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Decreased prepulse inhibition during nicotine withdrawal in DBA/2J mice is reversed by nicotine self-administration

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Abstract

We characterized spontaneous and mecamylamine-precipitated nicotine withdrawal using intravenous nicotine self-administration, the acoustic startle response, prepulse inhibition and somatic signs of withdrawal in DBA/2J mice. Nicotine dependence was induced by continuous nicotine infusion through osmotic minipumps. Nicotine self-administration was studied before and after the induction of dependence. The initial test revealed significant nicotine self-administration at the 0.048 μg/infusion dose. During the second self-administration test, saline-treated mice exhibited increased aversiveness of response-contingent infusions of high nicotine doses; these changes were not seen in the nicotine-treated animals reflecting tolerance to nicotine's effects. Neither mecamylamine administration nor spontaneous withdrawal affected the expression of somatic signs, except that increases in jumping were observed during spontaneous withdrawal. Finally, nicotine withdrawal increased general activity in the startle chambers when no stimuli were presented, possibly reflecting increased body tremor and/or agitation, and decreased prepulse inhibition reflecting a sensorimotor gating deficit; the last two effects were reversed by nicotine self-administration. Thus, nicotine withdrawal results in modest, but yet detectable, changes in the behavior of mice.

Keywords: Nicotine withdrawal; Self-administration; Acoustic startle; Prepulse inhibition; Somatic sign; 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). Withdrawal from chronic nicotine consumption in nicotine-dependent humans (i.e., smoking cessation) results in an abstinence syndrome (Hughes and Hatsukami, 1986; Hughes et al., 1991; Shiffman and Jarvik, 1976) which includes a number of somatic signs, such as bradycardia, insomnia, gastrointestinal discomfort and increased appetite (Hughes et al., 1991), as well as affective symptoms including 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, including the genetic, bases of nicotine dependence and for developing effective treatment strategies to facilitate nicotine abstinence. Mice are perhaps the most commonly used species for behavioral genetic studies (for review, Crawley et al., 1997). Thus, the purpose of the present studies was to characterize both the somatic and affective aspects of the nicotine withdrawal syndrome in mice because, although the nicotine withdrawal syndrome has been extensively characterized in rats, little is known about nicotine withdrawal in mice.

In rats, withdrawal from chronic continuous nicotine administration delivered through subcutaneous osmotic minipumps resulted in somatic signs, such as abdominal constrictions, facial fasciculation, increased eye blinks and ptosis (Epping-Jordan et al., 1998; Harrison et al., 2001; Hildebrand et al., 1997; Malin et al., 1992; Watkins et al., 2000b). This somatic syndrome also can be precipitated by administration of nicotinic acetylcholine receptor antagonists such as mecamylamine, hexamethonium, chlorisondamine and dihydro-β-erythroidine (Hildebrand et al., 1997; Malin et al., 1994, 1996, 1998; Markou and Paterson, 2001; Watkins et al., 2000b). Studies in nicotine-dependent mice indicated that mecamylamine could precipitate various withdrawal symp-

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toms (e.g., rearings, jumps, head, forelimb and body shakes, abdominal constrictions, chewing, facial and body tremor, scratching, genital licks, ptosis and piloerection) in Swiss Webster (Isola et al., 1999) and CD1 Swiss albino (Castane et al., 2002) mice. Nevertheless, somatic signs of nicotine withdrawal do not reflect the affective aspects of withdrawal (Watkins et al., 2000a,b). The assessment of the affective aspects of withdrawal is important because it is hypothesized that the affective symptoms of withdrawal contribute significantly more to craving, relapse and the maintenance of dependence than the somatic aspects of withdrawal (Kenny and Markou, 2001; Markou et al., 1998). One affective aspect of nicotine withdrawal in humans is increased anxiety (American Psychiatric Association, 1994; Hughes et al., 1994). In rats, studies also have 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). Additionally, the acoustic startle response and prepulse inhibition are purported to reflect reactivity to environmental stimuli that may be an aspect of anxiety-like responding and sensorimotor gating, respectively. 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). Interestingly, 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 following nicotine withdrawal. Faraday et al. (1998, 1999), using the same two rat strains, confirmed that the modulatory effects of nicotine withdrawal on both startle and prepulse inhibition response were strain-dependent. Thus, in the present study, both the startle response and prepulse inhibition were assessed during spontaneous nicotine withdrawal in mice.

To assess potential changes in the reinforcing effects of self-administered nicotine during the early withdrawal stages compared to the non-dependence state, a nicotine self-administration paradigm was used. In humans, most reports focused on cigarette smoking behavior, although some studies examined intravenous nicotine self-administration (Henningfield et al., 1983; Goldberg and Henningfield, 1996; Perkins, 1999; for review, Rose and Corrigall, 1997). In animals, intravenous nicotine self-administration was demonstrated in non-human primates (Goldberg et al., 1981; Sannerud et al., 1994), dogs (Risner and Goldberg, 1983), rats (Chiamulera et al., 1996; Corrigall and Coen, 1989; Donny et al., 1995, 1998; Markou and Paterson, 2001; Paterson and Markou, 2002; Shaham et al., 1997; Shoaib et al., 1997; Watkins et al., 1999) and mice (Martellotta et al., 1995; Picciotto et al., 1998; Rasmussen and Swedberg, 1998; Stolerman et al., 1999). Nicotine replacement therapy in humans (sublingual nicotine tablets: Molander et al., 2000; nicotine gum: Schneider et al., 1984; nicotine patch: Rose et al., 2001; Fagerstrom et al., 1993) was shown to attenuate the severity of withdrawal symptoms and partially reduced craving for nicotine in abstinent smokers. Meanwhile, to the best of our knowledge, there are no reports on nicotine self-administration during nicotine withdrawal in mice. The self-administration protocol used in our experiments has been specifically designed for relatively "acute" studies in mice, and thus does not require extensive training. Using this procedure, there have been successful demonstrations of self-administration of most major drugs of abuse, including nicotine (e.g., Paterson et al., 2003; Rasmussen and Swedberg, 1998; Semenova et al., 1995, 1999).

