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Strain-dependent Effects of Acute, Chronic, and Withdrawal from Chronic Nicotine on Fear Conditioning

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Abstract

The effects of nicotine on cognitive processes such as learning and memory may play an important role in the addictive liability of tobacco. However, it remains unknown whether genetic variability modulates the effects of nicotine on learning and memory. The present study characterized the effects of acute, chronic, and withdrawal from chronic nicotine administration on fear conditioning, somatic signs, and the elevated plus maze in 8 strains of inbred mice. Strain-dependent effects of acute nicotine and nicotine withdrawal on contextual fear conditioning, somatic signs, and the elevated plus maze were observed, but no association between the effects of acute nicotine and nicotine withdrawal on contextual fear conditioning were observed, suggesting that different genetic substrates may mediate these effects. The identification of genetic factors that may alter the effects of nicotine on cognition may lead to more efficacious treatments for nicotine addiction.

Keywords

Nicotine; Genetics; Addiction; Anxiety; Learning; Withdrawal

Introduction

Despite overwhelming evidence of the harmful effects of tobacco use, only a small percentage of smokers successfully quit smoking (McIlvain et al. 1995; Lee and Kahende 2007). Furthermore, current treatments for smoking cessation only produce a small increase in the likelihood of successfully quitting, which highlights the need for more effective strategies that facilitate smoking cessation (Cahill et al. 2007; Hughes et al. 2007; Silagy et al. 2004). An improved understanding of the genetic factors that alter risk for becoming addicted may aid in the development of new treatments for tobacco use. Research focusing on nicotine, the primary addictive component of tobacco (Benowitz 1988), has demonstrated that genetic variability can alter sensitivity to nicotine. Heritability studies in humans have

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demonstrated that genetic factors account for a large percentage of variability in smoking-related behaviors such as smoking initiation (~50–60%), nicotine dependence (~60–76%) and smoking cessation (~54–57%) (Li et al. 2003; Sullivan and Kendler 1999; Haberstick et al. 2007; Xian et al. 2003; Hardie et al. 2006). Furthermore, candidate gene and genome-wide association studies have identified single nucleotide polymorphisms (SNPs) in genes that encode dopamine receptors, nicotinic acetylcholine receptors (nAChRs), serotonin receptors, and proteins involved in nicotine metabolism that were associated with smoking phenotypes (see Ho and Tyndale 2007; Portugal and Gould 2008 for review). These data suggest that a wide array of genes are involved in determining sensitivity to the effects of nicotine, but there is much that remains unknown about the genetic factors that alter risk for nicotine addiction.

One approach to identify genetic factors that alter nicotine sensitivity is to characterize the behavioral effects of nicotine in inbred mice. Given that mice within an inbred strain are genetically identical to each other after twenty generations of breeding (Lyon and Searle 1989), differences in the behavioral effects of nicotine between two or more strains would indicate that variability in genetics can influence nicotine sensitivity. The effects of nicotine on physiological measures such as respiration, heart rate, and locomotor activity differ between multiple strains of inbred mice, and strain-dependent differences in tolerance to the effects of nicotine on physiological measures have also been reported (Collins et al. 1988; Marks et al. 1989a, 1991). Additional studies have identified between-strain differences in the effects of nicotine on reward, conditioned place preference, and antinociception (Robinson et al. 1996; Jackson et al. 2008; Damaj et al. 2007; Grabus et al. 2006). Furthermore, Marks and colleagues (1989b) have demonstrated that binding sites for nicotine vary between strains, suggesting that genetic variability may alter the functional properties of nAChRs. Together these data reveal that genetic variability can influence sensitivity to the behavioral effects of nicotine, but the genes that regulate these effects remain unknown. Furthermore, many of the behavioral effects of nicotine have not been studied in multiple inbred strains.

The characterization of strain differences in the behavioral effects of nicotine can be used in the development of subsequent quantitative trait loci (QTL) studies. QTL studies in mice have identified chromosomal loci associated with some behavioral effects of nicotine. A study using recombinant congenic strains (RCS) that were derived from C57BL/6J and A/J progenitor strains found loci on chromosomes 2, 7, 8, 11–14, 16 and 17 that were associated with the locomotor enhancing effects of nicotine (Gill and Boyle 2005). These QTL were tested in a follow-up study; the association between the locomotor activating effects of nicotine and loci on chromosomes 2, 11, 14, 16, and 17 was replicated using an F2 cross and/or chromosomal substitution strains derived from C57BL/6J and A/J mice (Boyle and Gill 2009). Furthermore, Li and colleagues (2007) used an F2 cross between C57BL/6J and C3H/HeJ mice to identify loci on chromosomes 1, 4, 7, and 15 that were associated with oral nicotine consumption. Interestingly, the QTL on chromosome 1 identified in this study is syntenic with chromosome 1 in humans and has been associated with smoking quantity (Wang et al. 2005). Thus, QTL studies have identified regions of the genome that are associated with the effects of nicotine on locomotor activity and nicotine consumption. Beyond the studies mentioned, few studies to date have used this approach in mice to identify chromosomal regions associated with the behavioral effects of nicotine.

Data from strain studies can also be informative for the development of genetically modified mice. Researchers developing new lines of genetically modified mice will use inbred mice or F1 hybrids that have high rates of fertilization and that have a greater number of implanted embryos that survive until birth (Auerbach et al. 2003). Although these factors are important to consider when developing genetically modified mice, other factors such as

genetic background should also be considered. For example, the behavioral effects of nicotine can be tested in knockout (KO) mice that lack a target gene, or in knockin (KI) mice that have had a target gene inserted that alters the function of the target gene. However, these data would be difficult to interpret if nicotine produced no change in behavior in wild-type mice. Thus, data from strain studies can indicate which strains and F1 hybrids show the greatest and least sensitivity to the effects of nicotine; these data can be used to select an inbred strain for developing new lines of genetically modified mice that are optimal for investigating genes that alter sensitivity to nicotine.

Although some behavioral effects of nicotine have been characterized in strain studies, it remains unknown whether genetic variability can alter the effects of nicotine on cognitive processes such as associative learning. Studies using C57BL/6J mice have demonstrated that nicotine alters contextual learning in Pavlovian fear conditioning. Acute nicotine enhances contextual fear conditioning but has no effect on cued fear conditioning (Davis et al. 2005, 2006, 2007; Gould and Higgins 2003; Gould and Wehner 1999; Wehner et al. 2004). However, Davis and colleagues (2005) reported that chronic nicotine does not alter fear conditioning, even though the dose chosen in this study produced equivalent plasma nicotine levels as an acute dose that enhanced contextual fear conditioning. Lastly, withdrawal from chronic nicotine impairs contextual but not cued fear conditioning (Davis et al. 2005; Davis and Gould 2007a; Portugal and Gould 2007; Portugal et al. 2008; André et al. 2008; Davis and Gould 2009). Although these effects have been well characterized in C57BL/6J mice, it remains unknown whether differences in genetics can alter sensitivity to the effects of nicotine on fear conditioning. Therefore, the present study investigated the effects of acute, chronic, and withdrawal from chronic nicotine on fear conditioning in eight inbred strains of mice; differences in the effects of nicotine between strains would reveal that genetic factors influence the effects of nicotine on fear conditioning. Nicotine withdrawal can produce multiple symptoms such as increased somatic symptoms (e.g. changes in grooming, scratching, or shaking), and increased anxiety that can be studied using the elevated plus maze (Jackson et al. 2008; Damaj et al. 2003). It is possible that different genetic substrates may alter the severity of each withdrawal-related change in behavior. Thus, the present study also investigated the effects of chronic nicotine and nicotine withdrawal on the elevated plus maze and somatic symptoms in 8 inbred strains of mice.

Methods

Subjects

Male A/J (A), BALB/cByJ (BALB), C3H/HeJ (C3H), C57BL/6J (B6), CBA/J (CBA), DBA/1J (D1), and DBA/2J (D2) mice were obtained from Jackson Laboratory (Bar Harbor, ME). 129/SvEv (129) mice were obtained from Taconic Farms (Germantown, NY). All mice were 8–12 weeks of age at the time of training and testing. These inbred mouse strains were selected because fear conditioning has been previously demonstrated in these strains (Balogh and Wehner 2003; Bolivar et al. 2001; Owen et al. 1997). Mice were maintained on a 12 h light/dark cycle (lights on at 7:00 a.m.) and were provided with unlimited access to food and water. All behavioral and surgical procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus

Mice were trained and tested in contextual fear conditioning in four identical conditioning chambers (17.8 × 19.1 × 38.1 cm) housed in sound attenuating boxes (Med Associates, St. Albans, VT). The chambers had a stainless steel grid floor that consisted of 18 metal rods, which were connected to a shock generator and scrambler. The side walls of each chamber were made of stainless steel and all other walls were made of clear Plexiglas. Speakers

mounted on the right wall of each chamber were used to administer a white noise conditioned stimulus (CS). Ventilation fans attached to the right wall of each sound attenuating box provided air exchange and background noise (69 dB). A computer using MED-PC software (Med-Associates, St. Albans, VT) was connected to each chamber and controlled stimulus administration. The testing of cued fear conditioning occurred in four identical altered chambers (20.3 × 22.9 × 17.8 cm) housed in sound attenuating boxes that are located in a different room from the training chambers. Each chamber had aluminum side walls, and all other walls were made of clear Plexiglas. Chamber floors were made of white plastic. Speakers mounted on the left wall of each chamber were used to administer the white noise CS, and ventilation fans attached to the sound attenuating box provided 69 dB background noise. A vanilla extract olfactory cue was added prior to testing to further distinguish these chambers from the training chambers.

