 2009) and the civilian population (Jovanovic et al., 2010) with PTSD fail to learn that stimulus B signals safety. Moreover, Craske et al. (2008) found that children whom show difficulties in learning safety signals are at a higher risk of developing anxiety disorders, when they become adults. There is also evidence showing that healthy individuals have impaired safety learning after anxiety induction, compared to healthy individuals whom did not receive the anxiogenic treatment (Liao and Craske, 2013). These studies suggest a causal relationship between impaired safety processing and anxiety disorders.

Tobacco smoking rates among PTSD patients (45.3%) are significantly higher than among non-clinical smokers (22.5%) (Ziedonis et al., 2008; Lasser et al., 2000). Numerous studies indicate a relationship exists between smoking and PTSD. Feldner et al. (2007) reviews this. For example, while prior smoking increases the chance of developing PTSD (Koenen et al., 2005), both the rate of smoking and initiation of smoking increase after a traumatic event that leads to PTSD (Breslau et al., 2003, 2004). Also, there is evidence suggesting that the severity of PTSD symptoms, such as hyper-arousal and avoidance of memories of the trauma, is positively correlated with smoking dependence severity (Thorndike et al., 2006). These results suggest that either smoking is a risk factor in developing PTSD or it functions as an attempt to reduce PTSD symptoms.

In line with studies associating PTSD and tobacco use (Breslau et al., 2003, 2004; Koenen et al., 2005), evidence from numerous reports suggest that acute nicotine administration enhances hippocampus-dependent contextual and trace fear conditioning, a widely used animal model for PTSD (Cordero et al., 2002; Yehuda and Antelman, 1993), while having no effect on hippocampus-independent cued fear conditioning (Davis et al., 2006; Gould, 2003; Gould and Higgins, 2003; Gould and Wehner, 1999; Gould et al., 2004). Moreover, evidence from numerous studies show that while chronic nicotine administration has no effect on hippocampus-dependent learning, withdrawal from chronic nicotine leads to deficits in contextual and trace fear conditioning (André et al., 2008; Davis et al., 2005;

Portugal and Gould, 2009; Portugal et al., 2012; Raybuck and Gould, 2009). Together, these studies suggest that nicotine alters fear-related memory processes, based on the administration regimen.

There are also multiple studies investigating the effects of acute nicotine on extinction of cued and contextual fear, a form of learning that requires inhibiting responses (Bouton, 2004; Konorski, 1967). For example, Elias et al. (2010) found that acute nicotine differentially affected cued fear extinction, based on the timing of the nicotine injections. According to the results of this study, while acute nicotine injections during extinction enhanced cued fear extinction, injections during both training and extinction delayed cued extinction learning. Recently, Kutlu and Gould (2014) investigated the effects of acute nicotine on extinction of contextual fear conditioning. The results demonstrated that acute nicotine administration during extinction, impairs extinction of both background and foreground contextual fear conditioning; moreover, these results from Kutlu and Gould (2014) show that nicotine does not affect generalized freezing, when tested in a novel context, which suggests that acute nicotine impairs extinction by enhancing the recall of contextual fear. A further analysis of Elias et al.'s (2010) results by Kutlu and Gould (2014) shows that Elias et al. also found impaired contextual extinction within the first extinction session. Overall, these results support the idea that nicotine may interfere with the treatment of PTSD by impairing safety learning and may contribute to the development of the disorder, through enhancing the retrieval of contextual fear memories.

In the present study, we investigated the effects of acute nicotine on contextual safety learning by employing a contextual safety discrimination paradigm. We hypothesized that, if nicotine impairs extinction of contextual fear conditioning, it may also impair contextual safety learning; and thus, nicotine-treated animals will be impaired in their ability to discriminate safe and dangerous contexts.