The purpose of the present study was to characterize the behavioral effects of nicotine withdrawal using a battery of tests in DBA/2J mice. The reinforcing effects of acute nicotine were assessed using the intravenous self-administration technique before and after a 14-day continuous nicotine infusion through osmotic minipumps; the somatic aspects of nicotine withdrawal reflecting "physical" dependence were assessed using a conventional behavioral observation checklist and general activity levels detected in the startle chamber when no acoustic stimuli were presented; sensorimotor reactivity and gating during spontaneous nicotine withdrawal were evaluated by measuring the startle response and prepulse inhibition, respectively. The DBA/2J strain of mice was selected because it has been shown previously that this strain readily acquires intravenous self-administration of drugs such as morphine (Kuzmin et al., 1996b; Semenova et al., 1995, 1999), cocaine (Kuzmin et al., 1996a, 1997) and nicotine (Paterson et al., 2003). Also, this strain is scoring moderately in a number of behavioral tests such as acoustic startle and prepulse inhibition and with regard to their sensitivity to nicotine (for review, see Crawley et al., 1997; Olivier et al., 2001). Studies on strain differences indicated that DBA/2J mice were among the strains resistant to the development of tolerance to nicotine-induced seizures (Miner and Collins, 1989), as well as effects of nicotine on Y-maze alternation and body temperature (Marks et al., 1989, 1991). These deficits could be related to the low nicotinic receptor binding levels in DBA/2J mice (Marks et al., 1989). Meanwhile, there have been no nicotine dependence studies in DBA/2J mice and, therefore, this strain seemed well suited for behavioral phenotyping of nicotine withdrawal using the specific battery of tests employed in the present studies.

2. Materials and methods

2.1. Animals

Adult experimentally naive DBA/2J male mice 10–12 weeks old and weighing 22–25 g were purchased from Harlan (Indianapolis, IN). Mice were housed in a humidity-(50–70%) and temperature-controlled (20–22 °C) animal facility on a 12:12 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 and mecamylamine hydrochloride (both from Sigma, St. Louis, MO) were dissolved in sterile 0.9% saline. Nicotine tartrate was delivered either intravenously at the unit doses of 0.016–0.48 μg/infusion (corresponding drug solution concentrations: 0.01–0.3 mg/ml salt; 0.0056–0.17 μg/inf nicotine free base; or 0.69–20.86 μg/kg/inf unit dose for the average 23-g mouse) or subcutaneously through osmotic minipumps at the daily dose of 18 mg/kg/day (6.31 mg/kg/day nicotine free base) for 14 days. Mecamylamine hydrochloride (0.1–3 mg/kg, salt) was injected subcutaneously (injection volume 10 ml/kg). Drug doses are based on the forms indicated above.

2.3. General procedures

Experiments were conducted in two replications during the period of April–October 2001. Upon arrival to the laboratory, animals were individually housed and acclimated for 7 days prior to the first experimental procedure. Then, over the course of 16 consecutive days, all mice were subjected to the intravenous nicotine self-administration test twice, once before implantation of the nicotine/saline minipumps (test 1) and once after the removal of the minipumps (test 2). During the period separating these two self-administration tests, mice were prepared with osmotic minipumps that delivered either saline or nicotine for 14 days. Signs of nicotine withdrawal were assessed twice; once after the administration of the nicotinic receptor antagonist mecamylamine (day 12 of nicotine/saline minipump infusion; precipitated withdrawal) and then again 24 h after minipump removal (spontaneous withdrawal). Finally, the startle test was conducted 25 min after the end of the second intravenous self-administration test, aiming to evaluate the effects of acutely self-administered saline/nicotine on startle reactivity and prepulse inhibition in mice that were withdrawn from chronic nicotine/saline exposure. All tests are individually described below, and the general time schedule is summarized in Table 1.

Table 1
Summary of the experimental design

Day(s)	Experimental procedure
1	spontaneous nose-poke rate during pretest i.v. nicotine self-administration-test 1
2	minipump implantation
9, 11	habituation to the behavioral observation arena
13	precipitated nicotine withdrawal test (observation of somatic signs)
15	minipump removal
16	spontaneous nicotine withdrawal test (observation of somatic signs) i.v. nicotine self-administration-test 2 acoustic startle test

2.4. Intravenous self-administration

Each of the three apparatuses (San Diego Instruments, San Diego, CA) consisted of four identical test cages (8 × 8 × 8 cm) for simultaneous testing of two pairs of mice per apparatus (see below). Test cages were made from opaque plastic and were covered with an opaque lid during the test. Each cage had a frontal wall hole (diameter = 1.6 cm) for nose-poking and a semi-circle slot (diameter = 0.5 cm) in the back wall for immobilizing the mouse's tail. During the test, the mice were partially immobilized by fixing their tails with adhesive tape to a horizontal surface; the mice were able to move their body and their limbs at all times although the body movements were somewhat restricted. The nose-poke responses were recorded by means of infrared sensors interfaced to an operating computer, which controlled the activation of the two-syringe infusion pumps (one pump for each mouse pair). The volume and duration of infusions were held constant at 1.6 μl and 1.0 s, respectively. During the infusion, the nose-poke responses were recorded but had no programmed consequences.

A preliminary test (pretest) was conducted for each mouse to record the free operant level of nose-poking. For this pretest, mice were placed into the test cages for 10 min, their tails were immobilized but needles were not inserted, and the nose-poke responses had no consequences. Based on these pre-tests, the mice were grouped in pairs so that both animals in a pair exhibited approximately equal numbers of spontaneous nose-poking activity.

