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Variation in Nicotine Consumption in Inbred Mice Is Not Linked to Orosensory Ability

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Abstract

Genetic studies of nicotine addiction in mice have utilized the oral self-administration model. However, it is unclear if strain differences in nicotine consumption are influenced by variation in bitter taste sensitivity. We measured both nicotine consumption and nicotine brief-access licking behavior in several commonly used inbred strains of mice that were previously shown to differ in nicotine consumption. A/J (A), C57BL/6J (B6), and DBA/2J (D2) mice were given a 2-bottle choice test with a single concentration of nicotine (75 μg/ml; nicotine vs. water). Mice of these strains were also tested with a range of nicotine concentrations (5–400 μg/ml) using a brief-access test, which measures orosensory response and minimizes postingestive effects. Although B6 mice consumed more 75-μg/ml nicotine than A or D2 mice in the 2-bottle test, these strains did not differ in level of aversion to nicotine when tested with the brief-access procedure. Strain differences in orosensory response to nicotine were not found; yet, differences emerged during the 2-bottle tests. This study provides evidence that variation in intake level of nicotine is likely not due to differences in taste or trigeminal sensitivity but likely due to postingestive factors.

Key words: A/J, aversion, C57BL/6J, DBA/2J, inbred, intake, mice, nicotine, orosensory, taste

Introduction

Genetic approaches to nicotine addiction in mice depend on an effective paradigm of drug administration (Mohammed 2000). The presentation of nicotine in an animal's drinking water, termed "oral self-administration," is a method that has seen increasing use for chronic nicotine administration in mice (Adriani et al. 2002, 2004; Klein et al. 2004; Abreu-Villaca et al. 2006; Marttila et al. 2006; Weiss et al. 2007). Likewise, 24- or 48-h preference tests with 2 or more drinking bottles (water vs. nicotine) have been used to gauge variation in amount of nicotine self-administration among inbred strains of mice or mice with genetic manipulations (Meliska et al. 1995; Robinson et al. 1996; Lee et al. 2004; Butt et al. 2005; Li et al. 2005, 2006). The advantages of the oral self-administration model for use with mice are as follows: First, providing the mice with ad libitum access to nicotine may mimic the low-level time-distributed administration found in human smokers (Rowell et al. 1983). Second, nicotine enters the bloodstream through drinking at concentrations comparable to human smoking (Rowell et al. 1983). Third, drinking tests can be easily and inexpensively set up in the animal's home cage, minimizing stress from handling, and allowing the simultaneous testing of a large sample (Boughter and Bachmanov 2007).

A drawback to the oral self-administration method is the fact that mice almost always avoid nicotine solutions relative to water (Meliska et al. 1995; Robinson et al. 1996). Because nicotine tastes bitter to humans, it may be assumed that the cause of avoidance is aversive taste, and nicotine is indeed a potent activator of taste neurons (Iwasaki and Sato 1981; Dahl et al. 1997). However, nicotine also activates trigeminal neurons in the oral cavity (Liu and Simon 1996, 1998; Dessirier et al. 2000). Stimulation of trigeminal receptors in oral or nasal mucosa may evoke stinging, burning, or pungent sensations (Thuerauf et al. 1999). Although strain differences in regard to nicotine preference and intake have been demonstrated, it is not clear if variation in either taste or trigeminal sensitivity may play a role.

As is the case for nicotine, there is substantial variation among standard inbred strains in regard to preference and intake of bitter-tasting stimuli (Lush 1984; Lush and Holland 1988; Whitney and Harder 1994; Bachmanov et al. 1996; Kotlus and Blizard 1998). Studies using

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taste-salient, brief-access tests, taste nerve physiology, and molecular techniques have indicated that strain variation in intake of bitter stimuli is typically linked to differences in taste ability (Shingai and Beidler 1985; Frank and Blizard 1999; Chandrashekar et al. 2000; Inoue et al. 2001; Boughter et al. 2002, 2005; Nelson et al. 2003, 2005). In order to make nicotine more palatable to mice, some investigators have mixed it in solution with a sweetener such as sodium saccharin (Robinson et al. 1996; Klein et al. 2004). One major limitation with this technique is that strains of mice also show marked variation in avidity for sweet-tasting compounds, including saccharin (e.g., Lush 1989; Capeless and Whitney 1995; Reed et al. 2004; Glendinning et al. 2005; Inoue et al. 2007), again making interpretation of consumption levels problematic.

