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Mild anxiogenic effects of nicotine withdrawal in mice

Sietse Jonkman, Brook Henry, Svetlana Semenova, Athina Markou*

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, USA

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Abstract

Increased anxiety is one of the symptoms of nicotine withdrawal that may lead to relapse. Previous studies have shown that nicotine withdrawal affects anxiety-like behavior in different tests of anxiety in humans and rats. However, relatively few studies have focused on the anxiogenic effect of nicotine withdrawal in mice. The present study investigated the effect of nicotine withdrawal on anxiety-like behavior in DBA/2J and C57BL/6J mouse strains in the light–dark box, acoustic startle response, and prepulse inhibition tests. An initial experiment showed that nicotine administration of 12 or 24 mg/kg/day (free base) for 14 days did not result in significant effects during withdrawal in startle, prepulse inhibition, or light–dark box, but there was a trend towards an anxiogenic effect in the light–dark box 24 h, but not 1 or 4 h, after cessation of nicotine administration. A subsequent study was therefore performed, with minipumps delivering saline, 24 mg/kg/day nicotine, or 48 mg/kg/day nicotine (free base), for 14 days. The pumps were removed, and the mice were tested 24 h after cessation of nicotine administration. Cessation of administration of 48 mg/kg/day nicotine free base in C57BL/6J mice resulted in increased anxiety-like behavior in the light–dark box, while the behavior of DBA/2J mice was unaffected. The acoustic startle response and prepulse inhibition were also unaffected in both strains. In conclusion, the present data show that nicotine withdrawal is mildly anxiogenic in C57BL/6J mice under the conditions used in the present experiments.

Keywords: Light–dark box; Anxiety; Nicotine; Startle; Withdrawal; Prepulse inhibition; (Mouse)

1. Introduction

The negative affective aspects of nicotine withdrawal have been hypothesized to contribute to tobacco dependence and to high rates of relapse to tobacco smoking (Kenny and Markou, 2001; Hughes and Hatsukami, 1986). Withdrawal from chronic nicotine administration in humans results in an abstinence syndrome (Hughes and Hatsukami, 1986; Hughes et al., 1991; Shiffman and Jarvik, 1976). This syndrome is characterized by somatic signs, such as bradycardia, insomnia, gastrointestinal discomfort, and increased appetite (Hughes et al., 1991), as well as affective symptoms, such as depressed mood, irritability, anxiety, frustration, difficulty concentrating, and craving for tobacco (American Psychiatric Association, 1994). Animal models of nicotine withdrawal are important tools for understanding the neurobiological bases, including the genetic bases, of nicotine dependence and for developing effective treatment strategies to facilitate nicotine abstinence.

Increased anxiety is one affective aspect of nicotine withdrawal in humans (American Psychiatric Association, 1994; Hughes et al., 1994). The light–dark box is a mouse-specific test of anxiety-like behavior that is based on the conflict between the tendencies to explore a novel environment and to avoid brightly lit places. Mice are placed in a novel environment that consists of a dark area and a brightly lit area. The time spent in the dark compartment and the latency to the first cross into the light area are considered measures of aversion to the brightly lit compartment. The number of transitions between the two sides of the box is considered an index of activity and exploration (for review, see Bourin and Hascoet, 2003). Thus, drugs that increase the time spent in the light compartment and decrease the latency to the first transition are considered anxiolytic, while drugs that produce the reverse pattern are considered anxiogenic

* Corresponding author. Tel.: +1 859 784 7244; fax: +1 859 784 7405.
E-mail address: amarkou@scripps.edu (A. Markou).

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(Bourin and Hascoet, 2003). One early study reported increased anxiety-like behavior in the light–dark box during spontaneous nicotine withdrawal after 14 days of injections (twice daily, 0.1 mg/kg intraperitoneally, free base) in BKW mice (Costall et al., 1989). The elevated plus-maze is another test of anxiety-like behavior that is also based on the concept of conflict (i.e., tendency to explore a novel environment and avoid open high places). A recent study reported increased anxiety-like behavior in the elevated plus-maze during withdrawal from chronic nicotine administration (14-day minipumps, 24 and 48 mg/kg/day nicotine free base, tested 24 h post-pump removal) in C57BL/6J and 129/SvEv mice (Damaj et al., 2003). In this study, C57BL/6J mice were more sensitive to the anxiogenic effect of nicotine withdrawal than 129/SvEv mice (Damaj et al., 2003). In rats, studies demonstrated that nicotine withdrawal is associated with anxiety-like responses in the elevated plus-maze test and the social interaction test (Cheeta et al., 2001; Irvine et al., 2001a,b).

