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Nicotine Withdrawal Disrupts Both Foreground and Background Contextual Fear Conditioning but not Pre-Pulse Inhibition of the Acoustic Startle Response in C57BL/6 Mice

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Abstract

Nicotine withdrawal is associated with multiple symptoms such as anxiety, increased appetite, and disrupted cognition in humans. Although animal models have provided insights into the somatic and affective symptoms of nicotine withdrawal, less research has focused on the effects of nicotine withdrawal on cognition. Therefore, in this study, C57BL/6 mice were used to test the effects of withdrawal from chronic nicotine on foreground and background contextual fear conditioning, which present the context as a primary or secondary stimulus, respectively. Mice withdrawn from 12 days of chronic nicotine (6.3 mg/kg/day) or saline were trained and tested in either foreground or background contextual fear conditioning; nicotine withdrawal-associated deficits in contextual fear conditioning were observed in both conditions. Mice were also tested for the effects of withdrawal on pre-pulse inhibition of the acoustic startle reflex (PPI), a measure of sensory gating, and on the acoustic startle reflex. Mice withdrawn from 12 days of chronic nicotine (6.3 or 12.6 mg/kg/day) or saline underwent one 30-minute PPI and startle session; no effect of withdrawal from chronic nicotine on PPI or startle was observed for either dose at 24 hours after nicotine removal. Therefore, mice were tested at different time points following withdrawal from 12.6 mg/kg/day chronic nicotine (8, 24, and 48 hours after nicotine removal). No effect of withdrawal from chronic nicotine was observed at any time point for PPI. Overall, these results demonstrate that nicotine withdrawal disrupts two methods of contextual learning but not sensory gating in C57BL/6 mice.

Keywords

learning; nicotine; withdrawal; addiction; sensory gating; acetylcholine

Introduction

Nicotine addiction is a world-wide public health crisis. Smoking leads to nearly 20% of deaths in developed countries, making it the single greatest avoidable cause of death [1]. In 2005, approximately 20.9% of U.S. adults were current cigarette smokers, the same percentage as in 2004 [9]; this signifies the end of an 8-year decline in smoking frequency among adults in the U.S. Most smokers know that cigarette smoking is detrimental to their health and nearly 35 million of them try to quit every year [50]. However, of the 42% of smokers that attempt to quit each year, less than 6% are successful [55]. One reason for the low rate of successful

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smoking cessation is that nicotine is highly addictive [50]. Thus, a better understanding of the mechanisms that underlie nicotine addiction may be useful in developing more effective smoking cessation treatments.

Nicotine withdrawal is associated with anger, anxiety, increased appetite, and agitation in humans [38]. Smokers who are withdrawn from nicotine also exhibit deficits in learning and working memory [4,41,44,84], report deficits in attention [18,39,74], and in sensory-gating (the ability to filter out extraneous stimuli) [46,66]. These cognitive impairments, in addition to the somatic and affective effects of withdrawal from nicotine, may be critical factors in the high rate of relapse to cigarette smoking [11,53]. Although animal models have been used to examine the somatic and affective symptoms of nicotine withdrawal, less research has focused on the effects of nicotine withdrawal on learning and memory. One behavioral paradigm that has been used to examine the effects of nicotine on learning and memory is contextual fear conditioning [see 31 for review], and studies have consistently shown that acute nicotine enhances contextual conditioning, chronic nicotine does not alter contextual conditioning and nicotine withdrawal disrupts contextual conditioning [16,13,15,32,64,65].

During contextual fear conditioning, an animal is trained to produce a conditioned response (CR) by associating a neutral conditioned stimulus (CS; which could be a discrete stimulus such as a tone or a compound stimulus such as a context), with an unconditioned stimulus (US), such as a foot shock. Previous studies examining the effects of nicotine on contextual fear conditioning have used a fear conditioning protocol in which an auditory CS is paired with a foot shock US, reducing the context to a background stimulus [69]. Following background contextual fear conditioning, the tone may be a stronger predictor of the shock than the context due to the close temporal association between presentation of the tone and the shock compared to the context, which is ubiquitous throughout the training session [57]; however, this overshadowing effect has not been seen in all studies [see 52]. When the foot shocks are administered without the auditory stimulus, in foreground conditioning, the context is more salient and becomes a foreground stimulus that elicits a CR [56,57]. Thus, it is unknown if nicotine withdrawal will disrupt foreground as well as background fear conditioning

Interestingly, acquisition and consolidation of background contextual fear conditioning may involve different cellular substrates than acquisition and consolidation of foreground contextual fear conditioning [62,77]. Trifilieff and colleagues [81] demonstrated that consolidation of foreground contextual fear conditioning recruits distinct early and late phases of protein synthesis in the hippocampus and amygdala, while background contextual fear conditioning recruits only the early phase. However, Trifilieff and colleagues used a conditioning paradigm in which the tone and shock were presented in an unpaired manner. Thus, the biphasic activation may be related to the unpaired presentation of the tone and shock rather than to the saliency of the context. Whereas different substrates may be involved in the two types of conditioning, background and foreground conditioning do involve some common neural substrates. For example, DBA/2J mice, which have reduced levels of hippocampal protein kinase C (PKC) activity [22,83] and altered hippocampal mossy fiber projections [12,33,71] relative to other inbred mouse strains, show deficits in learning regardless of whether the context is a foreground or a background stimulus [60]. In addition, hippocampal lesions disrupt both foreground and background fear conditioning [5,43,51,61]. Finally, studies in our lab have shown that acute nicotine administration enhances contextual fear conditioning regardless of whether the context is a foreground or a background stimulus [14,15,27,25,26, 24]. The current study examined if nicotine withdrawal disrupts processes common to both types of contextual conditioning.