Within 1 h after the pretest, pairs of mice were placed again into the experimental boxes, and needles (OD 0.04 cm) were inserted into the lateral tail veins of both animals of the pair. A silastic tubing catheter (Baxter Scientific, McGraw Park, IL) was attached to the needle and connected with the syringe. After a 5-min habituation to the test cages, intravenous deliveries of nicotine or saline were made contingent upon each nose-poke of one animal per pair (master mouse). Each nose-poke of the master mouse resulted in an infusion of 1.6 μl of the nicotine solution or saline to both the master mouse and the yoked control mouse. Nose-pokes of the yoked control were counted but had no programmed consequence. Test sessions lasted 30 min. Mice were returned to their home cage after the self-administration test. The experimental boxes were thoroughly cleaned and deodorized with alcohol pads (Dynatex, Brewster, NY) after each animal.

After the initial self-administration test sessions, half of the mouse pairs were prepared with nicotine-containing pumps, and the other half with saline-containing pumps

(see below). These two groups were matched for nose-poke activity and body weights. Mice were re-tested for nicotine self-administration 24 h after the minipump removal using exactly the same pair and nicotine unit dose assignments as during the initial test.

2.5. Osmotic minipump implantation and removal

Mice were anesthetized with an isoflurane/oxygen vapor mixture (1–3%) and 14-day osmotic minipumps (model 1002, Alza, Palo Alto, CA) were inserted subcutaneously using aseptic surgery techniques. Pumps were filled with either sterile 0.9% saline or nicotine solution at a concentration that resulted in 18 mg/kg/day nicotine tartrate (6.32 mg/kg/day nicotine free base) delivery for 14 days. 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 zink ointment USP (Alpharma USPD, Baltimore, MD) was applied to the incision area. On day 14, the pumps were surgically removed under isoflurane anesthesia.

2.6. Rating of somatic signs of nicotine withdrawal

The procedure was adopted from an earlier published report in mice (Isola et al., 1999) and the procedures are used widely in rats (e.g., Epping-Jordan et al., 1998; Harrison et al., 2001; Hildebrand et al., 1997; Malin et al., 1992; Watkins et al., 2000a). Mice were placed individually into a clear plastic cylindrical container (22 × 25 cm, diameter by height). Before the nicotine withdrawal somatic signs observation test, there were two 20-min habituation periods to the behavioral observation arena (experimental days 9 and 11; see Table 1). On the test day, the behavioral observation was 20 min in duration and was always preceded by a 10-min or 5-min habituation period (for precipitated and spontaneous withdrawal tests, respectively). The observation arenas were cleaned after each test (i.e., the sawdust bedding was changed). Recorded behavioral signs included rears, jumps, forelimb shakes, head shakes, body shakes and abdominal constrictions. On day 12 post-surgery (experimental day 13), mice were pretreated with mecamylamine (0.1–3 mg/kg) or saline 10 min prior to the behavioral observation. One day after the minipumps were removed (experimental day 16), spontaneous nicotine withdrawal signs were observed twice, immediately before and after the self-administration test.

2.7. Acoustic startle procedure

Apparatus and startle procedures were similar to those reported previously (Geyer and Swerdlow, 1998). Two acoustic startle apparatuses were used (SR-LAB, San Diego Instruments), 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 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 (nostim) 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 (PPI) values [based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus (Geyer et al., 1990)], 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 (120 ms onset-to-onset interval). Prepulse intensities were 4, 8 and 12 dB above the 70 dB background level, and each prepulse trial was designated as follows: 4 dB prepulse + pulse, 8 dB prepulse + pulse and 12 dB prepulse + pulse. The no stimulus trial consisted of background noise only and allowed the assessment by the piezoelectric accelerometer of general activity in the startle chamber when no acoustic stimuli were presented.

2.8. Data analyses

For nicotine self-administration, the data analyses were based on the assumption that the number of nose-pokes of the master mouse exceeded the corresponding value in the yoked control animal when the delivery line is loaded with reinforcing drug solution. The ratio (R) criterion was calculated for each pair of the experimental animals according to the formula: $R = \log (M_T/Y_T)\text{-}\log (M_{BL}/Y_{BL})$, with M_T the total number of nose-poke responses of the master mouse during the 30-min test, Y_T the total number of nose-poke responses of the yoked control mouse during the 30-min test, M_{BL} the total number of nose-poke responses of the master mouse during the 10-min pretest (baseline), Y_{BL} the total number of nose-poke responses of the yoked control mouse during the 10-min pretest (baseline). R-criterion data were subjected to logarithmic transformation in order to normalize the distribution. Further, the self-administration data also were analyzed using the Delta measure that was calculated as the difference in the number of nose-pokes emitted by the master and yoked mice during the 30-min test.

For startle experiments, the amount of PPI was calculated as a percentage score for each prepulse trial type: % PPI = 100-{[(startle response for prepulse + pulse)/(startle response for pulse-alone)] × 100}. 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 data were analyzed using SAS-STAT software (SAS Institute, Cary, NC). Data were subjected to distribution-free one-, two- and three-factorial analyses of variance (ANOVA; general linear models procedure). For different tests, varying combinations of the following independent variables were used as appropriate for the experimental design: nicotine unit dose (five dose levels), osmotic pump treatment (saline or nicotine), mecamylamine dose (four dose levels including saline) and prepulse intensity (three levels). Dunnett's and Tukey's tests were used whenever between-group pair-wise comparisons were indicated by the ANOVA results.

3. Results

3.1. Intravenous nicotine self-administration

There were no overall differences between treatment groups with regard to their performance during the 10-min pretest (group average range: 87–116 responses per test; Table 2). Further, the nose-poke activity of master and yoked control mice did not differ for any treatment group (group by response contingency interaction: $F(4,170) = 0.08$, n.s.), as these groups were matched for baseline nose-poking activity.