The acoustic startle response reflects reactivity to environmental stimuli that may be increased in an anxious state. Prepulse inhibition of the startle response reflects sensorimotor gating of environmental stimuli. In humans, nicotine withdrawal has been reported to decrease prepulse inhibition (Kumari and Grey, 1999; Postma et al., 2001), while it had no effect on startle amplitude (Kumari and Grey, 1999; Mueller et al., 1998; Postma et al., 2001). Nevertheless, non-human animal studies investigating the effects of nicotine withdrawal on startle and prepulse inhibition reported conflicting results. Specifically, Acri et al. (1991), using Sprague–Dawley rats, reported a decrease, while Helton et al. (1993), using Long–Evans rats, reported an increase in startle amplitude during nicotine withdrawal. In DBA/2J mice, nicotine withdrawal did not affect the magnitude of the acoustic startle response, while prepulse inhibition of startle was decreased (Semenova et al., 2003).

The purpose of the present study was to further characterize the affective aspects of spontaneous nicotine withdrawal in mice, using the light–dark box as a measure of anxiety-like behavior, the acoustic startle test as a measure of reactivity to environmental stimuli that may be increased when in an anxious state, and prepulse inhibition as a measure of sensorimotor gating. We used C57BL/6J mice to extend the findings by Damaj et al. to a different test of anxiety-like behavior (i.e., light–dark box) than the one previously used (i.e., elevated plus-maze), and DBA/2J mice to further explore potential mouse strain differences. There are strain differences in sensitivity to nicotine between DBA/2J and C57BL/6J mice (Crawley et al., 1997). We exposed DBA/2J and C57BL/6J mice to saline, 12 mg/kg/day nicotine, and 24 mg/kg/day nicotine (free base), and tested the animals in the light–dark box, acoustic startle, and prepulse inhibition test 1, 4, and 24 h after cessation of nicotine administration. Based on the tendency for anxiogenic-like effects seen in the mice 24 h after termination of administration of the highest nicotine dose, in the second experiment, we exposed DBA/2J and C57BL/2J mice to higher nicotine doses (saline, 24 mg/kg/day nicotine, and 48 mg/kg/day nicotine, free base) for 14 days, and then studied their behavior in startle, prepulse inhibition, and light–dark box 24–26 h after the cessation of nicotine administration.

2. Materials and methods

2.1. Subjects

Adult experimentally naive DBA/2J and C57BL/6J male mice 10–12 weeks old and weighing 22–25 g were purchased from Harlan (Indianapolis, IN). Mice were housed in a humidity-controlled (50–70%) and temperature-controlled (20–22 °C) animal facility on a 12 h:12 h light/dark cycle (lights off at 6 am) with ad libitum access to food and water except during testing. All tests and other manipulations were conducted during the dark phase of the cycle. The experiments were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. All experimental protocols and animal facilities were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care and the National Institutes of Health guidelines.

2.2. Drugs

Nicotine tartrate (Sigma-Aldrich, St. Louis, USA) was dissolved in sterile 0.9% saline and infused through subcutaneous osmotic minipumps (model 2002; Alzet Co., Palo Alto, CA) that delivered 0.5 μl of solution per hour. Minipumps were prepared to deliver saline, 12 mg/kg/day nicotine, 24 mg/kg/day nicotine, or 48 mg/kg/day nicotine (free base; equivalent to 68 and 137 mg/kg/day salt), for 14 days. The concentration of the solution in the pump was adjusted to the individual weights of the mice.