A paradigm used to model the effects of nicotine on attentional processes that may alter cognition is pre-pulse inhibition of the acoustic startle response (PPI). Like fear conditioning,

PPI is dependent on the hippocampus [3,36,49,54,67,85] and thus may serve as a control for general deficits in hippocampal function during nicotine withdrawal. PPI is a phenomenon in which a low threshold initial stimulus, called a pre-pulse, suppresses subsequent responding to a startling stimulus [40] and is used as a measure of sensory gating [2,23,79]. Acute nicotine enhances PPI in smokers and non-smokers in humans [17–19] and animals [2,30]. In humans, withdrawal from chronic nicotine is associated with deficits in PPI that can be reversed by an acute dose of nicotine [45]. In addition, deficits in PPI have been reported in DBA/2J mice during withdrawal from chronic nicotine [72]. However, DBA/2J mice have altered hippocampal structure and function compared to other mouse strains [12,22,33,71,83] and thus the effects of nicotine withdrawal on hippocampal processes may differ between DBA/2J mice and other strains that do not show the same hippocampal deficits, such as C57BL/6 mice. Furthermore, it is unknown if the same treatment that produces nicotine withdrawal deficits in contextual fear conditioning in C57BL/6 mice would also produce deficits in PPI or startle responding. Therefore, the aim of this study was to examine the effects of withdrawal from chronic nicotine on foreground and background contextual fear conditioning, and on PPI and startle in C57BL/6 mice in order to determine whether withdrawal produces similar deficits across hippocampus-dependent paradigms in this strain of inbred mice.

Methods

Subjects

Male C57BL/6 mice (8–16 per group; Jackson Laboratory, Bar Harbor, ME), weighing between 20–28g, were group housed, four to a cage, with *ad libitum* access to food and water. The C57BL/6 strain was selected because this strain demonstrates robust levels of contextual fear conditioning [58]; the effects of nicotine on contextual fear conditioning in this strain have been well characterized [13,29,26,24], and previous research indicates that this strain reliably shows PPI [80]. Separate mice were used for each experiment. The light-dark cycle was 12:12 h with lights on at 07.00 h, and all testing occurred between 08.00 and 17.00 h. Mice were tested between 8 and 12 weeks of age. The Temple University Institutional Animal Care and Use Committee approved all procedures.

Apparatus

For contextual fear conditioning, training and testing took place in four identical conditioning chambers (18 × 19 × 38 cm) housed in sound-attenuating boxes (Med Associates, St. Albans, VT, USA). Ventilation fans at the back of the boxes provided air exchange and background noise (69 dB) and a speaker mounted to the right wall of each chamber produced an 85-dB white noise CS. The front and back walls of the conditioning chambers were made of clear Plexiglas, and side walls were made of stainless steel. The grid floors were connected to a shock scrambler and generator. An IBM PC-compatible computer running MED-PC software controlled stimulus administration. The chambers were cleaned with 70% ethanol before each use.

The testing of pre-pulse inhibition occurred in two identical sound attenuating chambers (65 × 35 × 25 cm). Each chamber was equipped with a Radio Shack loudspeaker mounted 25 cm above the holding cylinder. Startle responses were recorded by a commercial startle reflex system (S-R Lab, San Diego Instruments, CA). Mice were placed in a Plexiglas holding cylinder mounted on a Plexiglas platform. A piezoelectric accelerometer located beneath the platform was used to transform startle responses into units based on force and latency of response.

Drugs and Administration

Nicotine hydrogen tartrate salt (Sigma Co., St Louis, MO, USA) was dissolved in physiological saline and administered via mini-osmotic pumps (model 1002; Alzet, Cupertino, CA). For contextual fear conditioning, pumps were loaded with a 6.3 mg/kg/day (freebase weight) nicotine solution. This dose was chosen based on prior research which demonstrated that 6.3 mg/kg/day nicotine produced plasma nicotine levels of approximately 13.00 ng/ml [13], which is within the range of plasma nicotine found in smokers, approximately 10–50 ng/ml [35], and based on previous research in our lab showing that withdrawal from 6.3 mg/kg/day chronic nicotine produces significant deficits in background contextual fear conditioning [16,13,65]. For pre-pulse inhibition, pumps were loaded with nicotine (6.3 or 12.6 mg/kg/day) or saline solutions. Withdrawal from the 6.3 mg/kg/day dose of nicotine has been shown to produce deficits in DBA/2J mice [72]. In a separate experiment, sodium hydrogen tartrate, 13.0 mg/kg, also dissolved in physiological saline (Sigma Co., St Louis, MO, USA), or saline, was administered via mini-osmotic pumps. This dose was based on the weight of the hydrogen tartrate salt administered in combination with nicotine during the first experiment, and was used to verify that withdrawal from hydrogen tartrate salt does not alter contextual fear conditioning.

General Procedures

Surgery—Mini-osmotic pumps that administered 0.25 µl/hour of solution were filled with 100 µl of nicotine, hydrogen tartrate, or saline solutions and inserted subcutaneously via an incision in the back of the mouse. Pumps were removed 12 days after the initial surgery. Surgeries were performed under sterile conditions with 5% isoflurane used as an anesthetic.

Contextual Fear Conditioning—Methods for training and testing mice in contextual fear conditioning were based on previous studies [15,26]. Mice were withdrawn from 12 days of chronic nicotine administration and received either foreground or background conditioning 24 hours later; control groups were withdrawn from chronic saline and received either foreground or background conditioning. Previous work has demonstrated that chronic nicotine treatment does not alter background contextual fear conditioning; thus the effects of chronic nicotine treatment are dissociable from the deficits in background contextual fear conditioning associated with withdrawal from chronic nicotine [13]. In a separate experiment, one group of mice withdrawn from chronic sodium hydrogen tartrate was trained in background conditioning and was compared to a group of mice withdrawn from chronic saline.

Background Training: There are multiple measures of conditioned fear that include changes in heart rate [48], freezing [6] and potentiated startle [7,47] this study used freezing as the behavioral measure of conditioned fear. During training, mice were placed in conditioning chambers for 5 minutes and 30 seconds and freezing was assessed. Freezing was defined as the absence of movement except for respiration during a 1 second period assessed every 10 seconds. At the start of training, baseline freezing behavior was recorded for 120 seconds. Next, the CS (85 dB white noise) was presented for 30 seconds and co-terminated with a 2-second 0.57 mA US foot shock. A second CS-US pairing was presented at 270 seconds. The mice remained in the chamber 30 seconds after the second CS-US presentation. Twenty-four hours after training, freezing to the context was assessed by placing mice in the training chamber for 5 minutes and scoring freezing behavior.