Methods

Subjects

Subjects were 7–9 week-old male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA). All subjects were group-housed in a colony room maintained on a 12-hour light/dark cycle, and had access to food and water *ad libitum*. All training and testing took place between 7:00 am and 6:00 pm. Behavioral procedures used in this study were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus

Behavioral training and testing took place in three different contexts: context A (CXA), context B (CXB) and context C (CXC). CXA was designated as the dangerous context, and it consisted of four identical conditioning chambers (18.8 X 20 X 18.3 cm) placed in sound-attenuating boxes (MED Associates, St. Albans, VT, USA). Ventilation fans were located at the back of the boxes, providing a background noise (65 decibels (dB)). A white noise (WN) conditioned stimulus (CS) of 85 dB was produced by a speaker that was located on the right wall of the conditioning chamber, and a light-based CS was presented through a light bulb

that was mounted on the left wall of the chamber. The front wall and ceiling of the chambers were composed of Plexiglas, the side walls were aluminum, and the floors were metal grids (0.20 cm and 1.0 cm apart), connected to a shock generator that produced a 2 s long, 0.57 mA foot-shock unconditioned stimulus (US). The stimuli were controlled by a computer (IBM-PC compatible) running MED-PC software.

CXB, which was designated as the safe context, also consisted of four identical testing chambers (23.5 X 22 X 25.3 cm). In addition to the dimensions, CXB was distinguished from CXA by plastic floors, Plexiglas walls and top, and a vanilla olfactory cue applied to paper towels placed in an open compartment below each chamber.

Finally, CXC, the novel context, consisted of four identical conditioning chambers (23.5 X 20.3 X 19.1 cm) distinguished from CXA and CXB by its dimensions and round aluminum sides; also, a black- and white-striped visual cue was attached to the back Plexiglas wall of these chambers. All chambers were cleaned with 70% ethanol between each subject.

Drugs and their administration

Nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.9% physiological saline (saline), or saline alone, were injected intra-peritoneally (i.p.) 2–4 mins prior to behavioral training and testing. The time course of these injections was chosen because the half-life of nicotine is approximately 10 minutes in mice and nicotine concentration reaches its peak between 2–4 mins (Petersen et al., 1984). Both the control saline and nicotine injection volumes were 10 mL/kg. Nicotine freebase concentrations were used; these were administered at the following doses: 0.0275, 0.045, 0.09 and 0.18 mg/kg.

Behavioral procedures

For all experiments, ‘freezing’, which is defined as the absence of movement except for respiration (Blanchard and Blanchard, 1969), was used as the dependent variable. A time-sampling technique was used to score freezing behavior, wherein each subject was observed every 10 seconds, for a duration of 1 second, and then scored as either freezing or not freezing. These freezing scores were converted to % freezing by the experimenters, whom were blind to the drug conditions of the mice.

As shown in Figure 1, in Experiment 1, subjects were trained in CXA and CXB once a day for three consecutive training days. The order of the CXA and CXB training sessions was pseudo-randomized in the following order, CXA-CXB (Day1), CXB-CXA (Day2) and CXA-CXB (Day3). During the training sessions in CXA, following a 60-s baseline period, two WN and two light presentations were given, pseudo-randomly. Both the WN and light presentations were 30 s in duration, co-terminated with a 2-s US, a 0.57 mA foot-shock. In the CXB training sessions, mouse subjects received four 30-s WN presentations, where the US presentations were omitted. For both CXA and CXB training sessions, the inter-trial interval (ITI) was 60 s. These CXA and CXB sessions were separated by a 1-h delay.

Testing occurred 24 h after the third day of training. The order of the test sessions was also pseudo-random, with one-half of the mice being tested in CXA first, and the other one-half tested in CXB first. During testing, freezing to the context was assessed for 120 s; followed

by a light presentation for 120 s, to measure contextual inhibitory control. Low freezing levels were an indicator of stronger inhibitory control. Each subject received either a saline or nicotine (0.0275, 0.045, 0.09, 0.18 mg/kg) injection, prior to both the training and testing sessions.

Experiment 2 aimed to examine the effects of nicotine on generalized freezing to a novel context. The training was identical to Experiment 1; however, subjects were tested in CXA as well as in a novel context, CXC, instead of in CXB.

In Experiment 3, all CS presentations during training were omitted. Consequently, during training, there were four unsignaled foot-shock US delivered in CXA; whereas no USs nor CSs were presented in CXB. The order and length of the training and test sessions were identical to Experiment 1.