2.3. Minipump implantation and removal

Mice were anesthetized with an isoflurane/oxygen vapor mixture (1–3%) and 14-day osmotic minipumps (model 2002; Alzet Co., Palo Alto, CA) were inserted subcutaneously using aseptic surgery techniques. The pump was placed parallel to the spine at the shoulder level with the flow moderator directed posteriorly. The wound was closed with 7 mm stainless steel wound clips (Becton Dickinson Primary Care Diagnostics, Sparks, MD), and antibacterial Bacitracin zinc ointment USP (Alpharma USPD Inc., Baltimore, MD) was applied to the incision area. Fourteen days later, the pumps were surgically removed under isoflurane anesthesia.

2.4. Light–dark box

The light–dark box was located in a dark room, and consisted of a black compartment (27 × 15 cm) that was not illuminated, and a white illuminated (40 W light bulb, located 1.5 m above the white compartment) compartment (27 × 29 cm). Both compartments were connected through an opening (10 × 10 cm). At the

beginning of the test, the mouse was placed in the dark compartment, with its head facing away from the opening. The behavior of the mouse was recorded on videotape for 5 min. The total time spent in the white compartment, the latency to the first transition, and the number of transitions were later scored from the tapes by an observer blind to the mouse's treatment. The mouse was considered to enter the white compartment when both front paws and hind paws entered the white compartment, and, similarly, it was considered to enter the dark compartment if both front paws and hind paws entered the dark compartment. In between trials, feces were removed and the surface was wiped with a wet paper towel. One day prior to the experiment, mice were handled for 5 min to habituate them to human handling.

2.5. Acoustic startle and prepulse inhibition

Three acoustic startle apparatuses were used (SR-LAB; San Diego Instruments, San Diego, CA), each consisting of a 5.1 cm (outside diameter) Plexiglas cylinder mounted on a Plexiglas platform and enclosed in ventilated sound-attenuated cubicles equipped with high-frequency loudspeakers. Movements within the cylinder were detected and transduced by a piezoelectric accelerometer attached to the platform, digitized, and stored by the operating computer.

After the mice were placed in the non-illuminated startle chambers, the 70-dB background noise was presented for a 5-min acclimation period and continued throughout the test session. During a testing session, all trial types were presented several times in a pseudorandom order for a total of 60 trials (12 pulse-alone trials, 12 no-stimulus trials, 12 4-dB prepulse+pulse trials, 12 8-dB prepulse+pulse trials, and 12 12-dB prepulse+pulse trials). In addition, six pulse-alone trials, which were not included in the calculation of prepulse inhibition values, were presented at the beginning and six more pulse-alone trials at the end of each test session to assess startle habituation throughout the session. The time between trials averaged 15 s (ranging from 12 to 30 s) and the total duration of a test session was approximately 25 min. The pulse-alone trial consisted of a 40-ms 120-dB pulse of broadband noise. The prepulse+pulse trials consisted of a 20-ms noise prepulse, a 100-ms delay, then a 40-ms 120-dB startle pulse. Prepulse intensities were 4, 8, and 12 dB above the 70-dB background level. The no-stimulus trial consisted of background noise only and allowed assessment by the piezoelectric accelerometer of general activity in the startle chamber when no acoustic stimuli were presented. Startle magnitude was calculated as the average response to all of the pulse-alone trials, excluding the first and last blocks of five pulse-alone trials. The amount of prepulse inhibition was calculated as a percentage score for each prepulse trial type: % PPI = 100 − ([(startle response for prepulse+pulse)/(startle response for pulse-alone)] × 100).

2.6. Experimental procedures

Behavior in the light–dark box was assessed before the acoustic startle test. This sequence of testing was selected to minimize potential influences of successive testing of the same subjects. Exposure to the light–dark box is not expected to have any effects on startle responding. There was an interval of 2 h between the two tests.