Table 2
Mean number of nose-pokes (± S.E.M.) per session during i.v. nicotine self-administration

Nicotine unit dose (μg/inf)	Osmotic pump content	Master/ Yoked	Pretest	Test 1	Test 2
0	Saline	Master	102 ± 18	132 ± 29	126 ± 30
		Yoked	105 ± 17	178 ± 28	155 ± 28
	Nicotine	Master	115 ± 18	128 ± 22	139 ± 36
		Yoked	114 ± 16	138 ± 18	151 ± 19
0.016	Saline	Master	110 ± 10	232 ± 45	156 ± 42
		Yoked	111 ± 14	117 ± 15	110 ± 19
	Nicotine	Master	96 ± 15	111 ± 19	60 ± 12
		Yoked	99 ± 16	194 ± 34	84 ± 10
0.048	Saline	Master	104 ± 11	187 ± 39	124 ± 16
		Yoked	104 ± 9	149 ± 33	123 ± 23
	Nicotine	Master	90 ± 8	209 ± 34	150 ± 50
		Yoked	95 ± 10	121 ± 34	94 ± 20
0.16	Saline	Master	88 ± 8	153 ± 34	91 ± 13
		Yoked	87 ± 9	96 ± 21	138 ± 30
	Nicotine	Master	92 ± 16	126 ± 28	76 ± 13
		Yoked	87 ± 14	116 ± 24	91 ± 18
0.48	Saline	Master	116 ± 10	76 ± 10	56 ± 7
		Yoked	113 ± 9	83 ± 15	153 ± 25
	Nicotine	Master	117 ± 16	62 ± 4	67 ± 10
		Yoked	108 ± 13	79 ± 25	87 ± 24

Notes. Pretest and Test 1 sessions were conducted prior to osmotic minipump implantation. Test 2 sessions were conducted 24 h after the pumps were removed. $N = 9$ for each data point. See text for details.

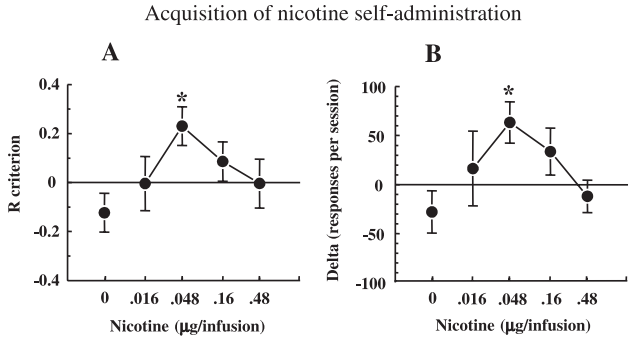

Fig. 1. Acquisition of nicotine self-administration in DBA/2J mice. Data (mean ± S.E.M.) are presented as R-criterion (panel A) and Delta-criterion values (difference between nose-poke activity of master and yoked control mice, panel B) for the 30-min test session. See text for details. $N = 18$ pairs for each data point. *$P < 0.05$ (Dunnett's test) compared to mice self-administering saline.

During the first 30 min self-administration test, there were no overall differences between mice that were subsequently prepared with saline- and nicotine-containing pumps (main effect of pump: $F(1,89) = 1.0$, n.s.). However, there was a significant interaction between the nicotine unit dose and the pump factors ($F(1,89) = 2.6$ and 3.5, $P < 0.05$, for data sets of R- and Delta-criteria presented in Fig. 1A and B, respectively) which was due to the mice that were subsequently treated chronically with saline self-administering more of the 0.016 μg/infusion unit dose than the nicotine-treated rats (Table 2). More specifically, because of the treatment randomization conducted prior to any nicotine experience, at the unit dose of 0.016 μg/infusion mice assigned to the subchronic saline treatment self-administered more nicotine (master vs. yoked: 232 ± 45 vs. 117 ± 15 nose-pokes per session) compared to the mice assigned to the subchronic nicotine treatment (master vs. yoked: 111 ± 19 vs. 194 ± 34 nose-pokes per session, see Table 2). Because the reason for this difference is not apparent and because the focus of the present experiments is on comparing mice implanted with saline- versus nicotine-containing pumps, animals assigned to the 0.016 μg/infusion treatment condition were excluded from the subsequent analyses (i.e., self-administration test 2).

During the initial self-administration test, nose-poke activity of mice significantly depended upon the nicotine unit dose (R-criterion: $F(3,71) = 2.8$, $P < 0.05$; Delta criteria: $F(3,71) = 3.8$, $P < 0.05$). Activity of mice receiving response-contingent infusions of nicotine exceeded that of yoked controls at the unit dose level of 0.048 μg/infusion (Fig. 1).

The second self-administration test (test 2) took place after the removal of the osmotic minipumps. This test revealed significant differences in nicotine self-administration between mice chronically treated with saline and

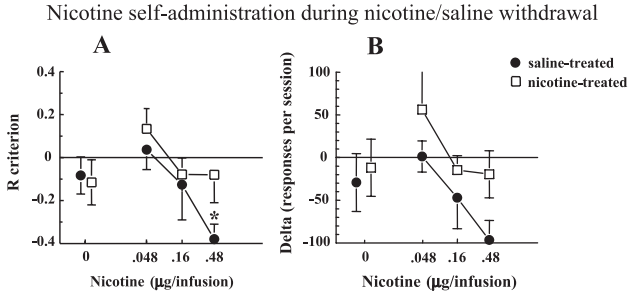

Fig. 2. Nicotine self-administration during nicotine/saline withdrawal. Mice were prepared with minipumps delivering either saline or nicotine (salt, 18 mg/kg/day) for 14 days. This second self-administration session took place one day after the minipump removal. Data (mean ± S.E.M.) are presented as R-criterion (panel A) and Delta-criterion values (difference between nose-poke activity of master and yoked control mice, panel B) for the 30-min test session. $N=9$ pairs for each data point. *$P<0.05$ (Dunnett's test) compared to mice self-administering saline.