The effects of nicotine withdrawal on anxiety were tested with an elevated plus-maze constructed of gray Plexiglas. Two open arms (7.6 × 30.6 cm), two closed arms (7.6 × 30.6 × 15.5 cm), and a center platform (7.6 × 7.6 cm) were elevated off the floor by a wooden platform measuring 62.8 cm in height. A 300 lux florescent ceiling light illuminated the room, and a solution of 70% ethanol was used to clean the elevated plus maze after the end of each session. Mice were tested for somatic signs during nicotine withdrawal in a clean cage made of clear plastic that was filled with bedding. The cage was placed on a counter and somatic signs were recorded in the same room that was used for the elevated plus maze test.

Behavioral procedures: fear conditioning

The procedure for fear conditioning was identical to those described in previous studies (Gould and Wehner 1999; Gould et al. 2004). Briefly, freezing was defined as the lack of all movement other than respiration (Blanchard and Blanchard 1969) and was scored using a time-sampling procedure in which mice were observed during 10 s intervals and were scored as either freezing or active (Gould and Wehner 1999). Training began with the activation of the house light and baseline activity was scored for 120 s. Next, a 85 dB white noise CS (30 s) was activated and co-terminated with a 0.57 mA footshock unconditioned stimulus (US; 2 s). Immediate freezing was scored for 120 s after the first CS-US pairing, and was followed by a second CS-US pairing. The training session ended with a 30 s interval during which freezing behavior was not recorded. The testing of contextual and cued fear conditioning occurred 24 h later. To test for contextual fear conditioning, mice were returned to the training chambers and freezing was scored for 5 min. Generalized freezing and cued fear conditioning was tested 1 h later in the altered context. Mice were placed in the altered context chambers and generalized freezing was scored for the first 3 min. After generalized freezing was scored, the CS was activated for 3 min and cued fear conditioning was scored during this period.

Somatic signs during nicotine withdrawal—Mice were tested for somatic signs of nicotine withdrawal using a procedure that has found increased symptoms in C57BL/6J mice withdrawn from 24 and 48 mg/kg/d nicotine (Damaj et al. 2003). Mice were placed in a home cage and somatic signs of nicotine withdrawal were recorded for 20 min. Consistent with previous literature (Damaj et al. 2003; Salas et al. 2004, 2007), somatic signs were recorded by counting each instance of the following behaviors: grooming, scratching, shaking, retropulsion, jumping, writhing, and head nodding. The total number of somatic signs was also calculated for each mouse. Video recordings of each session were made and were scored manually.

Elevated plus maze—To examine the effects of nicotine withdrawal on the elevated plus maze, mice were placed in the center portion of the maze and the total time spent in all parts of the maze (closed arms, open arms, and center) was recorded over a 5 min period. This procedure has been used in previous studies that reported increases in anxiety, as measured by decreased time spent in the open arms of the elevated plus maze, following withdrawal from 24 and 48 mg/kg/d nicotine (Jackson et al. 2008; Damaj et al. 2003). An entry into an arm was defined as instances where the mouse's head had entered an arm. Consistent with previous studies (Jackson et al. 2008; Damaj et al. 2003), increases in anxiety were measured as decreased time spent in the open arms of the elevated plus maze. However, increased time in the closed arms of the maze was also interpreted as increased anxiety because mice have limited access to any ledges in this area of the maze. Video recordings of each session were made and scored manually with the assistance of a custom-made computer program.

Drug administration and experimental design

For all experiments, nicotine hydrogen tartrate salt (Sigma Co., St. Louis, MO) was dissolved in physiological saline and administered via an intraperitoneal (i.p.) injection or by mini-osmotic pump (model 1002; Alzet, Cupertino, CA). All doses of nicotine are reported in freebase weight.

The effects of acute nicotine on fear conditioning—To investigate the effects of acute nicotine on contextual fear conditioning, mice were administered saline, 0.045, 0.09, or 0.18 mg/kg nicotine i.p. 5 min before both the training and testing of contextual and cued fear conditioning ($n = 8-14$ per group). The selection of acute nicotine doses was based on research demonstrating that 0.045 and 0.09 mg/kg nicotine enhances contextual fear conditioning in C57BL/6J mice (Davis et al. 2006; Gould and Higgins 2003; Portugal et al. 2008; Davis and Gould 2007b; Raybuck and Gould 2007).

The effects of nicotine withdrawal on somatic signs, elevated plus maze, and fear conditioning—To examine the effects of withdrawal from chronic nicotine on somatic signs, elevated plus maze, and fear conditioning, mini-osmotic pumps were filled with 3.0, 6.3, 12.0 mg/kg/d nicotine or saline ($n = 9-16$ per group). Pumps were inserted subcutaneously through an incision in the back of the mouse, and incisions were closed with wound clips, as previously described (Davis et al. 2005). Mice received chronic nicotine or saline treatment for 12 days. All mini-osmotic pumps were removed on day 12 and behavioral observation commenced on day 13. These doses of chronic nicotine were chosen because previous studies reported that withdrawal from 6.3 mg/kg/d chronic nicotine disrupts contextual fear conditioning in C57BL/6J mice (Davis et al. 2005; Portugal and Gould 2007; André et al. 2008; Raybuck et al. 2008). Initially, mice were evaluated for somatic signs. One hour later, withdrawal-related changes in anxiety were measured on the elevated plus maze. Next, the training of contextual and cued fear conditioning occurred 2 h later. Finally, the testing of contextual and cued fear conditioning occurred on day 14 (24 h after mice were trained).

The effects of chronic nicotine on somatic signs, elevated plus maze, and fear conditioning—Withdrawal from 12.0 mg/kg/d chronic nicotine disrupted contextual fear conditioning in five of the eight strains (see "Results" section). However, it was unclear whether this impaired contextual fear conditioning was due to chronic nicotine treatment or whether it was due to nicotine withdrawal. Thus, the effects of chronic nicotine on somatic signs, elevated plus maze, and fear conditioning were examined; mini-osmotic pumps were implanted subcutaneously and contained saline or 12.0 mg/kg/d nicotine ($n = 7-10$ per group). Mice received chronic nicotine treatment for 14 days, with behavioral training

occurring on day 13. The behavioral procedures were identical to those outlined in the nicotine withdrawal experiments above, except that mice were administered chronic nicotine or saline during somatic sign observations, elevated plus maze observations, and during the training and testing of fear conditioning.

Plasma nicotine collection

Previous research in humans has demonstrated that genetic variability in nicotine metabolism may alter risk for nicotine addiction (Pianezza et al. 1998; Schoedel et al. 2004), and differences in nicotine metabolism have been previously reported between different inbred strains of mice (Petersen et al. 1984). Given that strain-dependent differences in nicotine metabolism may influence sensitivity to the effects of nicotine on fear conditioning, an experiment was conducted to determine whether the intermediate doses of acute and chronic nicotine used in this study (0.09 mg/kg and 6.3 mg/kg/d, respectively) produced different plasma nicotine and cotinine levels between the eight inbred strains. For acute nicotine studies, mice from each inbred mouse strain were administered 0.09 mg/kg acute nicotine and blood samples were collected 10 min later. For chronic nicotine studies, mini-osmotic pumps containing 6.3 mg/kg/d nicotine were implanted subcutaneously and blood samples were collected 12 days after chronic nicotine administration.

Following collection, blood samples were transferred to lithium heparin tubes and were centrifuged to collect plasma. Plasma samples were analyzed by Dr. J. Randy James (Virginia Commonwealth University) using a Micromass Quattro II liquid chromatography/tandem mass spectrometer (LC/MS; Blue Lion Biotech, Snoqualmie, WA). The procedure for analyzing plasma nicotine was adapted from Naidong and colleagues (2001) and has been validated for selectivity, calibration model fit, sensitivity, accuracy, and precision.

Data analysis

Within-strain comparisons—Changes in fear conditioning following acute nicotine administration were assessed in each strain using one-way ANOVAs. Independent-samples t-tests were used for each strain to determine whether 12.0 mg/kg/d chronic nicotine altered fear conditioning, somatic signs, or elevated plus maze behavior. Finally, one-way ANOVAs were used to determine whether withdrawal from chronic nicotine altered fear conditioning, somatic signs, or elevated plus maze behavior within each inbred strain.

Between-strain comparisons of the effects of acute nicotine, chronic nicotine, and withdrawal from chronic nicotine—As expected, behavior in fear conditioning, somatic signs, and the elevated plus maze varied considerably between saline-treated mice from different inbred strains. Thus, in order to make comparisons of the effects of nicotine across strains, nicotine behavioral ratio scores were calculated for each strain by determining the percent change in the behavior of nicotine-treated mice relative to saline-treated mice $((\text{individual nicotine freezing score} - \text{mean saline freezing score}) / \text{mean saline freezing score} \times 100)$. Nicotine behavioral ratio scores were calculated for all groups of mice that received acute nicotine, chronic nicotine, or withdrawal from chronic nicotine. To determine whether the effects of acute, chronic, and withdrawal from chronic nicotine varied across strains, one-way ANOVAs were used to compare nicotine behavioral ratio scores from 0.045, 0.09, and 0.18 mg/kg acute nicotine, 12.0 mg/kg/d chronic nicotine, and withdrawal from 3.0, 6.3, and 12.0 mg/kg/d chronic nicotine.

Between-strain comparisons of saline-treated mice—Previous studies have demonstrated that behavioral phenotypes on tasks such as fear conditioning, acoustic startle, and Y-maze activity can vary considerably between different strains of inbred mice (Balogh and Wehner 2003; Bolivar et al. 2001; Owen et al. 1997; Logue et al. 1997). Therefore, it is

likely that between-strain differences in fear conditioning, somatic signs, and the elevated plus maze will exist that are independent of nicotine administration. One-way ANOVAs were used to compare the behavior of saline-treated mice on fear conditioning, somatic signs, and the elevated plus maze to determine between-strain differences in behavioral phenotypes for these tasks.