Foreground Training: During training mice were placed in the conditioning chambers for 5 minutes and 30 seconds. Baseline freezing behavior was recorded during the first 120 seconds of the session. At 148 seconds, a 2-second 0.57 mA foot shock US was presented. At 298 seconds, an additional 2-second foot shock US was presented. The mice remained in the chamber 30 seconds after the second US presentation. Twenty-four hours after training,

freezing to the context was assessed by placing the mice in the training chamber for 5 minutes, during which time freezing behavior was recorded.

PPI—The first PPI experiment examined the effects of 24 hour withdrawal from chronic nicotine. Mice withdrawn from chronic nicotine (6.3 mg/kg/day or 12.6 mg/kg/day) or saline were placed in sound attenuating chambers for one thirty-minute session. The PPI protocol was based on previous studies and is described in full detail elsewhere [28]. Each session started with a 5 minute acclimation to the 65 dB white noise acoustic background that was continuously presented throughout the session, followed by five 40 ms 120 dB startle pulses. An intertrial interval randomized from 10 to 20 s separated all the PPI and startle trials. Next, PPI trials were administered and PPI was recorded for pre-pulse intensities of 69, 73, and 81 dB. Each pre-pulse inhibition trial was administered ten times in random order. Each pre-pulse was 20 ms in length, followed by a 40 ms startle stimulus of 120 dB; the stimuli were separated by a 100 ms interstimulus interval. Trials of 120 dB startle pulses were randomly interspersed and used for calculation of PPI. Percent PPI was calculated using the formula: $[100 - (\text{response to pre-pulse} + 120 \text{ dB}) / (\text{response for } 120 \text{ dB alone}) \times 100]$. Acoustic startle trials followed the PPI trials. Startle trials consisted of 40 ms pulses at 0 (no stimulus), 90, 95, 100, 105, 110, 115, and 120 dB. Each trial type was presented five times in a randomized order. Data were sampled at 60 1-ms samples/sec and the maximum voltage in each trial was used as the measure of startle response.

The second PPI experiment examined the effects of withdrawal from chronic nicotine at multiple time-points. Mice were withdrawn from chronic nicotine (12.6 mg/kg/day) or saline, and were tested for PPI and startle at 8 hours, 24 hours, or 48 hours after withdrawal. The procedure used was the same as used in the first PPI experiment.

Statistical Analysis

Data from foreground and background contextual fear conditioning were analyzed with independent sample t-tests. In order to examine if differences between the group administered nicotine and the group administered saline were due to faster extinction of the freezing response in nicotine-treated animals during testing, the testing session data was split into 3 intervals, such that freezing was binned into the first, middle, and third 100-second intervals and differences in freezing between these bins were analyzed using a 3×2 repeated measures ANOVA, with interval as the within subjects factor and drug treatment (saline or nicotine) as the between subjects factor. Data for the dose response of nicotine withdrawal on PPI was evaluated with a 3×3 repeated measures ANOVA, with pre-pulse intensity (69, 73, or 81 dB) as a within subjects factor and drug treatment (withdrawal from 6.3 or 12.6 mg/kg/day nicotine, or withdrawal from chronic saline) as a between subjects factor. Startle data from the same experiment was assessed with an 8×3 repeated measures ANOVA, with startle stimulus intensity (0, 90, 95, 100, 105, 110, 115, or 120 dB) as a within subjects factor and drug treatment (withdrawal from 6.3 or 12.6 mg/kg/day nicotine, or withdrawal from chronic saline) as a between subjects factor. Even though no statistical difference was found with ANOVA, we further examined the startle data to detect if there were any changes in startle response at specific dB levels due to nicotine withdrawal. A sign test was used to test if the nicotine response was significantly above or below the saline response. Given that this non-parametric test is a fairly liberal test, it is likely to detect differences that are too small to detect with ANOVA.

To examine if withdrawal from chronic nicotine alters PPI at time-points other than the 24-hour point used in the initial experiment, PPI data acquired at 8, 24, and 48 hours after withdrawal from chronic nicotine were evaluated with a 3×2 repeated measures ANOVA, with pre-pulse intensity (69, 73, or 81 dB) as a within subjects factor and drug treatment

(withdrawal from chronic nicotine or saline) as a between subjects factor. Startle data was assessed with an 8×2 repeated measures ANOVA, with startle stimulus intensity (0, 90, 95, 100, 105, 110, 115, or 120 dB) as a within subjects factor and drug treatment (withdrawal from chronic nicotine or saline) as a between subjects factor. A sign test compared mice withdrawn from chronic nicotine to saline withdrawn mice for each dB level of startle, at all three time points. All tests were performed at the $p < 0.05$ level using SPSS (version 13.0) for all experiments.

Results

The effect of withdrawal from chronic nicotine administration on contextual fear conditioning

In the first experiment, independent samples t-tests showed a significant effect of drug on background contextual fear conditioning [$t(25) = 5.52, p < 0.001$] and on foreground contextual fear conditioning [$t(25) = 2.95, p < 0.05$]. In both cases, the groups withdrawn from chronic nicotine froze significantly less than the groups withdrawn from chronic saline (Figure 1). The results were further analyzed with a 3×2 repeated measures ANOVA with time (i.e. the first, second, and third interval during testing) as the within subject factor and drug condition as the between subjects factor to test if drug differences were due to faster extinction within the testing session. There was a significant effect of drug, replicating what was found with the t-tests, on background contextual fear conditioning [$F(1, 26) = 28.88, p < 0.05$] and foreground contextual fear conditioning [$F(1, 26) = 8.60, p < 0.05$]. There was also a significant effect of time on freezing during the first, second, and third interval in background contextual fear conditioning [$F(1, 26) = 7.93, p < 0.05$], such that there was lower freezing in the third interval of the testing session, but not in foreground contextual fear conditioning [$p > 0.05$]. Finally, there was no significant interaction of drug and time on freezing in background or foreground contextual fear conditioning ($p > 0.05$), suggesting that nicotine withdrawal was not altering extinction. There was no baseline freezing during training in any group or difference in immediate freezing during training in any group ($p > 0.05$; data not shown).