nicotine as indicated by a significant interaction between the factors nicotine unit dose and pump content (Fig. 2A and B; R-criterion: $F(3,71)=2.9$, $P<0.05$; Delta-criterion: $F(3,71)=2.4$, $P=0.07$). For the saline-treated mice, there was a significant main effect of nicotine unit dose ($F(3,35)=3.6$, $P<0.05$) mostly due to the impact of large differences between master and yoked control mice at the nicotine unit dose of 0.48 μg/infusion. In contrast, for the nicotine-withdrawn mice there was no significant main effect of nicotine unit dose ($F(3,35)=1.2$, n.s.). Pre-planned comparisons revealed significantly higher aversive reactions (e.g., negative values of R-criterion) in saline-withdrawing mice compared to nicotine-withdrawing mice during nicotine self-administration at the unit dose of 0.48 μg/infusion (see Fig. 2A and B). There were no overall differences between saline- and nicotine-withdrawn mice with regard to the rate of nose-poke activity and the cumulative doses of nicotine self-injected during this second test ($P>0.05$).

Table 3
Somatic signs of mecamylamine-precipitated nicotine withdrawal in DBA/2J mice

N	Parameter	Mecamylamine mg/kg	Saline-treated	Nicotine-treated
30	Jumps	0	0.94 ± 0.5	4.07 ± 1.77
20		0.1	1.20 ± 0.83	0.10 ± 0.07
20		1.0	1.05 ± 0.53	3.05 ± 2.03
20		3.0	0.63 ± 0.65	0.35 ± 0.31
30	Rears	0	82.77 ± 9.0	92.90 ± 11.21
20		0.1	75.75 ± 8.74	82.00 ± 10.98
20		1.0	69.60 ± 11.23	91.40 ± 9.27
20		3.0	45.16 ± 8.69	25.85 ± 6.29
30	Head shakes	0	0.39 ± 0.11	0.27 ± 0.13
20		0.1	0.20 ± 0.09	0.10 ± 0.07
20		1.0	0.55 ± 0.16	0.85 ± 0.27
20		3.0	0.37 ± 0.12	0.40 ± 0.14
30	Body shakes	0	0.68 ± 0.22	0.43 ± 0.12
20		0.1	0.80 ± 0.21	0.75 ± 0.30
20		1.0	1.35 ± 0.38	1.30 ± 0.48
20		3.0	0.74 ± 0.35	0.35 ± 0.15
30	Forelimb shakes	0	0.61 ± 0.20	0.87 ± 0.25
20		0.1	1.40 ± 0.44	2.30 ± 0.72
20		1.0	0.90 ± 0.31	2.00 ± 0.60
20		3.0	1.58 ± 0.55	1.7 ± 0.73
30	Abdominal	0	0.0 ± 0.0	0.0 ± 0.0
20	constrictions	0.1	0.0 + 0.0	0.0 ± 0.0
20		1.0	0.05 + 0.05	0.0 ± 0.0
20		3.0	0.21 + 0.10	0.35 ± 0.11

Data are presented as the mean ± S.E.M. N is the number of mice per group. There was no difference between dependent and non-dependent mice during mecamylamine-precipitated nicotine withdrawal.

Table 4
Somatic signs of spontaneous nicotine withdrawal in DBA/2J mice

Parameter	Saline-treated	Nicotine-treated
Jumps	2.7 ± 0.7	5.1 ± 1.0[a]
Rears	113.6 ± 4.8	115.7 ± 5.3
Head shakes	0.6 ± 0.1	0.8 ± 0.2
Body shakes	1.0 ± 0.1	1.0 ± 0.1
Forelimb shakes	2.0 ± 0.3	2.0 ± 0.2
Abdominal constrictions	0.0 ± 0.0	0.0 ± 0.0
Body weight	22.8 ± 0.4	23.2 ± 0.2

Data are presented as the mean ± S.E.M. $N=90$.
[a] $P<0.05$ (one-tailed t-test) compared to non-dependent mice.

3.2. Somatic signs of nicotine withdrawal

Twelve days after minipump implantation (experimental day 13), behavioral observations did not reveal any significant differences between nicotine- and saline-treated mice under baseline conditions (see Table 3). Moreover, injection of mecamylamine did not have any significant effects in either saline- or nicotine-treated mice (see Table 3).

Observations conducted after minipump removal during spontaneous nicotine/saline withdrawal revealed differences between mice exposed to chronic saline and nicotine administration. Nicotine-withdrawn mice demonstrated significantly higher jumping activity compared to saline-treated controls (Table 4). Nevertheless, nicotine pump removal did not affect the expression of rearings, head, body or forelimb shakes and had no effect on body weight (Table 4).

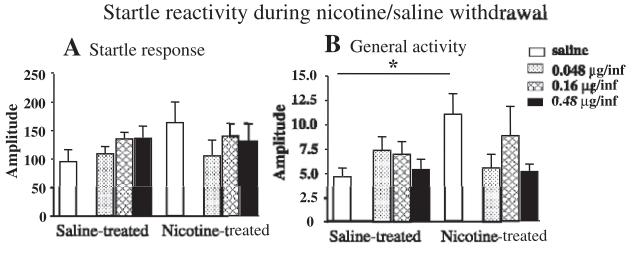

Fig. 3. Effects of nicotine on behavioral measures in the startle test during nicotine/saline withdrawal. Data (mean ± S.E.M.) are presented as amplitude of the startle response in response to 120 dB stimuli (panel A) and general activity during the no stimulus trials (panel B). $N=9$ for each data point. *$P<0.05$ (one-tailed t-test) compared to mice self-administering saline.