It has been suggested that taste sensitivity does not play a major role in differential nicotine intake among strains (Robinson et al. 1996; Butt et al. 2005; Li et al. 2006). In the current study, we directly test the orosensory response to nicotine in 3 commonly used inbred strains of mice using a brief-access test. Such tests have been previously used to assess variation in bitter taste sensitivity among inbred strains (Eriksson 1969; Nelson et al. 2003; Boughter et al. 2005). The salient feature of brief-access tests is the presentation of taste stimuli in a short-duration trial (≤5 s), which minimizes possible postingestive factors and produces a more faithful measure of the immediate sensory response (Grill et al. 1987; Spector 2000; Boughter et al. 2002; Glendinning et al. 2002). It is important to point out that although this assay is primarily used to measure taste ability, it does not discriminate between taste, potential trigeminal, or olfactory contributions to behavior; hence, here we use the term "orosensory response." We tested 3 strains, A/J (A), C57BL/6J (B6), and DBA/2J (D2), that have been previously shown to differ in preference and intake of a range of nicotine concentrations, with B6 mice displaying greater levels of consumption (Meliska et al. 1995; Robinson et al. 1996; Butt et al. 2005). In addition, we conducted 2-bottle preference tests to compare the consumption of nicotine relative to water. By utilizing both assays, we were able to compare behavioral responses to nicotine regarding orosensory response as well as long-term intake. The results of the study indicated that mice from all 3 strains possessed a similar orosensory response to a range of nicotine concentrations, suggesting that the higher preference seen in B6 mice is mediated by the postingestive or pharmacological effects of nicotine.

Materials and methods

Mice

A total of 65 naive mice from inbred strains C57BL/6J (B6), DBA2/J (D2), and A/J (A) were used in these experiments. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Similar numbers of mice from each strain (23 B6, 21 D2, and 21 A) and sex (38 males and 27 females) were used. For the 2-bottle experiment, 14 B6 (8 males and 6 females), 12 D2 (7 males and 5 females), and 12 A (6 males and 6 females) were tested. For the first brief-access experiment, 9 B6 (5 males and 4 females), 9 D2 (6 males and 3 females), and 9 A (6 males and 3 females) mice were tested. For the second brief-access experiment, 4 B6 (2 males and 2 females), 4 D2 (2 males and 2 females), and 4 A (2 males and 2 females) were tested, randomly selected from the group of animals that underwent 2-bottle testing; the experimenter did not know the results of individual mice from the 2-bottle test at this time.

Mice were approximately 2–4 months of age at the time of testing and prior to testing were group housed by sex in plastic shoebox cages (28 × 17.5 × 13 cm) with ad libitum chow and water. At least 24 h prior to either intake or brief-access testing, mice were singly housed in plastic shoebox cages with ad libitum chow. During intake tests, water was available in home cages as part of the testing paradigm. During brief-access tests, mice were water regulated as described below. Animals were treated according to a protocol approved by the University of Tennessee Health Science Center Institutional Animal Care and Use Committee.

Stimulus

L-Nicotine freebase [(–)-nicotine, TCI America, Portland, OR], diluted in deionized water, was used for all experiments. For 2-bottle intake tests, a concentration of 75 μg/ml was used. This concentration was deemed likely to result in strain differences in consumption based on pilot studies and previous reports (Meliska et al. 1995; Robinson et al. 1996; Klein et al. 2004). For brief-access tests, 6 concentrations of nicotine were administered: 5, 25, 50, 100, 200, and 400 μg/ml. Fresh solutions were mixed every 2 days.

Two-bottle tests

As previous studies have characterized strain differences in nicotine consumption over an extended concentration range, in this study, we collected long-term intake data using a single concentration deemed likely to result in strain differences (Meliska et al. 1995; Robinson et al. 1996). Two-bottle preference tests were used to measure the consumption of nicotine relative to water. Mice were tested individually in plastic home cages with stainless steel wire lids. Two graduated drinking cylinders were placed on the right side of the lid with sipper tubes protruding about 3.5 cm into the cage. Amounts consumed were measured after 24 h and the position of the cylinders switched to control for possible side preferences (Lush 1984). The time spent switching bottles was negligible. Amounts consumed were again recorded after 24 h and fresh solutions exchanged. Therefore, a 48-h "test" includes 2 sets of consumption readings, spaced 24 h apart, with the bottles switched after the first readings—the results are reported this way (as 48-h tests) due to the bottle

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switching and for consistency with prior 2-bottle taste studies (Whitney and Harder 1994). Therefore, a total of 8 consecutive 48-h tests were conducted, the first 2 with water and the final 6 with 75-μg/ml nicotine. Two B6 mice did not drink from either tube during the first 24 h with water (first day of the experiment). These data were not used but subsequent data from these mice were.