Finally, the Experiment 4 training and testing procedures were identical to Experiment 3, except the temporal delay between CXA and CXB during training was 6 hrs instead of 1 hr, a manipulation shown to reduce task difficulty in discrimination learning (Deisig et al., 2006; Sarason et al., 1956). In experiments 2, 3 and 4, the animals received either saline or nicotine at the dose of 0.09 mg/kg, before each training and testing session.

Statistical analysis

We examined the cued and contextual freezing levels in CXA, CXB and CXC during testing, using a repeated-measure analysis of variance (ANOVA). We analyzed the cued and contextual freezing separately. Planned comparison *t*-tests were used for post-hoc analysis, at $\alpha = 0.05$ level. All group sizes are indicated in the figure captions. All statistical analyses were run using statistical analysis software (SPSS 16.0).

Results

Experiment 1: The effects of acute nicotine injections on contextual safety learning

A repeated measures ANOVA yielded a significant drug-related (saline control; nicotine 0.0275, 0.045, 0.09 and 0.18 mg/kg) X context (CXA, CXB) interaction for the contextual freezing of mice during testing, $F(1,39) = 5.178$; $p = 0.002$ (Figure 2). Furthermore, individual planned *t*-tests showed a significant difference between the freezing response to CXA and CXB in the saline ($t(11) = 7.797$; $p < 0.001$), nicotine 0.0275 mg/kg ($t(7) = 4.867$; $p = 0.002$) and nicotine 0.045 mg/kg ($t(7) = 4.494$; $p = 0.003$) groups; but not in the nicotine 0.09 mg/kg ($t(7) = 1.293$; $p = 0.237$) and nicotine 0.18 mg/kg ($t(7) = 1.581$; $p = 0.158$) groups. Finally, both the nicotine 0.09 mg/kg and 0.18 mg/kg groups' CXB freezing significantly differed from saline controls ($t(18) = 2.710$; $p = 0.014$ and $t(18) = 5.080$; $p < 0.001$, respectively); while the nicotine 0.0275 mg/kg and 0.045 mg/kg CXB freezing did not ($t(18) = 0.271$; $p = 0.789$ and $t(18) = 0.089$; $p = 0.930$, respectively). These results suggested that while animals treated with saline and lower doses of nicotine were able to discriminate between safe and dangerous contexts, the animals receiving higher nicotine doses showed impaired contextual discrimination.

Interestingly, a separate repeated measures ANOVA did not yield a significant Drug X Context interaction for cued freezing to the light, during testing $F(1,39) = 1.094$; $p = 0.373$ (Figure 3). Moreover, planned t -tests indicated a significant difference between CXA and CXB in all groups (Saline, $t(11) = 4.408$, $p = 0.001$; Nicotine 0.0275 mg/kg, $t(7) = 2.693$, $p = 0.031$; 0.045 mg/kg, $t(7) = 5.851$, $p = 0.001$; 0.09 mg/kg, $t(7) = 3.629$, $p = 0.008$; and 0.18 mg/kg, $t(7) = 4.201$, $p = 0.004$). Together with the contextual safety learning effect, these results suggested that nicotine dose-dependently impairs contextual safety learning, while not affecting the contextual inhibitory control of the cue.

Experiment 2: The effects of acute nicotine injections on general freezing behavior

The results of Experiment 1 showed that acute nicotine impairs contextual safety learning by increasing freezing to the safe context; however, it is also possible that nicotine increases general freezing behavior, instead of impairing safety learning. To test this hypothesis, we trained animals in CXA and CXB, and then tested in CXA, as well as a novel context, CXC. We chose to use a nicotine dose (0.09 mg/kg) that effectively impaired safety learning in Experiment 1. A repeated measures ANOVA showed that the Drug (Saline control; Nicotine 0.09 mg/kg) X Context (CXA and CXC) interaction was not significant: $F(1,14) = 1.012$; $p = 0.332$ (Figure 4). Furthermore, individual planned t -tests showed a significant difference between the freezing response to CXA and CXC in both the saline ($t(7) = 8.403$; $p < 0.001$), and nicotine ($t(7) = 13.042$; $p < 0.001$) groups. Finally, the nicotine 0.09 mg/kg group's CXC freezing did not differ from the saline controls ($t(14) = 0.736$; $p = 0.474$). These results suggested that nicotine did not affect general freezing behavior, as both saline- and nicotine-administered animals showed significantly lower levels of freezing to the novel CXC.