Between-strain comparisons of plasma nicotine and cotinine—Plasma samples were collected from each strain of mice to determine whether plasma nicotine and cotinine varied between strains. A nicotine/cotinine ratio was also calculated and one-way ANOVAs were used to make between strain comparisons of plasma nicotine, cotinine, and the nicotine/cotinine ratio. The ratio of nicotine to cotinine is used as a measure of metabolic rate, with lower nicotine/cotinine ratios indicating a more rapid metabolism of nicotine or slower metabolism of cotinine (Rao et al. 2000). Furthermore, Pearson correlations were calculated to examine whether plasma nicotine, cotinine, or nicotine/cotinine ratio were associated with any of the behavioral measures.

Genetic correlations—Genetic correlations between two behavioral measures can reveal whether a common subset of genes can influence both behaviors (Plomin et al. 1990). In a genetic correlation, the association between two behavioral measures is compared using the group means from each strain. Inbred mice are considered genetically identical after 20 consecutive generations of brother/sister mating (Committee on Standardized Genetic Nomenclature for Mice 1989); thus, group means are used instead of individual scores because the variability within each strain is due to environmental factors and not due to differences in genetics. Pearson correlations were used to determine whether any behaviors recorded from saline-treated mice during fear conditioning, somatic signs or the elevated plus maze were associated with each other. Furthermore, nicotine behavioral ratio scores for contextual fear conditioning were calculated for mice treated with acute nicotine, chronic nicotine, and withdrawal from chronic nicotine. Pearson correlations were used to determine whether the effects of acute nicotine, chronic nicotine, and withdrawal from chronic nicotine on contextual fear conditioning were associated with each other.

Post hoc tests—Tukey HSD post hoc tests were conducted to test pair-wise comparisons. A Levene statistic was also used to test the assumption of homogeneity of variance for each ANOVA. Games-Howell post-hoc tests were used instead of a Tukey HSD if the assumption of homogeneity of variance was not met. All analyses were performed using SPSS version 18.

Results

The effects of acute nicotine on fear conditioning

The effects of acute nicotine (0.045, 0.09, or 0.18 mg/kg) on fear conditioning were investigated in 8 strains of inbred mice (Fig. 1). One-way ANOVAs were used to determine whether nicotine altered fear conditioning within each strain; a significant main effect of drug was found in 6 of the 8 strains for contextual fear conditioning (129: [$F(3, 38) = 13.17$, $p < 0.05$]; A: [$F(3, 36) = 4.53$, $p < 0.05$]; BALB: [$F(3, 38) = 3.89$, $p < 0.05$]; B6: [$F(3, 51) = 7.86$, $p < 0.05$]; D1: [$F(3, 52) = 4.89$, $p < 0.05$]; D2: [$F(3, 38) = 15.72$, $p < 0.05$]). Subsequent post-hoc tests revealed that three strains treated with 0.045 mg/kg nicotine (A, BALB, and B6 mice), two strains treated with 0.09 mg/kg nicotine (B6 and D2 mice), and three strains treated with 0.18 mg/kg nicotine (129, D1, and D2 mice) exhibited enhanced contextual fear conditioning when compared to saline controls within each respective strain ($p < 0.05$). Games-Howell post-hoc tests were used to compare nicotine-treated BALB and D1 mice to their respective saline controls; Tukey post-hoc tests were used for all other

strains. No significant effects of acute nicotine were observed for baseline freezing, immediate freezing, generalized freezing, or cued fear conditioning for all strains (data not shown; $p > 0.05$).

To compare the effects of acute nicotine between strains, nicotine behavioral ratio scores were calculated for each strain. One-way ANOVAs revealed significant differences between strains in the effects of 0.045 mg/kg nicotine [$F(7, 79) = 9.59, p < 0.05$], 0.09 mg/kg nicotine [$F(7, 76) = 3.07, p < 0.05$], and 0.18 mg/kg nicotine [$F(7, 80) = 10.44, p < 0.05$] on contextual fear conditioning. Furthermore, the effect of 0.18 mg/kg nicotine on immediate freezing was also significantly different between strains [$F(7, 80) = 2.40, p < 0.05$]. Games-Howell post-hoc comparisons found several differences between strains in the effects of acute nicotine on immediate freezing and contextual fear conditioning. For contextual fear conditioning, the rank order of strains was: BALB > A > B6 > 129 > CBA > D2 > C3H > D1 (0.045 mg/kg), D2 > BALB > B6 > 129 > D1 > C3H > A > CBA (0.09 mg/kg), and D2 > D1 > 129 > BALB > B6 > A > C3H > CBA (0.18 mg/kg). For immediate freezing, the rank order for mice treated with 0.18 mg/kg was B6 > A > D1 > 129 > D2 > C3H > CBA > BALB. The rank orders listed above range from the greatest enhancement to the least enhancement. For these rank orders and all subsequent rank orders, $x > y$ indicates that the mean of strain x is greater than all strains with lower nicotine behavioral ratio scores. However, rank orders do not indicate statistically significant differences between strains, and are used for descriptive purposes. Significant differences between strains are listed in Table 1a, and strain means for behavioral measures with between-strain differences are listed in Online Resource 1. The effects of acute nicotine on baseline freezing, generalized freezing, or cued fear conditioning did not differ between strains (data not shown; $p > 0.05$). Taken together, these results demonstrate that acute nicotine will enhance contextual fear conditioning in multiple strains of inbred mice, and that sensitivity to the effects of acute nicotine on contextual fear conditioning varies as function of genotype.

The effects of nicotine withdrawal on somatic signs, elevated plus maze, and fear conditioning: within-strain analyses

Mice were withdrawn from chronic nicotine (3.0, 6.3, or 12.0 mg/kg/d), then behavior in fear conditioning, somatic signs, and the elevated plus maze was investigated in 8 strains of inbred mice. For fear conditioning (Fig. 2), 5 of the 8 strains exhibited disrupted contextual fear conditioning following withdrawal from chronic nicotine (A: [$F(3, 48) = 6.70, p < 0.05$]; BALB: [$F(3, 40) = 9.48, p < 0.05$]; C3H: [$F(3, 39) = 19.52, p < 0.05$]; B6: [$F(3, 40) = 24.85, p < 0.05$]; D1: [$F(3, 40) = 9.32, p < 0.05$]). Tukey post-hoc tests revealed that A, C3H, and B6 mice showed disrupted contextual fear conditioning following withdrawal from 6.3 mg/kg/d chronic nicotine, whereas A, BALB, C3H, B6, and D1 strains withdrawn from 12.0 mg/kg/d chronic nicotine had deficits in contextual fear conditioning when compared to their respective saline controls ($p < 0.05$). A significant main effect of withdrawal was also found for generalized freezing in CBA mice [$F(3, 40) = 3.75, p < 0.05$], but Games-Howell post-hoc tests found no differences between groups (data not shown; $p > 0.05$). Nicotine withdrawal had no effects on baseline freezing, immediate freezing, or cued fear conditioning for all strains ($p > 0.05$). Overall, these data demonstrate that nicotine withdrawal produces deficits in contextual fear conditioning in multiple strains of inbred mice.

One-way ANOVAs were used to determine if nicotine withdrawal altered somatic signs (Online Resource 2) or elevated plus maze behavior (Online Resource 3) for each of the 8 inbred strains. Withdrawal from chronic nicotine produced significant changes in shaking for C3H and D2 mice (C3H: [$F(3, 39) = 2.94, p < 0.05$]; D2: [$F(3, 38) = 5.14, p < 0.05$]), total somatic signs for D1 and D2 mice (D1: [$F(3, 40) = 3.86, p < 0.05$]; D2: [$F(3, 38) = 3.99, p < 0.05$]), and grooming for D1 mice [$F(3, 40) = 11.42, p < 0.05$]). Tukey post-hoc

comparisons found that D2 mice withdrawn from 3.0 mg/kg/d nicotine exhibited significantly less shaking and total somatic signs relative to D2 mice withdrawn from saline ($p < 0.05$). Games-Howell post-hoc tests revealed that D1 mice exhibited significantly fewer instances of grooming during withdrawal from 3.0 mg/kg/d nicotine when compared to saline-treated D1 mice ($p < 0.05$). Additional pairwise comparisons for C3H and D1 mice were not significant ($p > 0.05$). No effects on scratching, retropulsion, jumping, writhing, and head nodding were observed ($p > 0.05$).

In the elevated plus maze, D2 mice exhibited changes in exploration of the closed arms [$F(3, 38) = 2.88, p < 0.05$] and the center of the maze [$F(3, 38) = 4.60, p < 0.05$] following withdrawal from chronic nicotine (Online Resource 3). Games-Howell post-hoc tests determined that D2 mice withdrawn from 12.0 mg/kg/d nicotine spent significantly more time in the closed arms and less time in the center area when compared to D2 saline controls ($p < 0.05$). Nicotine withdrawal had no effects on exploration of the open arms for all 8 strains ($p > 0.05$). Thus, nicotine withdrawal decreases somatic signs in C3H, D1, and D2 mice and increases anxiety-related behavior in the elevated plus maze in D2 mice. It is possible that withdrawal from higher doses of chronic nicotine than those currently tested may increase somatic signs and anxiety in the elevated plus maze in other inbred mouse strains. In support, Damaj and colleagues (2003) reported that C57BL/6J mice withdrawn from higher doses of chronic nicotine have increased somatic signs and decreased time in the open arms of the elevated plus maze.