For the first experiment, DBA/2J (n = 8 per dose) and C57BL/6J (n = 8 per dose) mice were surgically prepared with minipumps that delivered either saline, 12 mg/kg/day nicotine, or 24 mg/kg/day nicotine, free base, for 14 days (on day 1 of the experiment). After 14 days (on day 15 of the experiment), the pumps were surgically removed. All mice were tested in the acoustic startle and prepulse inhibition tests 24 h after cessation of nicotine administration. The C57BL/6J mice that had been exposed to saline and 24 mg/kg/day nicotine were also tested in the light–dark box, 1, 8, and 24 h after cessation of nicotine administration. In the second experiment, DBA/2J (n = 15 per dose) and C57BL/6J (n = 16 per dose) mice were surgically prepared with minipumps that delivered either saline, 24 mg/kg/day nicotine, or 48 mg/kg/day nicotine (free base) for 14 days. Fourteen days later, the pumps were surgically removed. Twenty-four hours after removal of the pumps, mice were tested in the light–dark box, and subsequently (approximately 2 h later) in the startle chambers. The 24-h time point after the removal of the pump was chosen based on prior work showing that withdrawal signs are present at this time point in mice (Semenova et al., 2003), and a report of an anxiogenic effect 24 h after cessation of nicotine administration in mice (Damaj et al., 2003). Light–dark box testing was conducted between 10:00 am and 1:00 pm.

2.7. Statistical analyses

For the light–dark box data (measures: time in the white compartment, latency to first crossing, number of transitions), a two-way analysis of variance (ANOVA) with Strain and Nicotine dose as between-subjects factors was performed on all three measures, followed by one-way ANOVAs (nicotine dose being the one factor) for each strain. The acoustic startle data (measures: startle magnitude and prepulse inhibition) were analyzed with a three-way ANOVA with Strain and Nicotine dose as the two between-subjects factors, and Block (for startle magnitude) and Intensity (for prepulse inhibition) as the respective within-subjects factors. Statistically significant results were followed by two-way and one-way ANOVAs as appropriate. For all experiments, statistically significant results were followed by Newman–Keuls post hoc analyses. The level of significance was set at 0.05.

Five of the DBA/2J mice that received the highest nicotine dose showed damage to the outer skin at the position of the flow moderator (away from the incision site). Probable cause of this damage was the high acidity of the nicotine solution at the highest dose. These five mice were subsequently excluded from all analyses. One of the three startle chambers was malfunctioning during the testing of the C57BL/6J mice. This equipment malfunction reduced the number of C57BL/6J animals in the startle and prepulse inhibition tests to 14 for the saline dose, to 13 for the 24 mg/kg/day, and to 11 in the 48 mg/kg/day dose groups.

3. Results

3.1. Light–dark box

In the first experiment, there were no significant differences between saline- and 24 mg/kg/day nicotine-treated C57BL/6J mice for any of the measures at either time point ($P > 0.05$). However, as seen in Fig. 1, there was a tendency for anxiety-like behavior in the 24 mg/kg/day nicotine group. Thus, the effects of cessation of administration of higher daily nicotine doses were investigated in the second experiment.

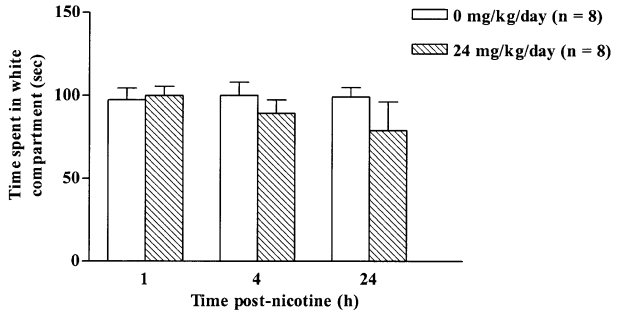

Fig. 1. Effects of saline/nicotine exposure (mg/kg/day, free base, for 14 days) on time spent in the white compartment 1, 4, and 24 h after cessation of nicotine administration in C57BL/6J. Data are presented as mean (±S.E.M.) number of seconds spent in the white compartment.

In the second experiment, nicotine withdrawal had no effect on time spent in the white compartment for DBA/2J mice, but significantly decreased time spent in the white compartment for C57BL/6J mice. There was a main effect of Strain ($F_{(1,82)}=95.82$, $P<0.01$) in the two-way ANOVA, but the effect of Nicotine dose on time spent in the white compartment was not significant ($P>0.05$). Based on our a priori hypothesis, a one-way ANOVA on the C57BL/6J data indicated a significant effect of Nicotine dose ($F_{(2,45)}=3.99$, $P<0.05$) on the time spent in the white compartment. Post hoc analyses indicated that exposure to the highest nicotine dose (48 mg/kg/day) significantly decreased the time spent in the white compartment compared to the saline-treated controls in C57BL/6J mice ($P<0.05$; see Fig. 2A). One-way ANOVA of the time spent in the white compartment of the DBA/2J mice did not reveal an effect of Nicotine dose ($P>0.05$).