Experiment 3: The effects of acute nicotine injections on contextual safety learning in the absence of a common cue

One possible explanation for our Experiment 1 results was that the common cue between CXA and CXB (i.e. WN) might lead to generalization of fear between contexts, and that nicotine enhanced this generalization; therefore, in Experiment 3 we omitted the CS presentations during training and testing, to test the effects of nicotine on pure contextual discrimination. A repeated measures ANOVA yielded a significant interaction between the drug (saline control and nicotine 0.09 mg/kg) X Context (CXA and CXB), as seen in Figure 5 ($F(1,14) = 7.431$; $p = 0.016$), which suggested that nicotine impairs safety learning, even in the absence of the common CS. Furthermore, while individual planned t -tests showed a significant difference between the freezing response to CXA and CXB in both the Saline ($t(7) = 6.138$; $p < 0.001$) and Nicotine ($t(7) = 3.742$; $p = 0.007$) groups, the Nicotine 0.09 mg/kg group's CXB freezing was significantly greater than the saline controls ($t(14) = 3.279$; $p = 0.005$), further indicating there was impaired safety learning. Our results indicated that, in the absence of the common cue between the dangerous CXA and safe CXB, nicotine still impaired contextual safety learning. That is, nicotine did not affect generalization of freezing behavior between contexts induced by the presentation of a common cue.

Experiment 4: Temporal control over the effects of acute nicotine injections on contextual safety learning

Our results from Experiment 2 and Experiment 3 suggested that nicotine impairs safety learning, but does not affect generalized freezing; and that the impairment does not require a common CS between contexts. Therefore, another possible explanation of these results was that nicotine strengthens the contextual fear learning; and consequently, impairs safety learning by increasing task difficulty when the temporal gap between training sessions are relatively short. To investigate this, we increased the temporal gap between the CXA and CXB sessions during training to 6 hrs. A repeated measure ANOVA did not yield a significant interaction between the drug (saline control or nicotine 0.09 mg/kg) X Context (CXA and CXB, $F(1,14) = 0.453$; $p = 0.512$), as seen in Figure 6. However, the main effects of drug and context were both significant ($F(1,14) = 5.238$; $p = 0.038$ and $F(1,14) = 42.942$, $p < 0.001$, respectively). Finally, the nicotine 0.09 mg/kg group's CXB freezing did not differ from saline controls ($t(14) = 0.772$; $p = 0.179$). These results suggested that when the temporal gap between CXA and CXB trials are longer, nicotine loses its impairing effects on contextual safety learning (in mice).

Discussion

Our results demonstrated that acute nicotine administration during training and testing dose-dependently impaired contextual safety discrimination in mice, while not affecting the contextual inhibitory control of the cue (Experiment 1), and that this impairment was not due to increased general freezing behavior (Experiment 2) nor generalization of the fear response between contexts, through the common cue (Experiment 3). These results are in line with our previous report demonstrating that acute nicotine impairs extinction of contextual fear conditioning, by enhancing recall of the fear memory (Kutlu and Gould, 2014). Notice that our results showed a nicotine-induced impairment of safety learning in CXB, but not an impairment of danger learning in CXA. This suggested that safety learning is more easily overridden by fear memories, while fear memories are stronger against interference from safety memories. In support of our results suggesting that following nicotine administration, the interference from a dangerous context is stronger than the interference from the safe context, there is evidence showing that conditioned fear is better retained; and therefore, more resistant to interference from newly-acquired memories in long intervals than in conditioned safety (Henderson, 1978).