The effects of nicotine withdrawal on somatic signs, elevated plus maze, and fear conditioning: between-strain analyses

As with previous experiments, nicotine behavioral ratio scores were calculated for each strain to compare the effects of withdrawal from chronic nicotine on fear conditioning between strains. Significant between-strain differences in the effects of nicotine withdrawal on contextual fear conditioning [$F(7, 77) = 8.47, p < 0.05$], generalized freezing [$F(7, 77) = 2.70, p < 0.05$], and cued fear conditioning [$F(7, 77) = 2.52, p < 0.05$] were found when mice were withdrawn from 6.3 mg/kg/d nicotine. Furthermore, significant between-strain differences in contextual fear conditioning [$F(7, 79) = 8.04, p < 0.05$] and generalized freezing [$F(7, 79) = 4.37, p < 0.05$] were also found when mice were withdrawn from 12.0 mg/kg/d nicotine. Games-Howell post-hoc tests found significant differences between strains in contextual fear conditioning and generalized freezing. For contextual fear conditioning, the rank order of strains was: D1 > BALB > 129 > CBA > D2 > A > B6 > C3H (6.3 mg/kg/d), and 129 > CBA > A > D2 > B6 > BALB > D1 > C3H (12.0 mg/kg/d). For generalized freezing, the rank order of strains was: 129 > A > C3H > D1 > CBA > D2 > BALB > B6 (6.3 mg/kg/d), and CBA > 129 > A > C3H > D2 > B6 > D1 > BALB (12.0 mg/kg/d). For cued fear conditioning, the rank order of strains was: BALB > D1 > B6 > 129 > A > CBA > C3H > D2 (6.3 mg/kg/d). The rank orders listed above range from the greatest nicotine behavioral ratio score values to the lowest nicotine behavioral ratio score values, and significant differences between strains are listed in Table 1b. Strain means for behavioral measures with between-strain differences are listed in Online Resource 4. Given that nicotine withdrawal disrupts contextual fear conditioning (and produces negative nicotine behavioral ratio score values), strains with lower nicotine behavioral ratio scores had more severe deficits in the behavioral measures listed above. No significant differences were found for baseline freezing and immediate freezing ($p > 0.05$).

Analysis of somatic sign nicotine behavioral ratio scores revealed significant main effects for grooming [$F(7, 72) = 3.02, p < 0.05$], shaking [$F(7, 72) = 2.44, p < 0.05$], jumping [$F(7, 72) = 2.93, p < 0.05$], and total somatic signs [$F(7, 72) = 2.71, p < 0.05$] following withdrawal from 3.0 mg/kg/d nicotine. Significant differences between strains were found in shaking [$F(7, 72) = 3.28, p < 0.05$], jumping [$F(7, 72) = 2.62, p < 0.05$], and head nodding

[$F(7, 72) = 2.17, p < 0.05$] when mice were withdrawn from 6.3 mg/kg/d nicotine. Finally, significant between-strain differences in scratching [$F(7, 71) = 3.15, p < 0.05$], jumping [$F(7, 71) = 3.99, p < 0.05$], and head nodding [$F(7, 71) = 3.68, p < 0.05$] were observed in mice that were withdrawn from 12.0 mg/kg/d nicotine. Games-Howell post-hoc tests identified several significant pairwise comparisons. The following are the rank orders for somatic signs: grooming (129 > CBA > BALB > B6 > A > C3H > D2 > D1; 3.0 mg/kg/d), shaking (129 > CBA > C3H > D1 > A > BALB > B6 > D2 (3.0 mg/kg/d), and 129 > BALB > C3H > CBA > D2 > D1 > A > B6 (6.3 mg/kg/d)), scratching (A > 129 > B6 > C3H > D2 > BALB > D1 > CBA; 12.0 mg/kg/d), and jumping (C3H > A > BALB > 129 > CBA > D1 > D2 > B6; 12.0 mg/kg/d). As with previously reported data, rank orders listed above range from the greatest nicotine behavioral ratio score values to the lowest nicotine behavioral ratio score values; higher nicotine behavioral ratio scores indicate that nicotine withdrawal increased somatic signs. Significant differences between strains are shown in Table 1b, and strain means for behavioral measures with significant between-strain differences are listed in Online Resource 4. Nicotine withdrawal did not alter retropulsion or writhing between strains. Although significant main effects of nicotine withdrawal were observed for jumping, head nodding, and total somatic signs, Games-Howell post-hoc tests were not significant ($p > 0.05$).

Elevated plus maze nicotine behavioral ratio scores were also analyzed to identify any between-strain differences in the effect of nicotine withdrawal on anxiety. Withdrawal from 6.3 and 12.0 mg/kg/d chronic nicotine produced significant between-strain differences in the time spent on the open arms (6.3 mg/kg/d: [$F(7, 69) = 2.59, p < 0.05$]; 12.0 mg/kg/d: [$F(7, 70) = 7.75, p < 0.05$]) and the closed arms (6.3 mg/kg/d: [$F(7, 69) = 2.28, p < 0.05$]; 12.0 mg/kg/d: [$F(7, 70) = 4.73, p < 0.05$]) of the plus maze. Subsequent Games-Howell post-hoc tests revealed significant differences between strains. The following are the rank orders for elevated plus maze: closed arms (129 > D2 > BALB > B6 > D1 > C3H > CBA > A; 12.0 mg/kg/d), and open arms (D1 > CBA > B6 > A > C3H > BALB > 129 > D2 (6.3 mg/kg/d), and A > D1 > CBA > C3H > B6 > BALB > 129 > D2 (12.0 mg/kg/d)). Rank orders range from the greatest nicotine behavioral ratio score values to the lowest nicotine behavioral ratio score values. Higher nicotine behavioral ratio scores for the closed arms in the elevated plus maze indicate that nicotine withdrawal increased time in the closed arms (increased anxiety), whereas high nicotine behavioral ratio scores for the open arms indicate that more time was spent in the open arms during nicotine withdrawal (decreased anxiety). Significant differences between strains are shown in Table 1b, and strain means for behavioral measures with between-strain differences are listed in Online Resource 4. There were no significant between-strain differences in the effects of nicotine withdrawal on time spent in the center of the maze ($p > 0.05$). Withdrawal from 6.3 mg/kg/d chronic nicotine also altered time spent in the closed arms, but Games-Howell post-hoc tests were not significant ($p > 0.05$). Taken together, these data demonstrate that the effects of nicotine withdrawal on fear conditioning, somatic signs, and elevated plus maze can vary between different strains of inbred mice.

The effects of chronic nicotine on somatic signs, elevated plus maze, and fear conditioning

The effects of chronic nicotine (12.0 mg/kg/d) on fear conditioning, somatic signs, and the elevated plus maze were investigated in 8 strains of inbred mice. For fear conditioning, independent-samples *t*-tests found no differences in baseline freezing, immediate freezing, contextual fear conditioning, generalized freezing, or cued fear conditioning for all 8 strains of inbred mice (data not shown; $p > 0.05$). In addition, no within-strain differences were found in all measures of somatic signs (grooming, scratching, shaking, retropulsion,

jumping, writhing, head nodding, and total) and elevated plus maze (time spent in open arms, closed arms, and center; $p > 0.05$).

Nicotine behavioral ratio scores were calculated for each strain to compare the effects of 12.0 mg/kg/d chronic nicotine between strains. Analysis of fear conditioning data with one-way ANOVAs found no differences in baseline freezing, immediate freezing, contextual fear conditioning, generalized freezing or cued fear conditioning ($p > 0.05$). No differences were found between strains for all measures of somatic signs (grooming, scratching, shaking, retropulsion, jumping, writhing, head nodding, and total) and elevated plus maze (time spent in open arms, closed arms, and center; $p > 0.05$). Overall, these data suggest that tolerance to the effects of acute nicotine on fear conditioning may develop during chronic nicotine use in all strains of inbred mice that showed enhanced contextual fear conditioning. Furthermore, these data suggest that changes in somatic signs, elevated plus maze, and fear conditioning observed during nicotine withdrawal are not a continuation of effects seen during chronic nicotine treatment.

Between-strain differences in saline-treated mice

The behavior of saline-treated mice from each strain was compared to determine whether strain-dependent differences in fear conditioning, somatic signs, and elevated plus maze exist that are independent of the effects of acute nicotine, chronic nicotine, or nicotine withdrawal. Comparisons of saline-treated mice from acute nicotine experiments found significant strain-dependent differences in immediate freezing [$F(7, 91) = 40.88, p < 0.05$], contextual fear conditioning [$F(7, 91) = 14.32, p < 0.05$], generalized freezing [$F(7, 91) = 45.33, p < 0.05$], and cued fear conditioning [$F(7, 91) = 14.34, p < 0.05$]. Games-Howell pairwise comparisons between strains revealed several differences between strains in immediate freezing, contextual fear conditioning, generalized freezing, and cued fear conditioning. These results are listed in Table 2a (left panel; $p < 0.05$). Baseline freezing did not differ between strains ($p > 0.05$).

Data from saline-treated mice from chronic nicotine and nicotine withdrawal experiments were also compared using one-way ANOVAs to identify differences in fear conditioning, somatic signs, and the elevated plus maze. Significant differences between strains were found in many of the behavioral measures of fear conditioning, somatic signs, and elevated plus maze. Furthermore, Games-Howell pairwise comparisons found numerous differences between strains. These results, along with significant f values, are listed in Table 2b (chronic saline) and Table 2c (saline withdrawal).

Finally, the rank order of saline-treated mice was largely consistent with a previous study that evaluated contextual fear conditioning in 6 of the 8 strains used in the present study (Owen et al. 1997). Specifically, Owen and colleagues (1997) reported the following rank order for contextual fear conditioning (from the greatest to least contextual fear conditioning): A/J > 129 > B6 = BALB > D2 > C3H, whereas for the present study the rank order for saline-treated mice was: 129 > A/J > B6 = BALB > D2 > C3H. Overall, these data demonstrate that differences in genetics can significantly influence fear conditioning, somatic signs, and performance in the elevated plus maze.