A t-test was also conducted comparing the effects of withdrawal from chronic sodium hydrogen tartrate (M = mean percent freezing; $M = 52.5$; N = group size, $N = 8$) to withdrawal from chronic saline ($M = 43.7$; $N = 8$) and no significant differences between groups were found for freezing to the context [$t(15) = 1.54, p > 0.1$]. There was no difference in baseline or immediate freezing during training (data not shown). Given that there was no deficit associated with withdrawal from chronic sodium hydrogen tartrate, the withdrawal deficits found in the first experiment can be attributed nicotine withdrawal and not to withdrawal from the inert ingredients in nicotine hydrogen tartrate salt.

The effect of withdrawal from chronic nicotine administration on PPI

For PPI, we first tested if there are dose-dependent effects of nicotine withdrawal on PPI at 24 hours. A 3×3 repeated-measures ANOVA with dB (69, 73, 81) as the within subjects factor and drug condition (0.0, 6.3, 12.6 mg/kg/day) as the between subjects factor revealed that there was a significant within-subjects effect of pre-pulse intensity level on PPI [$F(2, 35) = 35.06, p < 0.01$]; higher dBs produced greater PPI. However, there was no significant overall effect of drug condition on PPI, nor was there a drug condition by pre-pulse intensity interaction ($p > 0.05$; Figure 2).

We next examined the effects of withdrawal from the 12.6 mg/kg dose of nicotine at 8 hours, 24 hours, and 48 hours post-withdrawal with separate ANOVAs. A 3×2 repeated-measures ANOVA revealed that, at 8 hours following pump removal, there was a significant within-subjects effect of pre-pulse intensity level on PPI [$F(1,30) = 78.87, p < 0.001$], such that higher dBs produced greater PPI, but there was no effect of drug condition nor was there a drug

condition by pre-pulse intensity interaction ($p > 0.05$; Figure 3A). A second repeated-measures ANOVA revealed that, following 24 hours of nicotine withdrawal, there was a significant effect of pre-pulse intensity level on PPI [$F(1,30) = 128.57, p < 0.001$], such that higher dBs produced greater PPI; there was no significant main effect of drug condition, nor was there a drug by pre-pulse intensity interaction ($p > 0.05$; Figure 3B). Finally, a third repeated-measures ANOVA demonstrated that, after 48 hours of nicotine withdrawal, there was a significant effect of pre-pulse intensity on PPI [$F(1,30) = 108.91, p < 0.001$], such that higher dBs produced greater PPI, but there was no significant main effect of drug condition, nor was there a drug by pre-pulse intensity interaction ($p > 0.05$; Figure 3C). These results examining multiple time points suggest that nicotine withdrawal from a 12 day treatment of 12.6 mg/kg/day nicotine does not disrupt PPI in C57BL/6 mice.

The effect of withdrawal from chronic nicotine administration on the acoustic startle response

An 8×3 repeated measures ANOVA revealed that there was a significant within-subjects effect of startle stimulus intensity level on startle [$F(7,30) = 55.37, p < 0.001$]; higher dBs produced greater startle. However, there was no significant overall effect of drug condition on startle nor was there a drug condition by startle stimulus intensity interaction ($p > 0.05$; Figure 4). In order to detect potential differences that may not have been identified in the ANOVA, sign tests that compared mice withdrawn from both doses of chronic nicotine to saline treated mice were conducted; no differences were found between groups ($p > 0.05$). When examining mice withdrawn from the 12.6 mg/kg dose at different time points, 8×2 repeated-measure ANOVAs revealed that at 8 hours [$F(7,24) = 72.19, p < 0.001$], 24 hours [$F(7,24) = 86.64, p < 0.001$], and 48 hours [$F(7,24) = 87.05, p < 0.001$] after nicotine withdrawal there was a significant within-subjects effect of startle stimulus intensity level on startle with greater startle seen at higher dBs, but no effect of drug ($p > 0.05$) nor was there a drug condition by startle stimulus intensity interaction ($p > 0.1$; Figure 5). Sign tests that compared mice withdrawn from chronic nicotine and saline also found no differences at 8 hours and 24 hours after nicotine withdrawal ($p > 0.05$). However, sign tests revealed a significant difference between saline and nicotine-withdrawn mice at 48 hours after pump removal in 100 dB startle trials ($p < 0.05$).

Discussion

The present study demonstrated that nicotine withdrawal-associated deficits in contextual fear conditioning occur regardless of whether the context is a background or a foreground stimulus. However, withdrawal from doses of nicotine in the range of those that impair contextual fear conditioning does not produce deficits in PPI. The present study did not find any deficits in PPI in C57BL/6 mice withdrawn for 24 hours from 6.3 mg/kg/day of chronic nicotine; there were also no PPI deficits in mice withdrawn for 8, 24, or 48 hours from 12.6 mg/kg/day of chronic nicotine. These results are in agreement with findings from Jonkman and colleagues [42], who report that withdrawal from 14 days of either 24 or 48 mg/kg/day nicotine did not disrupt PPI in either C57BL/6 mice or DBA/2 mice. Thus, in the combined results between our study and the Jonkman and colleagues study [42], a full dose-response range for the effects of nicotine withdrawal on PPI has been addressed in C57BL/6 mice. However, DBA/2J mice may be sensitive to the effects of withdrawal from lower doses of nicotine. Semenova and colleagues [72] found that DBA/2J mice withdrawn from 14 days of 6.3 mg/kg/day nicotine showed deficits in PPI. Thus, because we saw no deficits at the 6.3 mg/kg/day dose in C57BL/6J mice, but Semenova and colleagues [72] saw an effect at this dose in DBA/2J mice, this suggests that there may be genetic differences in the effects of withdrawal from a lower dose of nicotine on PPI.

The effect of withdrawal from chronic nicotine on the acoustic startle response was also assessed in the present study. Withdrawal from chronic nicotine administration did not lead to any changes in the auditory startle response, in agreement with other findings in mice [42, 72]. However, Helton and colleagues [34] found that, in Long-Evans rats, nicotine withdrawal led to an increase in the auditory startle response. Therefore, the effects of nicotine withdrawal on startle response may be species specific and/or may be related to procedural and drug dose differences.