3.3. Acoustic startle, prepulse inhibition and general activity

The global ANOVA revealed no significant interaction between self-administered nicotine unit dose and saline/nicotine pump factors for startle amplitude (pulse trials; $F(4,89) = 1.3$, n.s.) and spontaneous activity (nostimtrials; $F(4,89) = 1.1$, n.s.). Nevertheless, visual inspection of the data depicted in Fig. 3 and pre-planned comparisons (one-tailed t-test) indicated that startle amplitude (pulse-trial condition) and general activity (nostim-trials condition) of nicotine-withdrawn mice were higher compared to the saline-withdrawn mice; and these effects of nicotine withdrawal appeared to be reversed by self-administered nicotine use these differences were no longer observed in mice that were allowed to self-administer nicotine.

Data on prepulse inhibition of the startle reflex are presented in Fig. 4. The global ANOVA indicated that prepulse inhibition was determined by the previous exposure to the nicotine-containing minipump, nicotine administered acutely during the self-administration test (test 2) and the magnitude of the prepulse intensity (interaction of these three factors: $F(6,112) = 2.4$, $P < 0.05$). As expected, the amplitude of the startle reflex was significantly decreased by the preceding stimulus presentation in a prepulse-intensity-dependent manner ($F(2,112) = 206.8$, $P < 0.01$).

Significant interactions between the acute (self-administration) and chronic (via osmotic pump) nicotine administration factors were observed for 8 and 12 dB prepulse intensities ($F(3,71) = 2.8$ and 4.9, $P < 0.05$ and 0.01, respectively; see Fig. 4). Further, post-hoc Tukey's test indicated that prepulse inhibition at 8 and 12 dB prepulse intensities was significantly impaired in nicotine-withdrawn mice compared to saline-withdrawn mice exposed to saline self-administration prior to the acoustic startle test (data points above '0'; $P < 0.05$). No such effect was seen in both nicotine- and saline-withdrawn mice allowed to self-administer nicotine prior to the startle test.

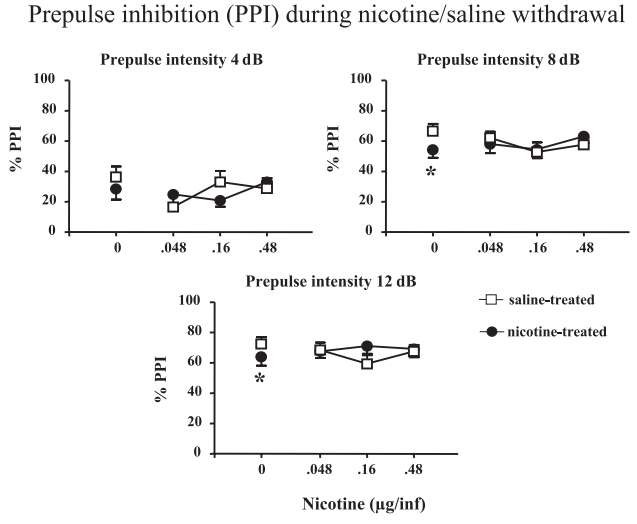

Fig. 4. Prepulse inhibition of startle reflex. Data are presented as mean (± S.E.M.) % prepulse inhibition for groups of mice that were first exposed to either saline or nicotine chronic infusion and then allowed to self-administer different doses of nicotine 25 min prior to the acoustic startle test. Within each startle test, prepulse inhibition was tested at three prepulse intensities, 4, 8 and 12 dB above background. $N = 9$ for each data point. *$P < 0.05$ (Tukey's test) compared to mice that received chronic infusion of saline through the pumps instead of nicotine and allowed to self-administer the same dose of nicotine in the self-administration procedure.

4. Discussion

The present study was designed to assess the effects of nicotine withdrawal on a variety of behavioral measures in DBA/2J mice. Earlier studies (Castane et al., 2002; Damaj and Martin, 2002; Isola et al., 1999) indicated that nicotine withdrawal produced mild but detectable changes in the behavior of other mouse strains than those used in the present study. More specifically, it has been reported that nicotine withdrawal is characterized by a constellation of abstinence signs such as increased rearing and jumping activity, shakes, abdominal constrictions, chewing, facial tremor and scratching. Our results did not confirm these findings, as neither mecamylamine injection in mice chronically treated with nicotine nor spontaneous withdrawal from nicotine (i.e., removal of the nicotine-containing pumps) affected the expression of abdominal constrictions, rearing, head, body or forelimb shakes. The only sign of withdrawal that was increased in nicotine-treated compared to saline-treated mice in the present study was jumping activity, and this increase was consistent with that seen previously in Swiss Webster mice (Isola et al., 1999). This overall discrepancy in experimental findings, except for jumping activity, is likely to be attributable to genetic differences in the mouse strains used and possibly differences in experimental design. Specifically, nicotine dependence was induced using different drug dosing regimens in the present and previous work (Isola et al., 1999). In the present study, nicotine was administered through subcutaneous osmotic minipumps at the daily doses of 18 mg/kg/day (salt) for 14 days. Isola et al. (1999) administered nicotine via four daily doses of 1–2.5 mg/kg each for 14 days. Damaj and Martin (2002) and Castane et al. (2002) administered nicotine through osmotic minipumps delivering 24 mg/kg/day of nicotine for 14 days and 10 mg/kg/day for 6 days, respectively. Thus, it appears that in the present study, mice received a high enough total nicotine dose that should have produced nicotine dependence comparable to or stronger than that seen in previous studies. Therefore, the dosing regimen is not a likely explanation for the differences in the expression of nicotine withdrawal.