Between-strain comparisons of plasma nicotine and cotinine

Between-strain differences in plasma nicotine, cotinine, and a nicotine/cotinine ratio were examined to determine whether nicotine metabolism varied between strains (Fig. 3). An analysis of plasma samples from mice treated with 0.09 mg/kg acute nicotine revealed significant main effects in plasma cotinine [$F(7, 68) = 9.45, p < 0.05$] and the nicotine/cotinine ratio [$F(7, 70) = 3.69, p < 0.05$]. Games-Howell post-hoc tests identified several

significant between-strain differences. For plasma cotinine, the rank order was D2 > BALB > 129 > D1 > B6 > A > C3H > CBA, whereas for the nicotine/cotinine ratio the rank order was CBA > C3H > A > D1 > 129 > BALB > B6 > D2. The rank orders listed above range from higher plasma cotinine and nicotine/cotinine ratio values to lower plasma cotinine and nicotine/cotinine ratio values. The nicotine/cotinine ratio is a measure of metabolic rate; higher nicotine/cotinine ratios indicate a slower metabolism of nicotine or a more rapid metabolism of cotinine (Rao et al. 2000). Plasma samples were also collected from mice that received 12 days of 6.3 mg/kg/d chronic nicotine; significant main effects were observed for plasma nicotine [$F(7, 72) = 7.59, p < 0.05$], plasma cotinine [$F(7, 72) = 51.60, p < 0.05$], and nicotine/cotinine ratio [$F(7, 72) = 13.64, p < 0.05$]. The following are the rank orders for nicotine metabolism measures following chronic nicotine treatment: plasma nicotine (D1 > A > D2 > CBA > B6 > C3H > 129 > BALB), plasma cotinine (129 > D1 > D2 > A > CBA > BALB > C3H > B6) and the nicotine/cotinine ratio (B6 > A > C3H > CBA > BALB > D1 > D2 > 129). Significant differences between strains are listed in Table 3, and strain means for plasma nicotine, plasma cotinine and nicotine/cotinine ratios are listed in Online Resource 5.

Pearson correlations were calculated to determine whether genetic variability in nicotine metabolism was associated with the effects of 0.09 mg/kg acute nicotine or withdrawal from 6.3 mg/kg/d chronic nicotine on fear conditioning, somatic signs, or elevated plus maze performance. For 0.09 mg/kg acute nicotine, plasma cotinine was significantly associated with baseline freezing ($r = 0.83; p < 0.05$) and immediate freezing ($r = 0.79; p < 0.05$). Furthermore, plasma cotinine obtained from mice treated with 6.3 mg/kg/d chronic nicotine was significantly associated with immediate freezing ($r = 0.84; p < 0.05$), head nodding ($r = 0.94; p < 0.05$), and time spent in the center of the elevated plus maze ($r = 0.88; p < 0.05$). The nicotine/cotinine ratio from mice that received 6.3 mg/kg/d chronic nicotine was significantly correlated with head nodding ($r = -0.77; p < 0.05$) and time spent in the center of the elevated plus maze ($r = -0.80; p < 0.05$). No other associations were significant ($p > 0.05$). When examining plots of these correlations (see Online Resource 6), it is clear that the association between plasma cotinine and baseline freezing is influenced by an extreme data point, whereas other scatterplots depict linear relationships. Together, these data indicate that the metabolism of nicotine and cotinine varied between strains, and genetic variability in nicotine metabolism was associated with some behavioral measures in fear conditioning, somatic signs, and the elevated plus maze, but not with differences in learning.

Genetic correlations

Genetic correlations were used to examine associations between somatic signs, fear conditioning, and the elevated plus maze to determine whether the phenotypes of these behavioral measures were controlled by the same genes. To examine whether common genes regulate these behavioral measures, independent of nicotine treatment, the strain means from saline-treated mice in the chronic nicotine and nicotine withdrawal experiments were compared with Pearson correlations. Strain means were used instead of individual scores because inbred mice are genetically identical and thus variability between individual scores is due to environmental factors and not due to differences in genetics. For the chronic saline dataset, time spent in the open arms of the elevated plus maze was significantly associated with immediate freezing ($r = -0.71; p < 0.05$) and time spent in the closed arms of the elevated plus maze ($r = -0.86; p < 0.05$). Time in the center of the elevated plus maze was significantly correlated with contextual fear conditioning ($r = 0.79; p < 0.05$) and head nodding ($r = 0.73; p < 0.05$). In addition, total somatic signs were significantly associated with grooming ($r = 0.73; p < 0.05$) and jumping ($r = 0.79; p < 0.05$). An association between shaking and scratching was also significant ($r = 0.75; p < 0.05$). For the withdrawal from chronic saline dataset, immediate freezing was significantly associated with time spent in the open arms of the plus maze ($r = -0.71; p < 0.05$), grooming ($r = 0.79; p < 0.05$), and shaking

($r = 0.76$; $p < 0.05$). Time spent in the closed arms of the elevated plus maze was significantly correlated with time spent in the open arms ($r = -0.80$; $p < 0.05$) and scratching ($r = 0.75$; $p < 0.05$). Furthermore, grooming was significantly associated with scratching ($r = 0.92$; $p < 0.05$), shaking ($r = 0.79$; $p < 0.05$), and total somatic signs ($r = 0.89$; $p < 0.05$); total somatic signs was also significantly associated with scratching ($r = 0.75$; $p < 0.05$). Plots of these correlations can be found in Online Resource 7 and 8. For the withdrawal from chronic saline dataset, the association between grooming and immediate freezing and the association between shaking and immediate freezing are influenced by extreme data points, whereas other scatterplots depict linear relationships. Furthermore, it should be noted that many correlational analyses were performed in this study, which increases the probability of false positive results. However, these data suggest that common genetic substrates may influence somatic sign behaviors, elevated plus maze behaviors, and immediate freezing.

To determine whether common genes regulate the effects of acute nicotine, chronic nicotine, and nicotine withdrawal on contextual learning, nicotine behavioral ratio scores for contextual fear conditioning were calculated for mice treated with all doses of acute, chronic, and withdrawal from chronic nicotine. Specifically, all possible comparisons were made, including associations between the most effective doses of acute nicotine (0.045 and 0.09 mg/kg nicotine), and withdrawal from 12 mg/kg/d chronic nicotine, which disrupted contextual fear conditioning in 5 of the 8 strains. There were no significant associations between the effects of acute nicotine, chronic nicotine and nicotine withdrawal on contextual fear conditioning ($p > 0.05$). Thus, these data suggest that different genetic substrates may mediate sensitivity to the effects of acute nicotine and the effects of nicotine withdrawal on contextual learning.

Discussion

It has been demonstrated that individual differences in genetics can account for a large percentage of smoking-related behaviors (Li et al. 2003; Sullivan and Kendler 1999; Haberstick et al. 2007; Xian et al. 2003; Hardie et al. 2006), and the identification of genes that alter risk for nicotine addiction may aid in the development of new treatments. However, it was unknown until now whether differences in genetics could alter the effects of nicotine on contextual learning. In the present study, the effects of acute, chronic, and withdrawal from chronic nicotine on fear conditioning were investigated in eight inbred mouse strains. Acute nicotine enhanced contextual fear conditioning in six of the eight strains, and the effective doses that produced enhancement varied between strains. Furthermore, strain-dependent differences in the amount of enhancement were observed, such that BALB mice exhibited the greatest increase in contextual fear conditioning following acute nicotine administration, whereas B6 mice showed the smallest significant increase in contextual fear conditioning. Withdrawal from 6.3 and/or 12.0 mg/kg/d chronic nicotine disrupted contextual fear conditioning in five strains, with C3H mice showing the greatest impairment, and A mice exhibited the smallest deficits that were still significantly different from saline treated mice. In contrast, chronic nicotine did not alter fear conditioning in all eight strains, suggesting that the effects observed during nicotine withdrawal were not due to the extended effects of chronic nicotine treatment. Acute nicotine and withdrawal from chronic nicotine had no effect on cued fear conditioning for all eight strains. Thus, the selective effects of nicotine on contextual but not cued fear conditioning demonstrates that nicotine alters contextual learning rather than altering other processes that may affect both types of learning such as locomotor activity.

Although these results demonstrate that differences in genetics can alter sensitivity to the effects of nicotine on contextual learning, these data also suggest that different genetic

substrates may mediate sensitivity to the effects of acute nicotine and sensitivity to nicotine withdrawal-related deficits in contextual fear conditioning. Acute nicotine and nicotine withdrawal produced significant changes in contextual learning for most of the inbred strains studied; genetic correlations revealed that there was no significant relationship between the effects of acute nicotine and nicotine withdrawal on contextual fear conditioning. For example, 129 mice showed enhancement of contextual fear conditioning when treated with the highest dose of acute nicotine, but nicotine withdrawal had no effect on contextual fear conditioning. Similarly, acute nicotine had no effect on C3H mice, whereas nicotine withdrawal dramatically impaired contextual fear conditioning. Thus, it is possible that there is little overlap in the genes that influence sensitivity to the effects of acute nicotine and nicotine withdrawal on contextual fear conditioning. Future QTL studies may help determine the degree of similarity between the genes that regulate the effects of acute nicotine and the genes that alter sensitivity to the effects of nicotine withdrawal.

The variability in the effects of nicotine on contextual fear conditioning between strains may be explained in part by differences in the behavioral effects of nicotine and strain-dependent differences in the acquisition and expression of fear conditioning. The behavioral effects of nicotine on B6 mice are well characterized, and previous studies have reported that B6 mice have increased sensitivity to the rewarding properties of nicotine (Robinson et al. 1996; Grabus et al. 2006), and show robust contextual fear conditioning compared to other inbred strains (Balogh and Wehner 2003; Bolivar et al. 2001; Owen et al. 1997). Thus, the combination of these two factors may partly account for the high sensitivity to the effects of nicotine on contextual fear conditioning observed in B6 mice. In contrast, nicotine had no effect on the CBA inbred strain in any of the behavioral tasks examined, and these data are consistent with a previous study reporting that CBA mice had low sensitivity to the effects of nicotine-induced antinociception (Damaj et al. 2007). The reduced sensitivity to nicotine-induced antinociception, along with lack of an effect of nicotine on many behavioral tasks suggests that CBA mice may be largely insensitive to the effects of nicotine for the doses examined. Future QTL studies using B6, CBA mice, and other strains may lead to the identification of genomic regions that are associated with sensitivity to nicotine.