Latency to first transition did not differ significantly between Nicotine doses for either strain. There was no significant effect of Nicotine dose in the two-way ANOVA ($P>0.05$), while the effect of Strain was significant ($F_{(1,82)}=13.70$, $P<0.01$). One-way ANOVAs were performed for both strains. The effect of Nicotine dose was not significant for either the DBA/2J ($P>0.05$) or the C57BL/6J mice ($P>0.05$; see Fig. 2B).

The number of transitions did not differ significantly between the Nicotine doses in both strains. There was no significant effect of Nicotine dose in the two-way ANOVA ($P>0.05$), while the effect of Strain was significant ($F_{(1,82)}=15.19$, $P<0.01$). Follow-up one-way ANOVAs indicated that the effect of Nicotine dose was not significant for either DBA/2J ($P>0.05$) or C57BL/6J mice ($P>0.05$; see Fig. 2C).

3.2. Acoustic startle

In the first experiment, there were no significant effects of Nicotine dose on either acoustic startle or prepulse inhibition (data not shown). In the second experiment, chronic nicotine exposure had no effect on startle amplitude in either strain (Table 1). The effect of Block in the three-way ANOVA was significant ($F_{(3,81)}=9.01$, $P<0.01$), reflecting a habituation effect. There was a significant interaction between Block and Strain ($F_{(1,81)}=3.51$, $P<0.05$), but there was no significant interaction between Block and Nicotine ($P>0.05$). DBA/2J mice appeared to exhibit increased startle amplitude with chronic nicotine exposure, but this effect was not statistically significant in the one-way ANOVA ($P>0.05$). One-way ANOVA of the C57BL/6J data revealed no effect of nicotine ($P>0.05$). Analysis of the mean startle amplitude also revealed no significant effect of Nicotine dose ($P>0.05$).

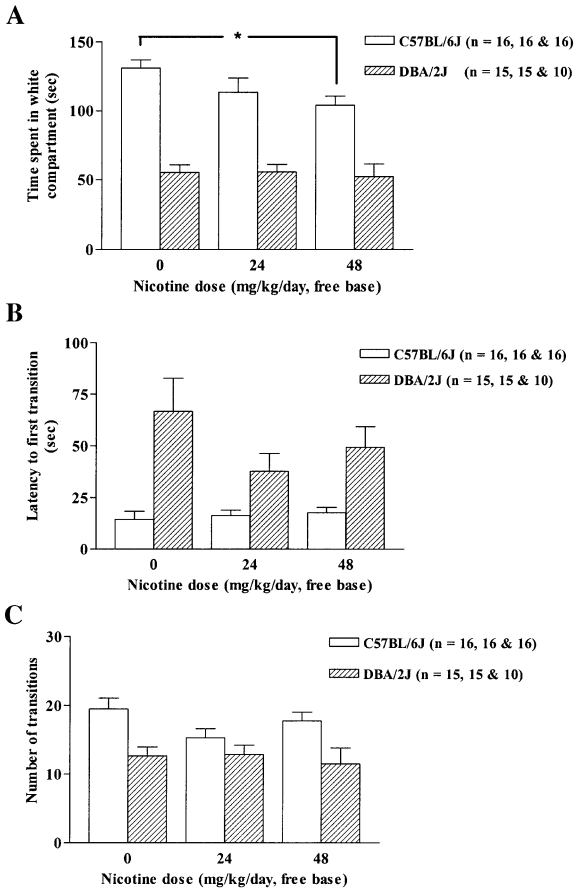

Fig. 2. (A) Effects of saline/nicotine exposure (mg/kg/day, free base, for 14 days) on time spent in the white compartment in C57BL/6J and DBA/2J mice. Data are presented as mean (±S.E.M.) number of seconds spent in the white compartment (*$P<0.05$). Mice were tested 24 h after cessation of nicotine administration. (B) Effects of saline/nicotine exposure (mg/kg/day, free base, for 14 days) on latency to first transition into the white compartment in C57BL/6J and DBA/2J mice. Data are presented as mean (±S.E.M.) number of seconds before entry into the white compartment. (C) Effects of saline/nicotine exposure (mg/kg/day, free base, for 14 days) on the number of transitions between light and dark compartments in C57BL/6J and DBA/2J mice. Data are presented as mean (±S.E.M) number of transitions.