The specificity of nicotine withdrawal-associated deficits for foreground and background contextual fear conditioning, but not PPI, suggests that nicotine withdrawal may alter neural structures or processes common to both foreground and background contextual fear conditioning, but not to PPI. Although these processes are unidentified, there are multiple substrates that could produce the differential effects of nicotine withdrawal on contextual fear conditioning and PPI. Potential candidates include hippocampal substrates involved in each task, genetic differences, nicotinic acetylcholinergic receptor (nAChR) subtypes involved in each task, and different underlying intracellular signaling molecules.

Although fear conditioning and PPI both depend upon the hippocampus [3,36,49,54,67,85], each task may differentially recruit subregions of the hippocampus [3,36,43,49,54,67,85]. For instance, contextual fear conditioning may depend more on the dorsal hippocampus than the ventral hippocampus [3,54,67]. In support, Lee and Kesner [49] demonstrated that all three subregions of the dorsal hippocampus (CA3, CA1, and dentate gyrus) contribute to the acquisition of contextual memory. PPI, on the other hand, may be more dependent on the ventral hippocampus [36]. For example, Zhang and colleagues [85] found that infusions of NMDA into the ventral hippocampus dose-dependently disrupted PPI; these changes did not occur when NMDA was infused into the dorsal hippocampus. Thus, the disparity in the effects of withdrawal from chronic nicotine on PPI and contextual fear conditioning may be due to the regions of the hippocampus involved in each task.

Withdrawal-associated changes in the functional properties of the nAChR subtypes involved in each task may also underlie the differential effects of withdrawal from chronic nicotine on contextual fear conditioning and PPI. Previous studies have demonstrated differences in desensitization, binding affinity, and cation permeability across various nAChR subtypes [10,21,59]. Therefore, different nAChR subtypes likely mediate the diverse effects of nicotine. Furthermore, it has been demonstrated that $\beta 2$ -containing nAChRs mediate the effects of acute nicotine and withdrawal from chronic nicotine on background contextual fear conditioning [16,65]. Given that the results of the present study found nicotine withdrawal-related deficits in both foreground and background contextual conditioning, it is possible that $\beta 2$ -containing nAChRs mediate this effect in foreground contextual fear conditioning as well. In contrast, the effects of nicotine on PPI may depend more upon $\alpha 7$ nAChRs [76]. Furthermore, a study examining inbred strains of mice has demonstrated a genetic correlation between sensory gating and $\alpha 7$ nAChR binding [75]. For example, DBA mice have the lowest level of $\alpha 7$ nAChR binding and the poorest sensory gating [75]. These results suggest that both genetic differences and differences in the nAChRs involved in each task may contribute to the differences in the effects of nicotine withdrawal on each task. In support, it has been shown that fear conditioning and PPI are not genetically correlated [51,58].

Differences in cell signaling pathways involved in contextual fear conditioning versus PPI could also lead to a greater effect of nicotine withdrawal on contextual fear conditioning. For example, it may be that nicotine withdrawal disrupts contextual fear conditioning through mechanisms downstream of nAChR activation. Downstream cell signaling molecules that are both involved in learning and memory and are linked to activation of nAChRs include extracellular signal-regulated kinase (ERK) and one of its target proteins cAMP-response

element-binding protein (CREB) [for review see 31,78]. Research has demonstrated that acute nicotine increases both ERK and CREB activation in the hippocampus [37,82] and withdrawal from chronic nicotine produces decreases in ERK and CREB signaling in several brain areas, such as the nucleus accumbens and the amygdala [8,63]. However, the effects of nicotine withdrawal on ERK and CREB signaling in the hippocampus remain unknown. Studies have demonstrated that both ERK and CREB are involved in foreground and background contextual fear conditioning [70,73,81], and that the acute effects of nicotine on background contextual fear conditioning critically depend on ERK [68]. Thus, withdrawal-associated changes in these signaling molecules could disrupt contextual fear conditioning. In contrast, these cell signaling molecules may not be critically involved in PPI; Falls and colleagues [20] found that PPI is not disrupted in CREB^{ΔΔ} mice. Thus, disruption of cell signaling via changes in ERK and/or CREB is one potential mechanism by which nicotine withdrawal could alter contextual fear conditioning without altering PPI.

Finally, studies testing for differences in levels of conditioning between foreground and background contextual fear conditioning have met with variable results. Some studies have shown that foreground conditioning results in stronger conditioning [62,77], while other studies have not found this effect [15,5,43,51,60,61]. Although no differences in freezing to the context were found between background and foreground conditioning in the current study, previous work in our lab has demonstrated that there are differences in the effects of foreground and background conditioning that are visible at one week, but not at 24 hours, post-training [32]. This and other evidence suggests that differences do exist in the neural mechanisms that underlie foreground and background contextual fear conditioning. However, these differences do not impact the effects of nicotine withdrawal on foreground and background conditioning, as both are similarly affected.

In summary, the results of the present study found that nicotine withdrawal has differential effects on learning and on sensory gating, as measured by contextual fear conditioning and PPI, respectively, in C57BL/6 mice. Nicotine withdrawal impaired contextual fear conditioning whether the context was a foreground or background stimulus; however, withdrawal deficits were not seen in PPI. This study suggests that nicotine withdrawal produces selective deficits in cognition, and expression of the various withdrawal symptoms may be influenced by multiple factors that could include genetics and nicotine treatment parameters. These results also suggest that further studies examining the influence of genetics and pharmacodynamics on nicotine withdrawal symptoms are warranted in order to advance our understanding of nicotine addiction and in order to find effective treatments for the many smokers who attempt to quit but ultimately relapse.