Strain-dependent differences in the brain regions that are required for contextual fear conditioning may also play an important role in altering sensitivity to the effects of nicotine on contextual learning. Previous studies have demonstrated that the effects of both acute nicotine and nicotine withdrawal on contextual fear conditioning are hippocampus-dependent and require 2 subunit-containing nAChRs (Davis et al. 2007; Wehner et al. 2004; Portugal et al. 2008; Davis and Gould, 2006, 2007b). Therefore, genetic variants that alter hippocampal and/or nAChR function may lead to changes in sensitivity to the effects of nicotine on contextual learning. In support, previous studies have shown that D2 mice have impaired hippocampal function and show poor contextual fear conditioning (Balogh and Wehner 2003; Paylor et al. 1994; Paylor et al. 1993). This impaired hippocampal function may partially explain why nicotine withdrawal has no effect on contextual fear conditioning in D2 mice, as performance in this task is already poor. Furthermore, D2 mice have reduced membrane-bound PKC levels in the hippocampus relative to B6 mice (Smith and Wehner 2002; Fordyce and Wehner 1992; Bowers et al. 1995). However, both strains show enhancement following treatment with acute nicotine, suggesting that differences in PKC levels may not alter sensitivity to the effects of acute nicotine on contextual learning, or that compensatory mechanisms have restored sensitivity to acute nicotine in D2 mice. The characterization of hippocampal function in other inbred strains may help explain strain-dependent differences in the effects of nicotine on contextual learning.

Nicotine withdrawal produces numerous changes in behavior that can include increased somatic signs and increased anxiety (Jackson et al. 2008; Damaj et al. 2003; Jackson et al.

2009; Malin et al. 1992). Therefore, the present study measured nicotine withdrawal-associated changes in somatic signs and the elevated plus maze before mice were trained in fear conditioning. Withdrawal-related decreases in somatic signs were observed in C3H, D1 and D2 mice withdrawn from 3.0 mg/kg/d, whereas increases in anxiety in the elevated plus maze were found in D2 mice withdrawn from 12.0 mg/kg/d nicotine. Furthermore, chronic nicotine administration did not alter somatic signs or elevated plus maze behavior, suggesting that these changes in somatic signs and the elevated plus maze were not carryover effects from chronic nicotine treatment. Although withdrawal from 3.0 mg/kg/d chronic nicotine reduced somatic signs in some strains, previous studies have reported increased somatic signs following withdrawal from high doses of chronic nicotine (24–48 mg/kg/d) (Jackson et al. 2008; Damaj et al. 2003). Therefore, it is possible that nicotine withdrawal has biphasic effects on somatic signs; withdrawal from lower doses may reduce somatic signs whereas withdrawal from high doses of nicotine may increase somatic signs. The reduction in somatic signs in the C3H, D1, and D2 strains does not mean that saline treated mice displayed somatic withdrawal symptoms but instead suggests that there may be baseline levels of somatic-related behaviors such as shaking and grooming, and C3H, D1, and D2 withdrawn from 3.0 mg/kg/d chronic nicotine show a reduction from baseline; these changes may not reflect a withdrawal state. Together, the data from nicotine withdrawal experiments suggest that each symptom of nicotine withdrawal may be mediated by different genetic substrates. For example, nicotine withdrawal disrupted contextual fear conditioning in 5 strains, whereas few changes were observed in somatic signs and the elevated plus maze during nicotine withdrawal. Studies using nAChR subunit KO mice have also demonstrated that different nicotine withdrawal symptoms are mediated by different nAChR subunits. For example, nicotine withdrawal-related increases in somatic signs are reduced in 5, 7, and 4 nAChR KO mice (Jackson et al. 2008; Salas et al. 2004, 2007). However, withdrawal-associated changes in contextual fear conditioning and the elevated plus maze are not observed in 2 KO mice (Jackson et al. 2008; Portugal et al. 2008).

The results of the nicotine withdrawal experiments also suggest that separate nicotine withdrawal symptoms may be sensitive to different doses of chronic nicotine. For example, previous studies with C57BL/6J mice have demonstrated that changes in somatic signs and the elevated plus maze emerge after withdrawal from higher doses of chronic nicotine (24–36 mg/kg/d) (Damaj et al. 2003; Jackson et al. 2009), whereas deficits in contextual fear conditioning are observed following withdrawal from lower doses of chronic nicotine (6.3 mg/kg/d, (Davis et al. 2005)). Thus, withdrawal from higher doses of chronic nicotine may be necessary for withdrawal-related changes in somatic signs and the elevated plus maze to emerge.

Currently, the genes that influence the effects of nicotine on fear conditioning, somatic signs, and the elevated plus maze are unknown. However, it is possible that genetic variability in the enzymes that metabolize nicotine may alter sensitivity to these behavioral effects of nicotine. For example, the gene encoding cytochrome P450 2A6 (*CYP2A6*), an enzyme that metabolizes nicotine into cotinine (Benowitz and Jacob 1994; Hukkanen et al. 2005), has been associated with smoking phenotypes in humans (Pianezza et al. 1998; Schoedel et al. 2004; Kubota et al. 2006) and a study investigating the ortholog of *CYP2A6* in mice (*Cyp2a5*) has shown that low levels of nicotine self-administration in male mice are associated with reduced nicotine metabolism and low *CYP2A5* protein levels (Siu et al. 2006). In the present study, plasma nicotine and cotinine samples were collected from mice treated with 0.09 mg/kg acute nicotine or 6.3 mg/kg/d chronic nicotine. Strain-dependent differences in plasma cotinine and the nicotine/cotinine ratio were observed in mice treated with acute nicotine, whereas chronic nicotine treatment produced changes in plasma nicotine, cotinine, and the nicotine/cotinine ratio. Furthermore, baseline and immediate freezing was associated with plasma cotinine from mice treated with acute nicotine, and

immediate freezing, head nodding, and time spent in the center of the elevated plus maze was associated with plasma cotinine and the nicotine/cotinine ratio in chronic nicotine treated mice. Together, these data suggest that genes involved in the metabolism of nicotine and cotinine may alter the effects of nicotine on measures of behavioral activity, but are not associated with the effects of nicotine on contextual learning.

In conclusion, the results of the present study are the first to identify strain-dependent differences in the effects of nicotine on fear conditioning, somatic signs, and the elevated plus maze. The effects of acute nicotine and nicotine withdrawal on contextual fear conditioning varied considerably between strains, but the lack of an association between the effects of acute nicotine and nicotine withdrawal on contextual fear conditioning suggests that different genes may mediate these two effects. Furthermore, the effects of nicotine withdrawal on somatic signs and the elevated plus maze, but not contextual fear conditioning, were associated with changes in nicotine metabolism. The characterization of the effects of nicotine on fear conditioning can be used in the development of QTL studies that can identify regions of the mouse genome associated with the effects of nicotine on contextual learning. For instance, an F2 cross between C3H/HeJ mice and C57BL/6J mice could be used to identify regions of the mouse genome associated with the effects of acute nicotine on contextual fear conditioning. The identification of genes that alter sensitivity to the effects of nicotine may lead to a better understanding of how individual differences in genetics contribute to nicotine addiction, and may lead to the development of smoking cessation treatments that are tailored to individual genotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- André JM, Gulick D, Portugal GS, Gould TJ. Nicotine withdrawal disrupts both foreground and background contextual fear conditioning but not pre-pulse inhibition of the acoustic startle response in C57BL/6 mice. *Behav Brain Res.* 2008; 190(2):174–181. [PubMed: 18367257]
- Auerbach AB, Norinsky R, Ho W, Losos K, Guo Q, Chatterjee S, Joyner AL. Strain-dependent differences in the efficiency of transgenic mouse production. *Transgenic Res.* 2003; 12(1):59–69. [PubMed: 12650525]
- Balogh SA, Wehner JM. Inbred mouse strain differences in the establishment of long-term fear memory. *Behav Brain Res.* 2003; 140(1–2):97–106. [PubMed: 12644283]
- Benowitz NL. Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addiction. *N Engl J Med.* 1988; 319(20):1318–1330. [PubMed: 3054551]
- Benowitz NL, Jacob P III. Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clin Pharmacol Ther.* 1994; 56(5):483–493. [PubMed: 7955812]
- Blanchard RJ, Blanchard DC. Crouching as an index of fear. *J Comp Physiol Psych.* 1969; 67(3):370–375.
- Bolivar VJ, Pooler O, Flaherty L. Inbred strain variation in contextual and cued fear conditioning behavior. *Mamm Genome.* 2001; 12(8):651–656. [PubMed: 11471061]
- Bowers BJ, Christensen SC, Pauley JR, Paylor R, Yuva L, Dunbar SE, Wehner JM. Protein and molecular characterization of hippocampal protein kinase C in C57BL/6 and DBA/2 mice. *J Neurochem.* 1995; 64(6):2737–2746. [PubMed: 7760054]