Second, previous studies were conducted in inbred ICR (Damaj and Martin, 2002), CD1 Swiss albino (Castane et al., 2002) and Swiss Webster (Isola et al., 1999) mice, while the present study used DBA/2J mice. There is some evidence indicating that somatic signs of naloxone-precipitated

morphine withdrawal were not as pronounced in DBA/2J as in C57BL/6 mice (Heyser et al., 2001). This observation indicates that genetic factors may be powerful determinants of the expression of the drug withdrawal syndrome. Importantly, genetic factors may determine the specific behavioral and somatic aspects of drug withdrawal expressed. Interestingly, Isola et al. (1999) suggested that the expression of nicotine withdrawal may not be uniform even within a single mouse strain. These investigators (Isola et al., 1999) identified two groups of nicotine-withdrawn mice that differed in terms of their behavioral display of withdrawal signs. One group exhibited primarily escape-like activity (rearing and jumping) while another group demonstrated stereotypic-like activity characterized by chewing, scratching, head shakes, body shakes and facial tremor. In general, our findings are consistent with previous reports indicating that DBA/2J mice exhibited medium to low responsiveness to the effects of nicotine compared to other mouse strains in the different behavioral paradigms (Marks et al., 1989, 1991; Miner and Collins, 1989).

Although no significant changes were detected in the expression of somatic signs of withdrawal in the present study, significant differences between saline- and nicotine-withdrawn animals were detected using two procedures that have not been applied previously in the study of nicotine withdrawal in mice; these are the intravenous nicotine self-administration and prepulse inhibition of the acoustic startle response procedures. The procedure used here for drug self-administration has been used extensively previously to evaluate the reinforcing properties of various abused drugs such as morphine, cocaine, ethanol and nicotine (Kuzmin et al., 1996a,b, 1997; Martellotta et al., 1995; Paterson et al., 2003; Rasmussen and Swedberg, 1998; Semenova et al., 1995, 1999). In the present study, nicotine self-administration was tested across a range of unit doses from 0.016 to 0.48 μg/infusion nicotine tartrate (corresponding to 0.0056–0.17 μg/inf nicotine free base). This range includes the doses that were shown to maintain significant nicotine self-administration in previous studies using similar self-administration techniques (Martellotta et al., 1995; Paterson et al., 2003; Rasmussen and Swedberg, 1998). Analyses of the raw nose-poke data, the derived R-criterion and Delta scores clearly indicated self-administration of nicotine at the 0.048 μg/inf unit dose (corresponding 2.09 μg/inf/kg salt or 0.73 μg/kg/inf free base nicotine). In human research, intravenous nicotine self-administration was studied at the doses ranging between 10 and 45 μg/kg/inf, a range that is close to that in rat studies (Henningfield et al., 1983; Goldberg and Henningfield, 1996; for review, see Rose and Corrigall, 1997). Further, data obtained from rat nicotine self-administration procedures also indicated that the nicotine dose–response curve is rather flat and the effective dose range is narrow (Corrigall and Coen, 1989; Donny et al., 1995; Watkins et al., 1999). Thus, the three-fold increments in the nicotine unit doses used in the present study were perhaps too large to detect significant nicotine self-administration at more than one unit dose level. Nevertheless, such a large range of nicotine doses was selected to: (a) ensure that a unit dose that would support significant self-administration in nicotine-naive animals was included (i.e., 0.048 μg/inf), and (b) facilitate the detection of potential shifts in the nicotine dose–response curve resulting from chronic exposure to nicotine.

Continuous nicotine infusion for 14 days produced only modest effects on nicotine self-administration behavior. There were no indications that nicotine's reinforcing effects were enhanced or diminished in nicotine-withdrawn mice. In general, there is no agreement in the literature on the role of drug withdrawal in maintaining self-administration behavior (Koob and Le Moal, 2001; Schulteis and Koob, 1996; Stewart and Wise, 1992). The compulsive nature of drug abuse often is attributed to the fact that drug self-administration enables an addict to escape from and avoid the severe withdrawal symptoms resulting from drug dependence (Koob and Le Moal, 2001). Accordingly, it was shown that dependent subjects show higher drug intake compared to non-dependent subjects (e.g., Schulteis and Koob, 1996). It is hypothesized also that drug withdrawal functions as a motivational state that enhances the incentive value of the drug, thereby increasing its reinforcing efficacy. Indeed, there is some evidence indicating that drug dependence and abstinence may enhance the relative reinforcing effects of the drug (e.g., caffeine; Garrett and Griffiths, 1998), facilitate drug intake at lower unit doses (e.g., heroin; Dai et al., 1989) and increase drug-seeking behavior after extended abstinence (e.g., cocaine; Semenova and Markou, in press; Weiss et al., 2001). To the best of our knowledge, there is no such evidence for nicotine. In fact, somewhat corroborating the results of the present study, it was previously shown that increased anxiety-like behavior upon withdrawal from nicotine does not seem to contribute to nicotine self-administration (Irvine et al., 2001b). The present study demonstrated that subchronic nicotine delivery through osmotic minipumps induced tolerance to acute nicotine as revealed by reduced aversiveness of the highest self-administered nicotine unit dose (0.48 μg/inf) in nicotine-withdrawn mice. This finding is somewhat parallel to human studies with nicotine patches indicating that nicotine replacement dose regimens (e.g., 14–63 mg/kg/day delivering by nicotine patch) that were about three times higher than those currently approved and yet did not result in significant subjective or physiological effects (for review, see Fagerstrom and Hughes, 2002). Thus, slow nicotine delivery rates may facilitate the induction of tolerance (e.g., Porchet et al., 1998).