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References

1. World Health Statistics 2006. World Health Organization; 2006.
2. Acri JB, Morse DE, Popke EJ, Grunberg NE. Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. *Psychopharmacology* 1994;114:369–374. [PubMed: 7838931]
3. Bast T, Zhang WN, Feldon J. Dorsal hippocampus and classical fear conditioning to tone and context in rats: effects of local NMDA-receptor blockade and stimulation. *Hippocampus* 2003;13:657–675. [PubMed: 12962312]
4. Bell SL, Taylor RC, Singleton EG, Henningfield JE, Heishman SJ. Smoking after nicotine deprivation enhances cognitive performance and decreases tobacco craving in drug abusers. *Nicotine Tob Res* 1999;1:45–52. [PubMed: 11072387]

5. Blanchard RJ, Fial RA. Effects of limbic lesions on passive avoidance and reactivity to shock. *Journal of comparative and physiological psychology* 1968;66:606–612. [PubMed: 5721489]
6. Blanchard RJ, Blanchard DC. Crouching as an index of fear. *Journal of comparative and physiological psychology* 1969;67:370–375. [PubMed: 5787388]
7. Brown JS, Kalish HI, Farber IE. Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *Journal of experimental psychology* 1951;41:317–328. [PubMed: 14861383]
8. Brunzell DH, Russell DS, Picciotto MR. In vivo nicotine treatment regulates mesocorticolimbic CREB and ERK signaling in C57Bl/6J mice. *Journal of neurochemistry* 2003;84:1431–1441. [PubMed: 12614343]
9. CDC. Tobacco use among adults-United States, 2005. *Morb Mortal Weekly Report*. 55. 2006. p. 1145–1148.
10. Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. Nicotinic receptor function: new perspectives from knockout mice. *Trends in pharmacological sciences* 2000;21:211–217. [PubMed: 10838608]
11. Corrigan WA. Nicotine self-administration in animals as a dependence model. *Nicotine Tob Res* 1999;1:11–20. [PubMed: 11072385]
12. Crusio WE, Genthner-Grimm G, Schwegler H. A quantitative-genetic analysis of hippocampal variation in the mouse. *Journal of neurogenetics* 1986;3:203–214. [PubMed: 3746523]
13. Davis JA, James JR, Siegel SJ, Gould TJ. Withdrawal from chronic nicotine administration impairs contextual fear conditioning in C57BL/6 mice. *J Neurosci* 2005;25:8708–8713. [PubMed: 16177040]
14. Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology* 2006;184:345–352. [PubMed: 15988571]
15. Davis JA, Porter J, Gould TJ. Nicotine enhances both foreground and background contextual fear conditioning. *Neuroscience letters* 2006;394:202–205. [PubMed: 16260086]
16. Davis JA, Gould TJ. beta2 subunit-containing nicotinic receptors mediate the enhancing effect of nicotine on trace cued fear conditioning in C57BL/6 mice. *Psychopharmacology* 2007;190:343–352. [PubMed: 17136517]
17. Della Casa V, Hofer I, Weiner I, Feldon J. The effects of smoking on acoustic prepulse inhibition in healthy men and women. *Psychopharmacology* 1998;137:362–368. [PubMed: 9676896]
18. Domino EF, Kishimoto T. Tobacco smoking increases gating of irrelevant and enhances attention to relevant tones. *Nicotine Tob Res* 2002;4:71–78. [PubMed: 11906683]
19. Duncan E, Madonick S, Chakravorty S, Parwani A, Szilagyi S, Efferen T, et al. Effects of smoking on acoustic startle and prepulse inhibition in humans. *Psychopharmacology* 2001;156:266–272. [PubMed: 11549228]
20. Falls WA, Kogan JH, Silva AJ, Willott JF, Carlson S, Turner JG. Fear-potentiated startle, but not prepulse inhibition of startle, is impaired in CREB α mutant mice. *Behavioral neuroscience* 2000;114:998–1004. [PubMed: 11085615]
21. Fenster CP, Rains MF, Noerager B, Quick MW, Lester RA. Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. *J Neurosci* 1997;17:5747–5759. [PubMed: 9221773]
22. Fordyce DE, Wehner JM. Determination of hippocampal protein kinase C using a frozen tissue method: comparison of synaptosomal and total activity in C57BL/6 and DBA/2 mice. *Biochemical and biophysical research communications* 1992;188:690–694. [PubMed: 1445314]
23. Geyer MA, Braff DL. Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophrenia bulletin* 1987;13:643–668. [PubMed: 3438708]
24. Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. *Behavioural brain research* 1999;102:31–39. [PubMed: 10403013]
25. Gould TJ, Lommock JA. Nicotine enhances contextual fear conditioning and ameliorates ethanol-induced deficits in contextual fear conditioning. *Behavioral neuroscience* 2003;117:1276–1282. [PubMed: 14674846]
26. Gould TJ, Stephen Higgins J. Nicotine enhances contextual fear conditioning in C57BL/6J mice at 1 and 7 days post-training. *Neurobiology of learning and memory* 2003;80:147–157. [PubMed: 12932430]