- Boyle AE, Gill KJ. Genetic analysis of the psychostimulant effects of nicotine in chromosome substitution strains and F2 crosses derived from A/J and C57BL/6J progenitors. *Mamm Genome*. 2009; 20(1):34–42. [PubMed: 19083055]
- Cahill K, Stead LF, Lancaster T. Nicotine receptor partial agonists for smoking cessation. *Cochrane database of systematic reviews (Online)*. 2007; (1):CD006103.
- Collins AC, Miner LL, Marks MJ. Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse. *Pharmacol Biochem Behav*. 1988; 30(1):269–278. [PubMed: 3174752]
- Committee on Standardized Genetic Nomenclature for Mice. Rules for nomenclature of inbred strains. In: Lyon, MF.; Searle, AG., editors. *Genetic variants and strains of the laboratory mouse*. Oxford University Press; New York: 1989. p. 632–635.
- Damaj MI, Kao W, Martin BR. Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. *J Pharmacol Exp Ther*. 2003; 307(2):526–534. [PubMed: 12970387]
- Damaj MI, Fonck C, Marks MJ, Deshpande P, Labarca C, Lester HA, Collins AC, Martin BR. Genetic approaches identify differential roles for $\alpha 4\beta 2^*$ nicotinic receptors in acute models of antinociception in mice. *J Pharmacol Exp Ther*. 2007; 321(3):1161–1169. [PubMed: 17371806]
- Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl)*. 2006; 184(3–4):345–352. [PubMed: 15988571]
- Davis JA, Gould TJ. Atomoxetine reverses nicotine withdrawal-associated deficits in contextual fear conditioning. *Neuropsychopharmacology*. 2007a; 32(9):2011–2019. [PubMed: 17228337]
- Davis JA, Gould TJ. $\beta 2$ subunit-containing nicotinic receptors mediate the enhancing effect of nicotine on trace cued fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl)*. 2007b; 190(3):343–352. [PubMed: 17136517]
- Davis JA, Gould TJ. Hippocampal nAChRs mediate nicotine withdrawal-related learning deficits. *Eur Neuropsychopharmacol*. 2009; 19(8):551–561. [PubMed: 19278836]
- 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(38):8708–8713. [PubMed: 16177040]
- Davis JA, Porter J, Gould TJ. Nicotine enhances both foreground and background contextual fear conditioning. *Neurosci Lett*. 2006; 394(3):202–205. [PubMed: 16260086]
- Davis JA, Kenney JW, Gould TJ. Hippocampal $\alpha 4\beta 2$ nicotinic acetylcholine receptor involvement in the enhancing effect of acute nicotine on contextual fear conditioning. *J Neurosci*. 2007; 27(40):10870–10877. [PubMed: 17913920]
- 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. *Biochem Biophys Res Commun*. 1992; 188(2):690–694. [PubMed: 1445314]
- Gill KJ, Boyle AE. Genetic basis for the psychostimulant effects of nicotine: a quantitative trait locus analysis in AcB/BcA recombinant congenic mice. *Genes Brain Behav*. 2005; 4(7):401–411. [PubMed: 16176386]
- Gould TJ, Higgins SJ. Nicotine enhances contextual fear conditioning in C57BL/6J mice at 1 and 7 days post-training. *Neurobiol Learn Mem*. 2003; 80(2):147–157. [PubMed: 12932430]
- Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. *Behav Brain Res*. 1999; 102(1–2):31–39. [PubMed: 10403013]
- Gould TJ, Feiro O, Moore D. Nicotine enhances trace cued fear conditioning but not delay cued fear conditioning in C57BL/6 mice. *Behav Brain Res*. 2004; 155(1):167–173. [PubMed: 15325790]
- Grabus SD, Martin BR, Brown SE, Damaj MI. Nicotine place preference in the mouse: influences of prior handling, dose and strain and attenuation by nicotinic receptor antagonists. *Psychopharmacology (Berl)*. 2006; 184(3–4):456–463. [PubMed: 16463055]
- Haberstick BC, Timberlake D, Ehringer MA, Lessem JM, Hopfer CJ, Smolen A, Hewitt JK. Genes, time to first cigarette and nicotine dependence in a general population sample of young adults. *Addiction (Abingdon, England)*. 2007; 102(4):655–665.
- Hardie TL, Moss HB, Lynch KG. Genetic correlations between smoking initiation and smoking behaviors in a twin sample. *Addict Behav*. 2006; 31(11):2030–2037. [PubMed: 16675152]

- Ho MK, Tyndale RF. Overview of the pharmacogenomics of cigarette smoking. *Pharmacogenomics J*. 2007; 7(2):81–98. [PubMed: 17224913]
- Hughes JR, Stead LF, Lancaster T. Antidepressants for smoking cessation. *Cochrane database of systematic reviews (Online)*. 2007; (1):CD000031.
- Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*. 2005; 57(1):79–115. [PubMed: 15734728]
- Jackson KJ, Martin BR, Changeux JP, Damaj MI. Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. *J Pharmacol Exp Ther*. 2008; 325(1):302–312. [PubMed: 18184829]
- Jackson KJ, Walters CL, Miles MF, Martin BR, Damaj MI. Characterization of pharmacological and behavioral differences to nicotine in C57Bl/6 and DBA/2 mice. *Neuropharmacology*. 2009; 57(4):347–355. [PubMed: 19619563]
- Kubota T, Nakajima-Taniguchi C, Fukuda T, Funamoto M, Maeda M, Tange E, Ueki R, Kawashima K, Hara H, Fujio Y, Azuma J. CYP2A6 polymorphisms are associated with nicotine dependence and influence withdrawal symptoms in smoking cessation. *Pharmacogenomics J*. 2006; 6(2):115–119. [PubMed: 16402086]
- Lee CW, Kahende J. Factors associated with successful smoking cessation in the United States, 2000. *Am J Public Health*. 2007; 97(8):1503–1509. [PubMed: 17600268]
- Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction (Abingdon, England)*. 2003; 98(1):23–31.
- Li XC, Karadsheh MS, Jenkins PM, Brooks JC, Drapeau JA, Shah MS, Lautner MA, Stitzel JA. Chromosomal loci that influence oral nicotine consumption in C57BL/6J $\times$ C3H/HeJ F2 intercross mice. *Genes Brain Behav*. 2007; 6(5):401–410. [PubMed: 17010155]
- Logue SF, Owen EH, Rasmussen DL, Wehner JM. Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: implications of genetic background for single gene and quantitative trait loci analyses. *Neuroscience*. 1997; 80(4):1075–1086. [PubMed: 9284061]
- Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA, Cunningham JS, Wilson OB. Rodent model of nicotine abstinence syndrome. *Pharmacol Biochem Behav*. 1992; 43(3):779–784. [PubMed: 1448472]
- Marks MJ, Stitzel JA, Collins AC. Genetic influences on nicotine responses. *Pharmacol Biochem Behav*. 1989a; 33(3):667–678. [PubMed: 2587608]
- Marks MJ, Romm E, Campbell SM, Collins AC. Variation of nicotinic binding sites among inbred strains. *Pharmacol Biochem Behav*. 1989b; 33(3):679–689. [PubMed: 2587609]
- Marks MJ, Campbell SM, Romm E, Collins AC. Genotype influences the development of tolerance to nicotine in the mouse. *J Pharmacol Exp Ther*. 1991; 259(1):392–402. [PubMed: 1920127]
- McIlvain H, Susman JL, Davis C, Gilbert C. Physician counseling for smoking cessation: is the glass half empty? *J Fam Pract*. 1995; 40(2):148–152. [PubMed: 7852938]
- Naidong W, Shou W, Chen YL, Jiang X. Novel liquid chromatographic-tandem mass spectrometric methods using silica columns and aqueous-organic mobile phases for quantitative analysis of polar ionic analytes in biological fluids. *J Chromatogr*. 2001; 754(2):387–399.
- 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(4):1087–1099. [PubMed: 9284062]
- Paylor R, Baskall L, Wehner JM. Behavioral dissociations between C57BL/6 and DBA/2 mice on learning and memory tasks: A hippocampal-dysfunction hypothesis. *Psychobiology*. 1993; 21(1):11–26.
- Paylor R, Tracy R, Wehner J, Rudy JW. DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. *Behav Neurosci*. 1994; 108(4):810–817. [PubMed: 7986374]
- Petersen DR, Norris KJ, Thompson JA. A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. *Drug Metab Dispos*. 1984; 12(6):725–731. [PubMed: 6150822]

- Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking. *Nature*. 1998; 393(6687):750. [PubMed: 9655391]
- Plomin, R.; DeFries, JC.; McClearn, GE. Quantitative genetic theory. W. H. Freeman and Co; New York: 1990.
- Portugal GS, Gould TJ. Bupropion dose-dependently reverses nicotine withdrawal deficits in contextual fear conditioning. *Pharmacol Biochem Behav*. 2007; 88(2):179–187. [PubMed: 17868796]
- Portugal GS, Gould TJ. Genetic variability in nicotinic acetylcholine receptors and nicotine addiction: converging evidence from human and animal research. *Behav Brain Res*. 2008; 193(1):1–16. [PubMed: 18571741]
- Portugal GS, Kenney JW, Gould TJ. beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiol Learn Mem*. 2008; 89(2):106–113. [PubMed: 17584502]
- Rao Y, Hoffmann E, Zia M, Bodin L, Zeman M, Sellers EM, Tyndale RF. Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. *Mol Pharmacol*. 2000; 58(4):747–755. [PubMed: 10999944]
- Raybuck JD, Gould TJ. Extracellular signal-regulated kinase 1/2 involvement in the enhancement of contextual fear conditioning by nicotine. *Behav Neurosci*. 2007; 121(5):1119–1124. [PubMed: 17907844]
- Raybuck JD, Portugal GS, Lerman C, Gould TJ. Varenicline ameliorates nicotine withdrawal-induced learning deficits in C57BL/6 mice. *Behav Neurosci*. 2008; 122(5):1166–1171. [PubMed: 18823172]
- Robinson SF, Marks MJ, Collins AC. Inbred mouse strains vary in oral self-selection of nicotine. *Psychopharmacology (Berl)*. 1996; 124(4):332–339. [PubMed: 8739548]
- Salas R, Pieri F, De Biasi M. Decreased signs of nicotine withdrawal in mice null for the beta4 nicotinic acetylcholine receptor subunit. *J Neurosci*. 2004; 24(45):10035–10039. [PubMed: 15537871]
- Salas R, Main A, Gangitano D, De Biasi M. Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the alpha7 nicotinic acetylcholine receptor subunit. *Neuropharmacology*. 2007; 53(7):863–869. [PubMed: 17920082]
- Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics*. 2004; 14(9):615–626. [PubMed: 15475735]
- Silagy C, Lancaster T, Stead L, Mant D, Fowler G. Nicotine replacement therapy for smoking cessation. *Cochrane database of systematic reviews (Online)*. 2004; (3):CD000146.
- Siu EC, Wildenauer DB, Tyndale RF. Nicotine self-administration in mice is associated with rates of nicotine inactivation by CYP2A5. *Psychopharmacology (Berl)*. 2006; 184(3–4):401–408. [PubMed: 16485141]
- Smith AM, Wehner JM. Aniracetam improves contextual fear conditioning and increases hippocampal gamma-PKC activation in DBA/2J mice. *Hippocampus*. 2002; 12(1):76–85. [PubMed: 11918291]
- Sullivan PF, Kendler KS. The genetic epidemiology of smoking. *Nicotine Tob Res*. 1999; 1(Suppl): 2S51–2S57. discussion S69–70.
- Wang D, Ma JZ, Li MD. Mapping and verification of susceptibility loci for smoking quantity using permutation linkage analysis. *Pharmacogenomics J*. 2005; 5(3):166–172. [PubMed: 15724146]
- Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, Beaudet A, Heinemann SF, Balogh SA. Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. *Neuroscience*. 2004; 129(1):11–24. [PubMed: 15489024]
- Xian H, Scherrer JF, Madden PA, Lyons MJ, Tsuang M, True WR, Eisen SA. The heritability of failed smoking cessation and nicotine withdrawal in twins who smoked and attempted to quit. *Nicotine Tob Res*. 2003; 5(2):245–254. [PubMed: 12745498]