There are several factors that determine whether "physical" dependence can be demonstrated to affect drug self-administration. First, previous experience with the effects of heroin during the withdrawal period is necessary for subsequent heroin-seeking behavior to be enhanced when dependent rats once again experience withdrawal (Hutcheson et al., 2001). Second, the acute withdrawal state, like the one

assessed in the present study, may produce non-specific decrements in responding for the drug. For instance, ethanol intake was suppressed in the presence of withdrawal symptoms (Winger, 1988), while cocaine-seeking behavior was decreased during early cocaine withdrawal (Arroyo et al., 1998). Importantly, in the present study, nicotine withdrawal did not affect the operant level of nose-poking (i.e., as seen in nicotine withdrawn mice allowed to self-administer saline).

It is also of interest that saline-withdrawn mice seemed to avoid the response-contingent infusions of high unit doses of nicotine. At 0.48 μg/infusion (20 μg/kg), master mice made fewer nose-poke responses than yoked control animals. It is unlikely that at this unit dose level nicotine was simply impairing operant performance because the response rates of yoked control animals were not different from those for yoked controls receiving lower unit doses of nicotine or saline. In other words, the difference between master and yoked mice exposed to high unit doses of nicotine are mainly attributable to decreased operant output in master mice that possibly reflects increased aversive effects of high doses of nicotine. The limited behavioral and drug history that are characteristic of this procedure were probably among the main reasons why these aversive effects of nicotine were revealed in the second self-administration session for the mice chronically treated with saline previously. Indeed, no such effects were observed in mice that had been exposed to continuous nicotine infusion via osmotic minipumps, possibly reflecting the development of tolerance to the aversive effects of higher nicotine doses after chronic nicotine infusion. In earlier studies, development of tolerance was demonstrated for the anxiogenic effects of nicotine using the social interaction and the elevated plus maze paradigms in rats (Cheeta et al., 2001; Irvine et al., 2001a,b). The development of tolerance also was demonstrated for the ability of nicotine to lower brain stimulation reward thresholds during continuous nicotine delivery through osmotic minipumps (salt, 9 mg/kg/day for 7 days) using the intracranial self-stimulation procedure in rats (Harrison et al., 2001; Skjei and Markou, in press; however, see Bozarth et al., 1998a,b) and to facilitate dopamine metabolism in brain reward areas (Pietila et al., 1996).

Another important finding of this study was that cessation of chronic nicotine administration led to a modest but significant impairment of prepulse inhibition of the acoustic startle response that was readily reversed by self-administered nicotine. Impaired prepulse inhibition in nicotine-withdrawn mice replicates earlier findings in ethanol-withdrawn rats (Rassnick et al., 1992) but is in contrast to the lack of prepulse deficits in animals withdrawn from psychostimulant (Adams et al., 2001; Murphy et al., 2001; Russig et al., in press) or opiate drugs (Fendt and Mucha, 2001). Startle reactivity itself was not affected by nicotine withdrawal; there was only a tendency for increased startle amplitude in nicotine-withdrawn mice. This result is consistent with previous studies in humans (Kumari and Grey, 1999; Postma et al., 2001) and in contrast to studies in rats showing strain-dependent decreases or increases of startle during nicotine withdrawal (see below). This pattern of results again illustrates strain-specific expression of the nicotine withdrawal syndrome. Even within a single research paradigm, different measures (startle amplitude vs. prepulse inhibition) assess different processes that are under differential genetic control. Startle and prepulse inhibition have been studied in a number of mouse strains (for review, see Crawley et al., 1997). Among all, the DBA/2J strain was generally characterized by rather poor acoustic startle response and moderate prepulse inhibition (for review, see Crawley et al., 1997; Paylor and Crawley, 1997). Extending these observations, the effects of nicotine withdrawal on acoustic startle and prepulse inhibition also may be strain-dependent. For instance, 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 following nicotine withdrawal. Faraday et al. (1998, 1999), in a direct comparison of these strains, confirmed that the modulatory effects of nicotine in both startle and PPI were strain-dependent. Further, the results of our study showed that the small tendency for increases in startle reactivity and the significant decreases in prepulse inhibition seen during nicotine withdrawal were reversed by self-administered nicotine suggesting that these effects could be attributed to nicotine withdrawal itself. Impaired prepulse inhibition after discontinued nicotine administration complements the previously published data showing that acute nicotine administration enhanced the prepulse inhibition response in both experimental animals (Acri et al., 1994; Curzon et al., 1994) and humans (Kumari et al., 1996, 1997). Finally, the results showed that the general activity in the startle chamber, when no stimuli were presented was significantly increased in nicotine-withdrawn compared to saline-withdrawn mice. Again, these increases in general activity were reversed by self-administered nicotine. Increased general activity in the startle chamber may be associated with increased agitation or body tremor. This finding is consistent with earlier results showing increased body tremor in nicotine-withdrawn mice (Castane et al., 2002).

In conclusion, behavioral observations of DBA/2J mice withdrawn from continuous subcutaneous infusion of nicotine (18 mg/kg/day for 14 days) did not reveal increases in several somatic signs of nicotine withdrawal reported earlier for other strains of mice (Castane et al., 2002; Damaj and Martin, 2002; Isola et al., 1999). In this study, nicotine withdrawal was associated with modest increases in jumping activity, increased general activity in the startle chambers when no stimuli were presented, and impairment of prepulse inhibition of the acoustic startle reflex; the last two effects were reversed with nicotine self-administration further supporting the hypothesis that these two effects are induced by abrupt cessation of nicotine administration after chronic nicotine exposure. Increased general activity in the startle chambers possibly reflects increased body tremor or agita-

tion that may reflect an additional somatic sign of nicotine withdrawal. The impairment of prepulse inhibition reflects a sensorimotor gating deficit reversible by nicotine administration. Finally, taken together with the previous reports, the present results suggest strain-specific expression of the behavioral syndrome characterizing nicotine withdrawal.

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