Brief-access tests

Brief-access testing procedures were conducted using an MS-160 computer-controlled lickometer (Dilog Instruments, Inc., Tallahassee, FL) and were similar to those recently described (Nelson et al. 2003; Boughter et al. 2005). Briefly, mice were placed in a test chamber (30 × 14.5 × 16 cm) with a stainless steel mesh floor and could access taste stimuli or water via a small opening at the front of the chamber. A trial began when a shutter opened to allow access to a stainless steel drinking tube and ended after a defined period when the shutter closed. The shutter closed in a constant movement, about 5 cm in 1 s; mice easily moved away from the spout during this time. In-between trials, a stepping motor placed 1 of up to 16 drinking tubes in front of the access opening. Licks were counted via a high-frequency AC contact circuit. The circuit was made upon contact of the tongue to the metal sipper tube, sending <60 μA of current through the tongue, an imperceptible amount (Contreras et al. 1995; Glendinning et al. 2002).

Water-deprived mice were first trained to lick water in the lickometer and then tested for 5 consecutive days with a 6-concentration series of nicotine. Mice were water deprived for 23 h prior to the first day of the experiment, and with the exception of a 5-min water supplement administered in the home cage at the end of each testing day, mice were restricted to water and solutions consumed during the testing sessions. Under this schedule, mice were able to maintain body weight at about 80% of their original weight (measured prior to testing each day—data not shown).

On the first training day, mice were placed in the test chamber and given access to water for 20 min. On the second training day, access was restricted to 5-s trials, presented randomly from 4 tubes of water. Mice initiated a trial with a single lick on the tube, and after 5 s, the shutter would close. The shutter would reopen after a 7.5-s interval, and the mouse could initiate a subsequent trial. If a mouse did not lick within 120 s of the shutter opening, the shutter would close and the next trial begun. Mice could initiate up to 16 trials during the training session.

Testing occurred on days 3–7. The trials were 5 s in length, with an intertrial interval of 7.5 s and a time limit of 120 s. Five seconds were previously determined to be an optimal trial length in brief-access experiments in mice using the bitter-tasting compound quinine (Glendinning et al. 2002). Six concentrations of nicotine plus water were delivered using a randomized block design. Twenty-four trials were divided into 3 blocks of 8; within each block, each concentration of nicotine plus 2 water trials were presented in random order. In sum, each test session provided 3 possible data points per nicotine concentration and 6 for water trials. The order of all trials was randomized anew for each mouse, and the position of bottles on the lickometer was randomized each day. Finally, mice of all 3 strains were also tested in a random order each day.

Data analysis

Data from the 2-bottle tests are reported in the form of both 48-h preference ratio (PR) and 48-h dose (milligrams/kilograms). PRs (amount of solution consumed/total amount consumed) were determined for each mouse and averaged together per strain. For the brief-access tests, the number of licks for each nicotine concentration, plus water trials, was averaged across the 5 test sessions for each individual mouse. As B6 mice have been shown to possess a slower rate of licking compared with other strains, including D2 (Wang and Fowler 1999; Boughter et al. 2007), these data are reported as lick ratios (LRs; average number of licks to stimulus/average number of licks during water trials) in order to minimize effects of variation in baseline water licking. Concentration–response functions were then fit with a 2-parameter logistic function:

$$f(x) = \frac{1}{1 + (x/c)^b}$$

where x is the concentration of stimulus, c is the concentration evoking half-maximal avoidance (i.e., LR = 0.5), and b is the slope. Fitting such curves provides a single parameter (c) that is sensitive to shifts in the concentration–response function, as potentially resulting from strain differences. For group comparisons, c values were log transformed; strain values presented are therefore geometric means.

All relevant variables were analyzed using a general linear model: either repeated-measures analysis of variance (ANOVA) (concentration or day) with between-subjects factors (strain and sex) or factorial ANOVA (strain and sex) (Statistica software, Statsoft, Inc., Tulsa, OK). Post hoc tests (Scheffe) were performed when appropriate. The statistical rejection criterion for all tests was set a priori at the 0.05 level for main effects.