Fig. 1. Strain-dependent differences in the effects of acute nicotine on contextual fear conditioning (a) and cued fear conditioning (b). Acute nicotine enhanced contextual fear conditioning in 6 of 8 strains. Error bars indicate $\pm$ SEM, (*) indicates $p < 0.05$ compared to saline treated mice from each respective strain

Fig. 2.

Strain-dependent differences in the effects of nicotine withdrawal on contextual fear conditioning (**a**) and cued fear conditioning (**b**). Nicotine withdrawal disrupted contextual fear conditioning in 5 of 8 strains. Error bars indicate SEM, (*) indicates $p < 0.05$ compared to saline treated mice from each respective strain

Fig. 3. Strain-dependent differences in plasma nicotine and cotinine following 0.09 mg/kg acute or 6.3 mg/kg/d chronic nicotine administration. **a** Acute plasma cotinine was significantly different between D2 mice and all strains other than 129 (*). BALB mice also exhibited higher plasma cotinine levels relative to A (#) and C3H mice (\$). **b** Chronic plasma nicotine levels differed between D1 and BALB mice (*). Chronic plasma cotinine levels differed between 129 mice and all strains other than D1 (#), between D1 mice and all strains other than 129 (^), between D2 and all strains other than A mice (\$), and between CBA and C57 mice (&)

Table 1

Between-strain comparison of difference scores

(A) Acute nicotine		(B) Nicotine withdrawal		
Fear conditioning		Fear conditioning		Elevated plus maze
Immediate freezing		Contextual fear conditioning		Closed arms
0.18 mg/kg	BALB vs. 129 ^{***} , A ^{***} , D1 ^{***} , D2 ^{***}	6.3 mg/kg/d	C3H vs. 129 ^{***} , A ^{***} , BALB ^{***} , CBA ^{***} , D1 ^{***} , D2 ^{***}	12 mg/kg/d
	CBA vs. 129 ^{***} , A ^{***} , D1 ^{***} , D2 ^{***}		B6 vs. 129 ^{***} , BALB ^{***} , CBA ^{***} , D1 ^{***} , A vs. 129 ^{***}	D2 vs. A ^{***} , B6 ^{***} , CBA ^{***} , C3H ^{***}
Contextual fear conditioning		Contextual fear conditioning		Open arms
0.045 mg/kg	BALB vs. 129 ^{***} , C3H ^{***} , CBA ^{***} , D1 ^{***} , D2 ^{***}	12 mg/kg/d	129 vs. BALB ^{***} , B6 ^{***} , D1 ^{***}	6.3 mg/kg/d
	A vs. D1 ^{***} , D2 ^{***} , C3H ^{***}		C3H vs. 129 ^{***} , A ^{***} , B6 ^{***} , CBA ^{***} , D2 ^{***}	D1 vs. D2 ^{***}
	B6 vs. D1 ^{***} , C3H ^{***}			
0.09 mg/kg	D2 vs. 129 ^{***} , A ^{***} , C3H ^{***} , CBA ^{***}	Generalized freezing		
	B6 vs. A ^{***} , CBA ^{***}	6.3 mg/kg/d	B6 vs. A ^{***}	
0.18 mg/kg	129 vs. A ^{***} , C3H ^{***} , B6 ^{***} , CBA ^{***}	12 mg/kg/d	BALB vs. 129 ^{***} , A ^{***}	
	D1 vs. A ^{***} , C3H ^{***} , B6 ^{***} , CBA ^{***}		D1 vs. 129 ^{***} , A ^{***}	
	D2 vs. C3H ^{***} , CBA ^{***}	Cued fear conditioning		
		12 mg/kg/d	BALB vs. D2 ^{***}	

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$

Table 2

Between-strain comparison of saline treated mice

(A) Acute saline		(B) Chronic saline		(C) Saline withdrawal	
Fear conditioning		Fear conditioning	Somatic signs	Elevated plus maze	Somatic signs
Immediate freezing		Immediate freezing	Grooming	Closed arms	Grooming
$[F(7, 91) = 40.88, p < 0.05]$		$[F(7, 71) = 23.78, p < 0.05]$	$[F(7, 72) = 10.27, p < 0.05]$	$[F(7, 65) = 3.42, p < 0.05]$	$[F(7, 98) = 15.81, p < 0.05]$
129 vs. CBA ^{***} , C3H ^{***} , BALB ^{***}		129 vs. CBA ^{***} , C3H ^{***} , BALB ^{***} , B6 ^{***}	C3H vs. B6 ^{**}	BALB vs. B6 [*] , D2 ^{**}	B6 vs. 129 ^{***} , BALB ^{***} , C3H ^{***} , CBA ^{***}
A vs. BALB ^{**} , B6 [*] , CBA ^{**}		A vs. BALB ^{**} , C3H [*] , B6 ^{**} , CBA ^{**}	D1 vs. 129 ^{***} , A ^{***} , BALB [*] , C3H ^{***} , CBA ^{***}	Open Arms	D1 vs. BALB [*]
D1 vs. BALB ^{**} , C3H [*] , B6 [*] , CBA ^{**}		D1 vs. BALB ^{**} , C3H ^{***} , B6 ^{***}	D2 vs. 129 [*] , C3H ^{***}	$[F(7, 65) = 4.30, p < 0.05]$	D2 vs. 129 ^{**} , BALB ^{***} , C3H ^{***} , CBA [*]
D2 vs. all ^{***} except 129		D2 vs. all ^{**} except 129, D1	Scratching	BALB vs. 129 [*] , B6 [*] , D2 ^{***}	Scratching
Contextual fear conditioning		Contextual fear conditioning	$[F(7, 72) = 2.16, p < 0.05]$	C3H vs. D2 [*]	$[F(7, 94) = 6.69, p < 0.05]$
$[F(7, 91) = 14.32, p < 0.05]$		$[F(7, 71) = 29.43, p < 0.05]$	No post-hoc differences	Center	Open arms
129 vs. BALB [*] , C3H ^{***} , B6 ^{***} , CBA ^{**} , D1 ^{***} , D2 ^{***}		129 vs. all ^{**}	Shaking	$[F(7, 65) = 10.85, p < 0.05]$	$[F(7, 94) = 10.18, p < 0.05]$
A vs. BALB [*] , C3H ^{***} , CBA ^{**} , D1 ^{***} , D2 ^{***}		A vs. all ^{**} except B6	$[F(7, 72) = 2.58, p < 0.05]$	129 vs. all ^{**} except 129	D1 vs. BALB ^{***} , C3H ^{***} , CBA ^{***}
B6 vs. D1, C3H		B6 vs. D1 ^{***} , D2 ^{***} , C3H ^{***}	No post-hoc differences	A vs. C3H ^{**}	D2 vs. BALB ^{***} , C3H ^{***} , CBA ^{***}
Generalized Freezing		Generalized Freezing	Jumping	C3H vs. B6 [*] , BALB [*]	B6 vs. BALB ^{***} , C3H ^{***} , CBA ^{***}
$[F(7, 91) = 45.33, p < 0.05]$		$[F(7, 71) = 49.55, p < 0.05]$	$[F(7, 72) = 4.93, p < 0.05]$	Generalized Freezing	129 vs. BALB ^{**} , C3H ^{***}
A vs. all ^{**}		A vs. all ^{***}	CBA vs. 129 [*] , BALB [*] , A [*] , D2 [*]	$[F(7, 104) = 79.81, p < 0.05]$	Center
				A vs. all ^{***}	Jumping
					$[F(7, 94) = 10.36, p < 0.05]$

[illegible]

*
 $p < 0.05$;
***
 $p < 0.01$;
****
 $p < 0.001$

Table 3

Between-strain comparisons of nicotine metabolism

Acute nicotine	Chronic nicotine
Plasma cotinine	Plasma nicotine
D2 vs. all ^{**} except 129	BALB vs. D1 [*]
BALB vs. A ^{**} , C3H ^{**}	
Nicotine/cotinine ratio	Plasma cotinine
D2 vs. 129 ^{**} , A ^{**} , C3H ^{**}	129 vs. all ^{****} except D1
	B6 vs. CBA [*]
	D1 vs. all ^{**} except 129
	D2 vs. all ^{**} except A
	Nicotine/cotinine ratio
	129 vs. A ^{**} , BALB ^{**} , C3H ^{**} , B6 ^{****} , CBA ^{****} , D1 [*] , D2 ^{**}
	D1 vs. C3H [*] , B6 [*]
	D2 vs. A [*] , C3H ^{****} , B6 ^{****} , CBA [*]

^{*} $p < 0.05$;
^{**} $p < 0.01$;
^{***} $p < 0.001$