In the present study, we showed that when there was a 1-h delay between CXA and CXB sessions, the saline-administered control animals learned the safety discrimination successfully; however, our results also demonstrated that the nicotine-treated animals could not learn safety discrimination with a 1-h gap, but could learn the contextual safety discrimination when the temporal gap between training sessions was 6 h (Experiment 4). This finding suggested that acute nicotine may impair safety learning by strengthening contextual fear memory; and thus, increasing task difficulty when the temporal gap is relatively short, and that this effect disappeared when the task was made easier, with spaced discrimination trials. Supporting this interpretation, there is evidence demonstrating that acute nicotine enhances contextual fear conditioning (Gould and Wehner, 1999). Similarly,

there is also evidence showing that both cued and contextual fear generalization is stronger when training intensity is increased, by both using a more salient shock and increasing the number of shocks used during training (Baldi et al., 2004; Laxmi et al., 2003), which suggests that stronger conditioning may increase discrimination difficulty, through increased generalization during training. Similarly, King (1969) found that fear generalization between different visual and olfactory cues was the strongest with a 1-h delay between training and testing, and that the strength of generalization decreased with longer retention delays. This suggested that spacing discrimination trials can decrease task difficulty induced by strong fear generalization between contexts. In support of this, previous studies show that spaced training trials result in better discrimination and reversal learning (Deisig et al., 2006; Sarason et al., 1956) than massed trials. Therefore, the lack of nicotine's effect on contextual safety learning, when the temporal gap between CXA and CXB sessions were increased to 6 h, may be attributed to discrimination learning becoming an easier task with spaced trials. Finally, it is important to note that in Experiment 2, nicotine did not increase generalization, but this was generalization to a novel context that the mice were first exposed to at testing. The results of Experiment 4 and prior work suggested that during training, nicotine may impair discrimination between contexts and/or enhance generalization between the contexts that the mice are exposed to during training.

Previous studies show that the hippocampus is required for contextual safety learning (Desmedt et al., 2003; Frankland et al., 1998). For example, Desmedt et al. (2003) found that lesions of the hippocampus impaired the acquisition of contextual discrimination. More recently, Wu and Hen (2014) found that neurogenesis in the dorsal hippocampus is necessary for successful contextual safety learning. As discussed above, multiple studies have found that nicotine enhances hippocampus-dependent learning (Davis et al., 2006; Gould, 2003; Gould et al., 2004; Gould and Higgins, 2003; Gould and Wehner, 1999). Therefore, the nicotine-induced impairment of contextual safety learning observed in the present study may seem to contradict previous studies reporting enhancement of hippocampus-dependent tasks by acute nicotine administration; however, importantly, both the present study and Kutlu and Gould (2014) showed that impaired safety learning is a product of enhanced fear memories by nicotine. Thus, our results are in line with the previous reports that show a robust enhancement of hippocampus-dependent fear memory, following acute nicotine administration.

As discussed previously, there is evidence from human studies indicating that the rate of smoking initiation, which is analogous to acute nicotine administration in animals, is higher among PTSD patients (Fu et al., 2007; Gabert-Quillen et al., in press; Koenen et al., 2005). This suggests that smoking may be an attempt to relieve PTSD symptoms. In support of this, several studies show that the presence of PTSD symptoms, such as hyper-arousal, is a predictor for nicotine dependence (Beckham et al., 2005; Feldner et al., 2007; Greenberg et al., 2012; Thorndike et al., 2006). The hyper-arousal symptom is shown to strongly influence the other PTSD symptoms, such as re-experiencing of the trauma events and avoidance of trauma-related memories; and thus, hyper-arousal may lead to maintenance of these symptoms (Marshall et al., 2006; Schell et al., 2004). In concert with the results from Kutlu and Gould (2014), the present study's results suggested that nicotine may contribute to the symptoms associated with PTSD, by making it more difficult to discriminate between

dangerous and safe contexts, a process that is already malfunctioning in this disorder. Therefore, while smoking has been shown to alleviate certain symptoms of PTSD, there is strong evidence suggesting that smoking may strengthen the anxiety-context associations. Moreover, this mouse study has suggested that the strong, maladaptive associations initially formed by a trauma event may worsen anxiety, as that context becomes difficult to extinguish as a result of smoking.