27. Gould TJ. Nicotine produces a within-subject enhancement of contextual fear conditioning in C57BL/6 mice independent of sex. *Integr Physiol Behav Sci* 2003;38:124–132. [PubMed: 14527181]
28. Gould TJ, Bizily SP, Tokarczyk J, Kelly MP, Siegel SJ, Kanen SJ, et al. Sensorimotor gating deficits in transgenic mice expressing a constitutively active form of Gs alpha. *Neuropsychopharmacology* 2004;29:494–501. [PubMed: 14694347]
29. 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;155:167–173. [PubMed: 15325790]
30. Gould TJ, Rukstalis M, Lewis MC. Atomoxetine and nicotine enhance prepulse inhibition of acoustic startle in C57BL/6 mice. *Neuroscience letters* 2005;377:85–90. [PubMed: 15740842]
31. Gould TJ. Nicotine and hippocampus-dependent learning: implications for addiction. *Molecular neurobiology* 2006;34:93–107. [PubMed: 17220532]
32. Gulick D, Gould TJ. Interactive effects of ethanol and nicotine on learning in C57BL/6J mice depend on both dose and duration of treatment. *Psychopharmacology*. 2007
33. Heimrich B, Schwegler H, Crusio WE. Hippocampal variation between the inbred mouse strains C3H/HeJ and DBA/2: a quantitative-genetic analysis. *Journal of neurogenetics* 1985;2:389–401. [PubMed: 4078651]
34. Helton DR, Modlin DL, Tizzano JP, Rasmussen K. Nicotine withdrawal: a behavioral assessment using schedule controlled responding, locomotor activity, and sensorimotor reactivity. *Psychopharmacology* 1993;113:205–210. [PubMed: 7855182]
35. Henningfield JE, Keenan RM. Nicotine delivery kinetics and abuse liability. *Journal of consulting and clinical psychology* 1993;61:743–750. [PubMed: 8245272]
36. Howland JG, MacKenzie EM, Yim TT, Taepavarapruk P, Phillips AG. Electrical stimulation of the hippocampus disrupts prepulse inhibition in rats: frequency- and site-dependent effects. *Behavioural brain research* 2004;152:187–197. [PubMed: 15196786]
37. Hu M, Liu QS, Chang KT, Berg DK. Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways. *Molecular and cellular neurosciences* 2002;21:616–625. [PubMed: 12504594]
38. Hughes JR, Gust SW, Skoog K, Keenan RM, Fenwick JW. Symptoms of tobacco withdrawal. A replication and extension. *Archives of general psychiatry* 1991;48:52–59. [PubMed: 1984762]
39. Hughes JR, Higgins ST, Bickel WK. Nicotine withdrawal versus other drug withdrawal syndromes: similarities and dissimilarities. *Addiction (Abingdon, England)* 1994;89:1461–1470.
40. Ison JR, McAdam DW, Hammond GR. Latency and amplitude changes in the acoustic startle reflex of the rat produced by variation in auditory prestimulation. *Physiology & behavior* 1973;10:1035–1039. [PubMed: 4717640]
41. Jacobsen LK, Krystal JH, Mencl WE, Westerveld M, Frost SJ, Pugh KR. Effects of smoking and smoking abstinence on cognition in adolescent tobacco smokers. *Biological psychiatry* 2005;57:56–66. [PubMed: 15607301]
42. Jonkman S, Henry B, Semenova S, Markou A. Mild anxiogenic effects of nicotine withdrawal in mice. *European journal of pharmacology* 2005;516:40–45. [PubMed: 15922326]
43. Kim JJ, Rison RA, Fanselow MS. Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral neuroscience* 1993;107:1093–1098. [PubMed: 8136063]
44. Kleinman KM, Vaughn RL, Christ TS. Effects of cigarette smoking and smoking deprivation on paired-associate learning of high and low meaningful nonsense syllables. *Psychological reports* 1973;32:963–966. [PubMed: 4704781]
45. Kumari V, Checkley SA, Gray JA. Effect of cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. *Psychopharmacology* 1996;128:54–60. [PubMed: 8944406]
46. Kumari V, Gray JA. Smoking withdrawal, nicotine dependence and prepulse inhibition of the acoustic startle reflex. *Psychopharmacology* 1999;141:11–15. [PubMed: 9952059]
47. Leaton R, Borszcz G. Potentiated startle: its relation to freezing and shock intensity in rats. *J Exp Psychol Anim Behav Process* 1985;11:421–428.
48. LeDoux JE, Sakaguchi A, Reis DJ. Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. *J Neurosci* 1984;4:683–698. [PubMed: 6707732]

49. Lee I, Kesner RP. Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. *Hippocampus* 2004;14:301–310. [PubMed: 15132429]
50. Leshner A. Addiction research can provide scientific solutions to the problem of cigarette smoking. NIDA. 1998
51. Logue SF, Paylor R, Wehner JM. Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behavioral neuroscience* 1997;111:104–113. [PubMed: 9109628]
52. Lolordo V, Williams D, McPhee J. Overshadowing of situational cues with variable but not fixed intertrial intervals. *Anim Learn Behav* 2001;29:143–152.
53. Mansvelder HD, De Rover M, McGehee DS, Brussaard AB. Cholinergic modulation of dopaminergic reward areas: upstream and downstream targets of nicotine addiction. *European journal of pharmacology* 2003;480:117–123. [PubMed: 14623355]
54. Matus-Amat P, Higgins EA, Barrientos RM, Rudy JW. The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *J Neurosci* 2004;24:2431–2439. [PubMed: 15014118]
55. McIlvain H, Susman JL, Davis C, Gilbert C. Physician counseling for smoking cessation: is the glass half empty? *The Journal of family practice* 1995;40:148–152. [PubMed: 7852938]
56. Odling-Smee FJ. Background stimuli and the inter-stimulus interval during Pavlovian conditioning. *The Quarterly journal of experimental psychology* 1975;27:387–392. [PubMed: 1196588]
57. Odling-Smee FJ. The overshadowing of background stimuli: some effects of varying amounts of training and UCS intensity. *The Quarterly journal of experimental psychology* 1978;30:737–746. [PubMed: 734041]
58. Owen EH, Logue SF, Rasmussen DL, Wehner JM. Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: implications of genetic background for single gene mutations and quantitative trait loci analyses. *Neuroscience* 1997;80:1087–1099. [PubMed: 9284062]
59. Papke RL, Sanberg PR, Shytle RD. Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. *The Journal of pharmacology and experimental therapeutics* 2001;297:646–656. [PubMed: 11303054]
60. Paylor R, Tracy R, Wehner J, Rudy JW. DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. *Behavioral neuroscience* 1994;108:810–817. [PubMed: 7986374]
61. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral neuroscience* 1992;106:274–285. [PubMed: 1590953]
62. Phillips, RG.; LeDoux, JE. Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. 1. Cold Spring Harbor, NY: Learning & memory; 1994. p. 34–44.
63. Pluzarev O, Pandey SC. Modulation of CREB expression and phosphorylation in the rat nucleus accumbens during nicotine exposure and withdrawal. *Journal of neuroscience research* 2004;77:884–891. [PubMed: 15334606]
64. Portugal GS, Gould TJ. Bupropion dose-dependently reverses nicotine withdrawal deficits in contextual fear conditioning. *Pharmacology, biochemistry, and behavior* 2007;88:179–187.
65. Portugal GS, Kenney JW, Gould TJ. beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiology of learning and memory* 2008;89:106–113. [PubMed: 17584502]
66. Postma P, Kumari V, Sharma T, Hines M, Gray JA. Startle response during smoking and 24 h after withdrawal predicts successful smoking cessation. *Psychopharmacology* 2001;156:360–367. [PubMed: 11549236]
67. Quinn JJ, Loya F, Ma QD, Fanselow MS. Dorsal hippocampus NMDA receptors differentially mediate trace and contextual fear conditioning. *Hippocampus* 2005;15:665–674. [PubMed: 15959918]
68. Raybuck JD, Gould TJ. Extracellular signal-regulated kinase 1/2 involvement in the enhancement of contextual fear conditioning by nicotine. *Behavioral neuroscience* 2007;121:1119–1124. [PubMed: 17907844]

