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Chronic nicotine differentially alters spontaneous recovery of contextual fear in male and female mice

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a devastating disorder with symptoms such as flashbacks, hyperarousal, and avoidance of reminders of the traumatic event. Exposure therapy, which attempts to extinguish fear responses, is a commonly used treatment for PTSD but relapse following successful exposure therapy is a frequent problem. In rodents, spontaneous recovery (SR), where extinguished fear responses resurface following extinction treatment, is used as a model of fear relapse. Previous studies from our lab showed that chronic nicotine impaired fear extinction and acute nicotine enhanced SR of contextual fear in adult male mice. In addition, we showed that acute nicotine's effects were specific to SR as acute nicotine did not affect recall of contextual fear conditioning in the absence of extinction. However, effects of chronic nicotine administration on SR are not known. Therefore, in the present study, we investigated if chronic nicotine administration altered SR or recall of contextual fear in adult male and female C57BL/6J mice. Our results showed that chronic nicotine significantly enhanced SR in female mice and significantly decreased SR in males. Chronic nicotine had no effect on recall of contextual fear in males or females. Female sham mice also had significantly less baseline SR than male sham mice. Overall, these results demonstrate sex differences in SR of fear memories and that chronic nicotine modulates these effects on SR but nicotine does not alter recall of contextual fear.

1. Introduction

Post-traumatic stress disorder (PTSD) is precipitated by a traumatic event, after which the person is unable to properly extinguish negative emotional responses associated with cues and contexts related to that event. Exposure therapy is a common treatment for PTSD that attempts to extinguish maladaptive fear responses by repeatedly presenting the patient with trauma-associated cues and contexts in a safe environment. While exposure therapy is an effective form of treatment, relapse commonly occurs. Anxiety disorders have a 19–62% rate of relapse of fear symptoms following successful treatment with the type of disorder and the different metrics used to define "relapse" influencing the rate [1]. Relapse seems to occur because of a failure of extinction memories to suppress the original fear memory. Certain populations may be more vulnerable to PTSD relapse. For example, individuals with PTSD are more likely to smoke than the healthy population; 45.3% of PTSD patients smoke compared to 22.5% of the healthy population [2], and daily smoking rates increased with reported severity of symptoms [3]. Individuals may initially use nicotine as a form of self-medication for its anxiolytic effects but with a transition to chronic use, adaptations may occur resulting in a loss of anxiolytic effects and a worsening of symptoms.

PTSD patients show several fear-related symptoms such as hypervigilance, re-experiencing trauma-related memories and avoidance of trauma-associated cues and contexts, which elicit behavioral symptoms such as exaggerated startle response [4]. Some aspects of these behavioral outcomes may be captured in rodent fear response (e.g., freezing) exhibited in the presence of a cue or context associated with fearful stimulus (e.g., a mild footshock). Utilizing fear conditioning models, previous studies investigated the effects of nicotine exposure on acquisition, extinction, and recovery of fear memories. These studies showed that acute nicotine enhanced contextual fear conditioning, indicating that nicotine may be a modulatory factor in the development of PTSD [5]. Extinction treatment is also affected by nicotine in rodents. Studies from our lab have shown that adult male mice given acute (0.18 mg/kg) and chronic (12.6 mg/kg) nicotine have deficits in contextual fear extinction [6,7]. We also ran a study looking at acute nicotine's effect on contextual SR in male mice and found that nicotine significantly enhanced SR. Using *c-fos* immunohistochemistry, we showed that mice that had received nicotine and undergone contextual

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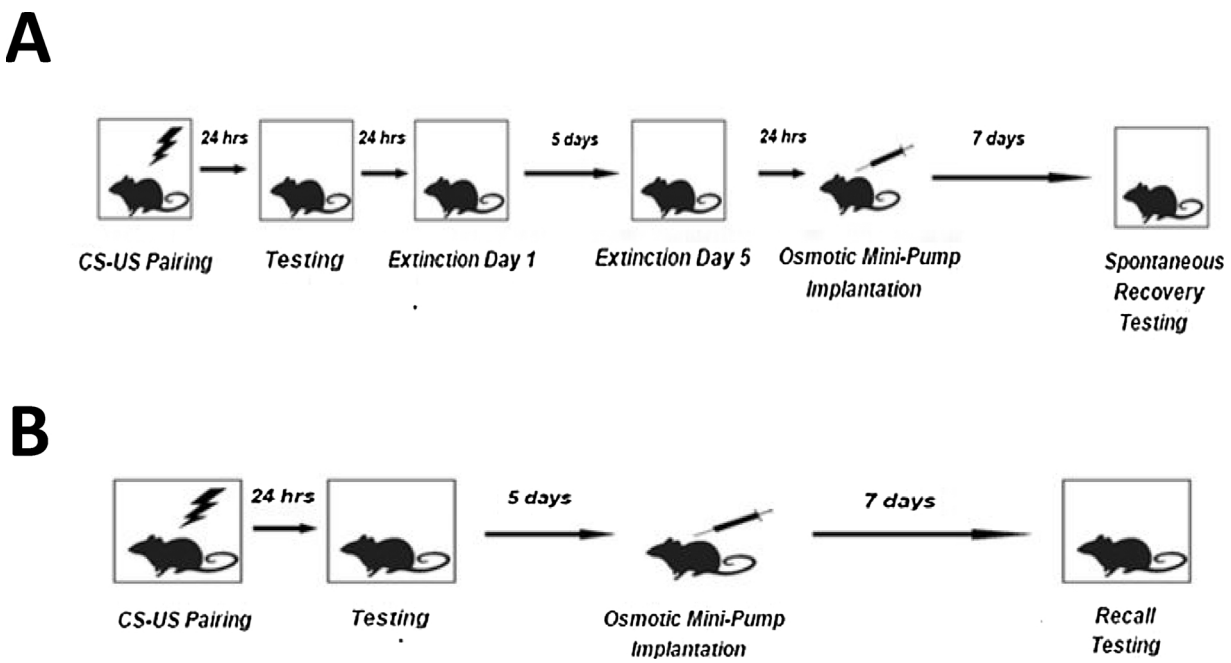