Results

Two-bottle tests

PR means per strain for 6 consecutive 48-h 2-bottle tests with 75-μg/ml nicotine are displayed in Figure 1, along with water baselines collected during the first two 48-h periods. A (n = 12), B6 (n = 14), and D2 (n = 12) mice did not differ in PR (~0.5) when both tubes contained only water. All mice strongly avoided 75-μg/ml nicotine to water over periods 3 and 4. B6 mice began to diverge from A and D2 mice during

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period 5 and displayed less aversion to nicotine than the other strains during periods 6–8. A repeated-measures ANOVA with factors for strain and sex indicated an overall effect of strain [$F(2,31) = 9.17$, $P < 0.001$] but not test period or sex ($P > 0.1$). However, significant strain × sex [$F(2,31) = 4.37$, $P < 0.05$] and test period × strain [$F(10,155) = 4.03$, $P < 0.0001$] interactions were found. Post hoc analyses (Scheffe, $P < 0.05$) confirmed that B6 mice (overall mean PR = 0.22) displayed significantly less aversion to nicotine than A or D2 mice (mean PR = 0.10).

Mean consumption data for nicotine and water for the 3 strains are provided in Table 1. Notably, mice from the A strain consumed less total fluid than B6 or D2 mice throughout the duration of the testing. There was a significant effect of strain on total consumption, collapsed across test periods [$F(2,35) = 20.16$, $P < 0.00001$], and post hoc tests (Sheffe, $P < 0.05$) indicated that A mice differed from the other 2 strains, whereas B6 and D2 mice displayed similar levels of consumption ($P > 0.5$). PRs, averaged over the 48-h periods and showing the maximum strain difference (i.e., periods 6–8), are displayed for males and females of each strain in Figure 2A. From these data, it is evident that the overall strain difference in level of aversion seen in Figure 1 is predominantly due to attenuated avoidance of nicotine among B6 female mice [Figure 2A; strain × sex interaction, $F(2,32) = 4.87$, $P < 0.05$]. B6 female mice ($n = 6$) displayed significantly less aversion to nicotine compared with males or females of the other strains ($P < 0.05$), although they did not significantly differ from B6 males ($n = 8$, $P > 0.12$). When only males were compared, B6 males (mean PR = 0.18) tended to have a higher PR than D2 or A males (mean PRs = 0.09 and 0.08, respectively), although this difference was not significant [$F(2,18) = 2.91$, $P = 0.08$]. Daily (24 h) dose (milligrams/kilograms) during this same time period is displayed in Figure 2B. During this portion of the test, B6 females displayed significantly greater nicotine consumption than B6 males and A and D2 mice of either sex [Figure 2B; strain × sex interaction, $F(2,32) = 7.21$, $P < 0.01$; Scheffe, $P < 0.05$]. When only males were compared, B6 males (mean 24-h dose = 2.84 mg/kg) had a higher 24-h dose than D2 or A males [mean doses = 1.55 and 1.08, respectively; $F(2,18) = 4.47$, $P < 0.05$].

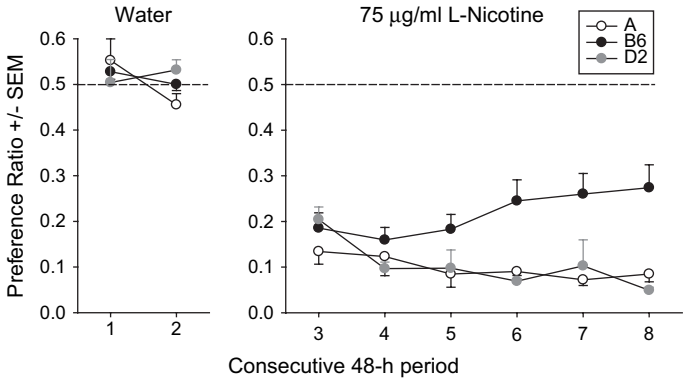

Figure 1 Forty-eight-hour PRs (mean ± standard error) for A, B6, and D2 mice during the water-only pretest (left) and across 6 consecutive periods with 75-µg/ml L-nicotine versus water. The dashed line denotes a PR of 0.5, indicating equal consumption from both bottles.