Table 1
Effects of saline/nicotine exposure on acoustic startle response in C57BL/6J and DBA/2J mice

Strain	Nicotine dose (mg/kg/day)	*n*	Startle amplitude
DBA/2J	Saline	16	38.3±6.5
	24	16	44.3±7.2
	48	11	50.0±12.2
C57BL/6J	Saline	14	248.8±25.8
	24	13	240.7±21.7
	48	11	239.2±30.6

Data are presented as mean startle amplitude (±S.E.M.).

Table 2
Effects of saline/nicotine exposure on prepulse inhibition in C57BL/6J and DBA/2J mice

Strain	Nicotine dose (mg/kg/day)	*n*	% PPI (4 dB)	% PPI (8 dB)	% PPI (12 dB)
DBA/2J	Saline	16	−8.0±7.2	12.6±8.7	33.1±8.7
	24	16	−3.8±5.9	1.7±7.8	19.9±5.8
	48	11	−4.3±11.0	7.2±7.2	23.2±5.7
C57BL/6J	Saline	14	−7.3±5.8	16.9±5.4	29.4±6.7
	24	13	0.8±5.6	25.9±5.1	45.6±4.6
	48	11	−5.5±6.7	21.3±2.4	42.9±3.6

Data are presented as mean values (±S.E.M.). PPI is prepulse inhibition of the startle response expressed as percentage of the startle response. Decibel values represent the decibels of the prepulse acoustic stimulus above the background noise level.

3.3. Prepulse inhibition

Chronic exposure to nicotine did not significantly affect prepulse inhibition for either mouse strain (Table 2). The three-way ANOVA showed that prepulse Intensity had a significant effect on prepulse inhibition ($F_{(2,80)}=72.55$, $P<0.01$), but there was no interaction with either Strain ($P>0.05$) or Nicotine ($P>0.05$). Two-way ANOVA of the DBA/2J data confirmed that prepulse Intensity had a significant effect on prepulse inhibition ($F_{(2,80)}=18.6$, $P<0.01$), but there was no interaction with Nicotine treatment ($P>0.05$). Two-way ANOVA of the C57BL/6J data also showed a significant effect of prepulse Intensity on prepulse inhibition ($F_{(2,70)}=93.7$, $P<0.01$), but no interaction with Nicotine dose ($P>0.05$).

4. Discussion

The results of this study demonstrated that withdrawal from nicotine administration increased anxiety-like behavior of C57BL/6J mice in the light–dark box, while the behavior of DBA/2J mice in the light–dark box was not altered during withdrawal. The fact that the number of transitions in the light–dark box of C57BL/6J mice was not affected during withdrawal suggests that the effect was genuinely anxiogenic. These data suggest that nicotine withdrawal has mild anxiogenic effects in C57BL/6J mice.