Overall, our study results have several implications for the effects of smoking initiation on PTSD treatment. Namely, our results suggested that the increased rate of smoking initiation may adversely affect safety learning in PTSD patients; however, it is important to note that while our present results indicated there is an impairing effect of acute nicotine on contextual safety learning, the effects of chronic nicotine on safety learning are unknown.

Previously, Beckham et al. (2007) found that smoking a cigarette following re-introduction of traumatic imagery reduced negative affective symptoms, such as re-experiencing in the PTSD patients that are chronic smokers. This suggests that for PTSD patients who are already chronic smokers, cigarette smoking may function as a mean to reduce PTSD-related anxiety and fear. As is discussed above, numerous studies in animals demonstrate that while acute nicotine enhances contextual fear conditioning (Davis et al., 2006; Gould and Higgins, 2003; Gould and Wehner, 1999), chronic nicotine administration has no effect; and withdrawal from chronic nicotine has a detrimental effect on contextual fear (André et al., 2008; Davis et al., 2005; Portugal and Gould, 2009; Portugal et al., 2012; Raybuck and Gould, 2009). Therefore, for future studies, it is essential to investigate the effects of chronic and withdrawal from chronic nicotine on contextual safety learning, to fully understand the effects of nicotine on safety learning in PTSD.

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Figure 1.

The schematic experimental design of Experiment 1. Black and red boxes represent Context A and Context B. The syringes represent nicotine or saline injections, the thunderbolt symbol indicates presentation of foot-shocks, and the light and tone pictures represent the different visual and auditory stimuli presented in each context.

Figure 2.

Experiment 1: The effects of acute nicotine injections on contextual safety learning. Learned safety was impaired in the groups that received 0.09 and 0.18 mg/kg nicotine; while the animals that received saline and nicotine at 0.0275 and 0.045 mg/kg showed normal contextual safety learning (Saline, $n = 12$; Nicotine 0.0275 mg/kg, $n = 8$; Nicotine 0.045 mg/kg, $n = 8$; Nicotine 0.09 mg/kg, $n = 8$; and Nicotine 0.18 mg/kg, $n = 8$). Error bars indicate SEM and asterisks represent differences at the $p < 0.05$ level.

CX: Context; g: kilograms (in this case, of mouse body weight); mg: milligrams (in this case, of the drug nicotine); SEM: standard error of the mean.

Figure 3.

Experiment 1: The effects of acute nicotine injections on cued freezing. Nicotine did not affect cued freezing based on contextual information. Error bars indicate SEM and asterisks represent differences at the $p < 0.05$ level.

CX: Context; SEM: standard error of the mean.

Figure 4.

Experiment 2: The effects of acute nicotine injections on generalized freezing behavior in mice. Saline or nicotine injections did not affect generalized freezing, when tested in a novel context, CXC (Saline group $n = 8$ and Nicotine 0.09 mg/kg, $n = 8$). Error bars indicate SEM and asterisks represent differences at the $p < 0.05$ level.

CX: Context; SEM: standard error of the mean.

Figure 5.

Experiment 3: The effects of acute nicotine injections on contextual safety learning in the absence of a common cue. In comparison to saline-treated mice, nicotine-injected animals showed impaired contextual safety learning, when the common cue presentations in CXA and CXB during training were omitted (Saline group $n = 8$; Nicotine 0.09 mg/kg, $n = 8$). Error bars indicate SEM and asterisks represent differences at the $p < 0.05$ level. CX: Context; SEM: standard error of the mean.

Figure 6.

Experiment 4: Temporal control over the effects of acute nicotine injections on contextual safety learning. The effects of nicotine on contextual safety was absent, when the temporal gap was increased between CXA and CXB during training (Saline group $n = 8$; Nicotine 0.09 mg/kg, $n = 8$). Error bars indicate SEM and asterisks represent differences at the $p < 0.05$ level.

CX: Context; SEM: standard error of the mean.