Fig. 1. Schematic description of experimental design *Panel A*: SR subjects were trained and tested in contextual fear conditioning, underwent extinction training for five days, and the following day underwent either osmotic mini-pump implantation or sham surgery. After a delay of 7 days, subjects were tested for SR. *Panel B*: Recall subjects were trained and tested in contextual fear conditioning, had a delay of 5 days, and then underwent either osmotic mini-pump implantation or sham surgery. Seven days later, subjects were tested for recall.

extinction had altered levels of recent neuronal activity in the infralimbic cortex, ventral hippocampus, and basolateral amygdala, regions of the brain implicated in extinction learning and memory retrieval, relative to saline and homecage controls while nicotine administration alone had no effect [8]. This indicates that acute and chronic nicotine use could lead to changes in extinction and spontaneous recovery, which could cause relapse.

However, the effects of chronic nicotine on SR and the effects of nicotine on SR in females are not known. While acute nicotine is a model of smoking initiation, chronic nicotine is more analogous to long-term nicotine use in humans. The current study investigated the effects of chronic nicotine on SR in male and female mice.

2. Materials and methods

2.1. Subjects

Subjects were naïve adult (8–10 week) male and female C57BL/6J mice. Mice were group-housed with access to food and water ad-libitum. Procedures used in this study were approved by the Temple University Institutional Animal Care and Use Committee.

2.2. Apparatus

Behavioral experiments took place in four identical chambers (18.8 × 20 × 18.3 cm) located in sound attenuating boxes. Ventilation fans produced the background noise (65 dB) and the white noise (85 dB) conditioned stimulus (CS) was delivered by a speaker. The chambers were composed of Plexiglas and the chamber floors were metal grids (0.20 cm in diameter and 1.0 cm apart) connected to a shock generator, which delivered a 2-s, 0.57-mA foot shock unconditioned stimulus (US).

2.3. Behavioral procedures

Freezing, defined as lack of all movement except respiration, was the dependent variable. Mice were scored using a time-sampling method in which they were observed every 10 s for a duration of 1 s. Experimenters were blind to group assignments. Following our previous studies showing nicotine-induced impaired contextual fear extinction [6,7], in both Experiment 1 and 2, mice were trained in background contextual fear conditioning, in which they were placed in the conditioning chambers and baseline freezing was measured for 120 s. Mice then received two white noise–foot shock pairings where the white noise co-terminated with the 2-sec foot shock with a 120-second interval between pairings. All mice remained in the chamber for another 30 s following the final CS-US pairing. The next day, all mice were placed back in the training conditioning chamber for 5 min to score freezing to the context in the absence of the foot shock. For the five following days, mice underwent contextual extinction trials within the training chambers in which their freezing was again scored in the absence of foot shocks. The day after extinction, the experimental group underwent osmotic mini-pump surgeries with a 12.6 mg/kg dose of nicotine while the control group received sham surgeries. On the eighth day following the surgeries, the mice were tested for SR by being placed back into the same chambers and scoring their freezing levels. In Experiment 2, separate groups of mice underwent the same procedure except they were not given the five extinction sessions(Fig. 1).

2.4. Osmotic mini-pump surgeries

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) dissolved in saline was administered subcutaneously through osmotic mini-pumps (Alzet, Model 1002, Durect, Cupertino, CA). Mini-pumps were surgically inserted through an incision on the lower back of the mouse while mice were under 3% isoflurane anesthetic. Surgeries were performed under sterile conditions. Mice were either implanted with subcutaneous mini-pumps delivering a nicotine solution (12.6 mg/kg/day) or received sham surgeries the day after the final extinction session, 7 days prior to SR testing. The nicotine doses are reported as freebase weights and were chosen based on the previous research form our laboratories showing deficits in contextual fear learning during extinction testing with this dose [7].