DBA/2J mice showed more anxiety-like behavior in the light–dark box than C57BL/6J mice under baseline conditions, which is consistent with reported strain differences in other behavioral tests (Crawley et al., 1997). The effect of nicotine withdrawal was also different between the two strains. Nicotine withdrawal did not affect anxiety-like behavior of DBA/2J mice at the doses tested, while it had a small but significant anxiogenic effect on C57BL/6J mice. Time spent in the white compartment was unchanged during withdrawal for DBA/2J mice, but significantly decreased for C57BL/6J mice during withdrawal from the highest nicotine dose. The latency to the first crossing into the white compartment was unchanged during nicotine withdrawal for both strains. The total number of transitions between compartments was also unchanged for both strains, indicating that nicotine withdrawal did not affect general locomotor activity. These results are consistent with the results of another study using twice-daily injections of 0.1 and 1.0 mg/kg nicotine that demonstrated an increase in anxiety-like behavior in the light–dark box in BKW mice during withdrawal (Costall et al., 1989). The elevated plus-maze is another test of anxiety-like behavior that, similarly to the light–dark box, is based on the conflicting motivations to explore a novel environment and to avoid anxiety-provoking circumstances such as open spaces. Consistent with the results of the present study, both C57BL/6J and 129/SvEv mice undergoing withdrawal after cessation of continuous administration of 24 and 48 mg/kg/day nicotine for 14 days showed increased anxiety-like behavior in the elevated plus-maze (Damaj et al., 2003). Further, rat studies demonstrated that nicotine withdrawal led to increased anxiety-like behavior in the elevated plus-maze and the social interaction test (Cheeta et al., 2001; Irvine et al., 2001a,b). Thus, the current finding that nicotine withdrawal produced a mild anxiogenic-like effect in C57BL/6J mice is consistent with previous findings in the literature. The finding that C57BL/6J mice are relatively sensitive to the effects of nicotine in an exploration/avoidance test is also consistent with findings from Damaj et al. (2003), who demonstrated that C57BL/6J mice were more sensitive to the effect of nicotine withdrawal in the elevated plus-maze than 129/SvEv mice.

In the startle test, DBA/2J mice exhibited lower startle amplitude than C57BL/6J mice under baseline conditions. This strain difference in reactivity to the acoustic startle test has been well documented (Crawley et al., 1997; Spielewoy and Markou, 2004). Nicotine withdrawal did not have a statistically significant effect on the startle amplitude of either strain. The only other study of acoustic startle response during nicotine withdrawal in mice also reported no significant effect on the startle response in DBA/2J mice (Semenova et al., 2003). Studies of the effect of nicotine withdrawal on startle amplitude using rats have yielded conflicting results, with decreased startle amplitude in Sprague–Dawley rats (Acri et al., 1991), but increased startle amplitude in Long–Evans rats (Helton et al., 1993). Considering these conflicting results, it is not surprising that there was no clear increase or decrease in startle amplitude during nicotine withdrawal in the present study.

Prepulse inhibition did not differ between the two strains under baseline conditions. Further, nicotine withdrawal did not have an effect on prepulse inhibition values for either strain, indicating that sensorimotor gating was not affected by nicotine withdrawal. A previous study that investigated prepulse inhibition during nicotine withdrawal in mice reported decreased prepulse inhibition using lower nicotine doses than in the present study (6 mg/kg/day, free base) in DBA/2J mice (Semenova et al., 2003). Another recent study reported no effects of acute nicotine administration on prepulse inhibition in C57BL/6J or DBA/2J mice (Spielewoy and Markou, 2004). In rats, some studies of nicotine

withdrawal found decreases in prepulse inhibition (Acri et al., 1991), while others found increases (Helton et al., 1993). Thus, the existing literature on the effects of nicotine withdrawal on prepulse inhibition reports mixed results.

As discussed above, nicotine withdrawal had different effects in the two mouse strains. These differential effects of nicotine withdrawal may be attributable to differences in pharmacokinetic and distribution factors, and differing nicotinic acetylcholine receptor distribution and metabolism in the hippocampus, caudate nucleus, and frontal–parietal cortex of these mice (Durkin et al., 1977; Stevens et al., 1996). Further, there were significant effects of strain in both the light–dark box and the startle test under baseline conditions. C57BL/6J mice display less anxiety-like behavior in the light–dark box than DBA/2J mice under baseline conditions, and nicotine withdrawal only affected the behavior of the C57BL/6J mice. It is therefore conceivable that the lack of effect in DBA/2J mice under these conditions is partly due to a ceiling effect, where higher baseline values of anxiety-like behavior are not increased during nicotine withdrawal, while lower values are.

In conclusion, the present data show that withdrawal from chronic nicotine administration of the reported doses and length of exposure affected anxiety-like behavior in the light–dark box in C57BL/6J but not DBA/2J mice. The increase in anxiety-like behavior in C57BL/6J mice was not accompanied by decreases in locomotion, and thus may probably a genuine anxiety-related effect. The acoustic startle response and prepulse inhibition were unaffected in either strain. It is possible that a longer nicotine exposure regimen and the use of repeated acute injections could lead to larger effects of nicotine withdrawal in mice.

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