69. Rescorla, R.; Wagner, A. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Black; Prokasy, editors. *Classical Conditioning II: Current Research and Theory*. New York: Appleton-Century-Crofts; 1972. p. 64-99.
70. Sananbenesi F, Fischer A, Schrick C, Spiess J, Radulovic J. Phosphorylation of hippocampal Erk-1/2, Elk-1, and p90-Rsk-1 during contextual fear conditioning: interactions between Erk-1/2 and Elk-1. *Molecular and cellular neurosciences* 2002;21:463–476. [PubMed: 12498787]
71. Schopke R, Wolfer DP, Lipp HP, Leisinger-Trigona MC. Swimming navigation and structural variations of the infrapyramidal mossy fibers in the hippocampus of the mouse. *Hippocampus* 1991;1:315–328. [PubMed: 1669312]
72. Semenova S, Bespalov A, Markou A. Decreased prepulse inhibition during nicotine withdrawal in DBA/2J mice is reversed by nicotine self-administration. *European journal of pharmacology* 2003;472:99–110. [PubMed: 12860478]
73. Shalin SC, Zirrgiebel U, Honsa KJ, Julien JP, Miller FD, Kaplan DR, et al. Neuronal MEK is important for normal fear conditioning in mice. *Journal of neuroscience research* 2004;75:760–770. [PubMed: 14994337]
74. Snyder FR, Davis FC, Henningfield JE. The tobacco withdrawal syndrome: performance decrements assessed on a computerized test battery. *Drug and alcohol dependence* 1989;23:259–266. [PubMed: 2752917]
75. Stevens KE, Freedman R, Collins AC, Hall M, Leonard S, Marks MJ, et al. Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. *Neuropsychopharmacology* 1996;15:152–162. [PubMed: 8840351]
76. Stevens KE, Kem WR, Mahnir VM, Freedman R. Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology* 1998;136:320–327. [PubMed: 9600576]
77. Stiedl O, Palve M, Radulovic J, Birkenfeld K, Spiess J. Differential impairment of auditory and contextual fear conditioning by protein synthesis inhibition in C57BL/6N mice. *Behavioral neuroscience* 1999;113:496–506. [PubMed: 10443777]
78. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *Journal of neurochemistry* 2001;76:1–10. [PubMed: 11145972]
79. Swerdlow NR, Geyer MA. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophrenia bulletin* 1998;24:285–301. [PubMed: 9613626]
80. Tarantino LM, Gould TJ, Druhan JP, Bucan M. Behavior and mutagenesis screens: the importance of baseline analysis of inbred strains. *Mamm Genome* 2000;11:555–564. [PubMed: 10886023]
81. Trifilieff, P.; Herry, C.; Vanhoutte, P.; Caboche, J.; Desmedt, A.; Riedel, G., et al. Foreground contextual fear memory consolidation requires two independent phases of hippocampal ERK/CREB activation. 13. Cold Spring Harbor, NY: *Learning & memory*; 2006. p. 349-358.
82. Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *The European journal of neuroscience* 2004;19:1826–1836. [PubMed: 15078556]
83. Wehner JM, Sleight S, Upchurch M. Hippocampal protein kinase C activity is reduced in poor spatial learners. *Brain research* 1990;523:181–187. [PubMed: 2400904]
84. Xu J, Mendrek A, Cohen MS, Monterosso J, Simon S, Brody AL, et al. Effects of acute smoking on brain activity vary with abstinence in smokers performing the N-Back task: a preliminary study. *Psychiatry research* 2006;148:103–109. [PubMed: 17088048]
85. Zhang WN, Bast T, Feldon J. Prepulse inhibition in rats with temporary inhibition/inactivation of ventral or dorsal hippocampus. *Pharmacology, biochemistry, and behavior* 2002;73:929–940.

Figure 1.

Fear conditioning results. Twenty-four hours of withdrawal from 6.3 mg/kg/day chronic nicotine produced deficits in freezing to the context when the context was either a foreground (A) and background (B) stimuli. There was no baseline freezing in any groups (Data not shown). Data are shown as mean $\pm$ SEM. * = $p < 0.05$. Foreground: Saline N = 15, Nicotine N = 13; Background: N = 14 for both groups.

Figure 2.

Dose-response analysis for withdrawal from chronic nicotine on PPI. There were no significant effects of 24 hours of withdrawal from chronic administration of 6.3 or 12.6 mg/kg/day nicotine on PPI. Data are shown as mean $\pm$ SEM. Saline N = 14, 6.3 mg/kg/day nicotine N = 14, 12.6 mg/kg/day nicotine. N = 10.

Figure 3.

Time-response analysis for withdrawal from chronic nicotine on PPI. There were no significant effects of withdrawal from chronic administration of the 12.6 mg/kg/day dose of nicotine on PPI measured at A) 8 hours B) 24 hours or C) 48 hours post withdrawal. Data are shown as mean $\pm$ SEM. N = 16 for all groups.

Figure 4.

Dose-response analysis for withdrawal from chronic nicotine on the acoustic startle response. There were no significant effects of 24 hours of withdrawal from chronic administration of 6.3 or 12.6 mg/kg/day nicotine on startle. Data are shown as mean $\pm$ SEM reported in San Diego Instruments' units reflecting maximum voltage change (V MAX). Saline N = 14, 6.3 mg/kg/day nicotine N = 14, 12.6 mg/kg/day nicotine N = 10.

Figure 5.

Time-response analysis for withdrawal from chronic nicotine on the acoustic startle response. There were no significant effects of withdrawal from chronic administration of 12.6 mg/kg of nicotine on startle measured at A) 8 hours, B) 24 hours or C) 48 hours post withdrawal. Data are shown as mean $\pm$ SEM reported in San Diego Instruments' units reflecting maximum voltage change (V MAX). N = 16 for all groups.