Brief-access tests

A, B6, and D2 mice (n = 9/strain) were tested with a 6-concentration series of nicotine over a period of 5 consecutive days. Mice from all 3 strains reduced licking to nicotine in a concentration-dependent fashion (Figure 3A). Repeated-measures ANOVA with factors for strain and sex indicated a main effect of concentration [$F(5,105) = 90.99$, $P < 0.00001$], but the strains did not differ from one another in level of avoidance [$F(2,21) = 0.19$, $P > 0.5$]. There were no effects of sex [$F(1,21) = 2.03$, $P > 0.15$], and there were no significant interactions (F values ≤ 0.85, $P > 0.5$).

Table 1 Mean water, solution, and total consumption per strain (milliliters) during the 2-bottle preference tests

Strain		Water 1	Water 2	Nicotine 1	Nicotine 2	Nicotine 3	Nicotine 4	Nicotine 5	Nicotine 6
B6	Total water consumed (ml)	6.42	5.89	8.75	9.97	9.76	9.86	8.94	8.60
	Total solution consumed (ml)	5.78	5.87	1.99	1.89	2.17	3.19	3.21	3.21
	Total amount consumed (ml)	*12.20*	*11.76*	*10.74*	*11.86*	*11.93*	*13.76*	*12.15*	*11.81*
D2	Total water consumed (ml)	5.58	5.47	8.50	10.79	10.79	11.87	11.00	12.07
	Total solution consumed (ml)	5.72	6.01	2.02	1.11	0.98	0.87	1.20	0.62
	Total amount consumed (ml)	*11.30*	*11.48*	*10.52*	*11.90*	*11.77*	*13.07*	*12.20*	*12.69*
A	Total water consumed (ml)	2.89	5.04	7.78	7.62	8.12	8.75	8.03	8.30
	Total solution consumed (ml)	3.93	3.97	1.11	0.99	0.78	0.84	0.61	0.84
	Total amount consumed (ml)	*6.82*	*9.01*	*8.89*	*8.61*	*8.90*	*9.59*	*8.64*	*9.14*

Solution was water for the first two 48-h periods, followed by 75-µg/ml nicotine for 6 consecutive 48-h periods. The strains (B6, D2, and A) did not vary significantly in body weight at the start or end of testing [$F(2,35) \leq 1.37$, $P > 0.27$]; therefore, consumption values were not adjusted for body weight.

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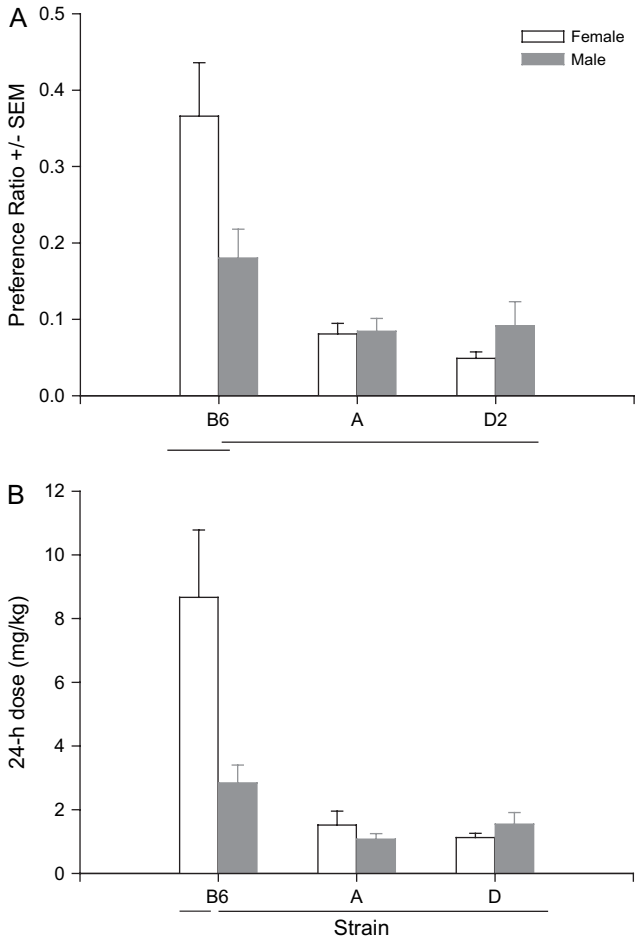

Figure 2 Forty-eight-hour PRs **(A)** and dose **(B)** averaged across the final 3 test periods shown in Figure 1. Data (mean ± standard error) are separated into females (black bars) and males (white bars) for each strain. Horizontal lines at the bottom of each graph indicate group differences in post hoc tests ($P < 0.05$).

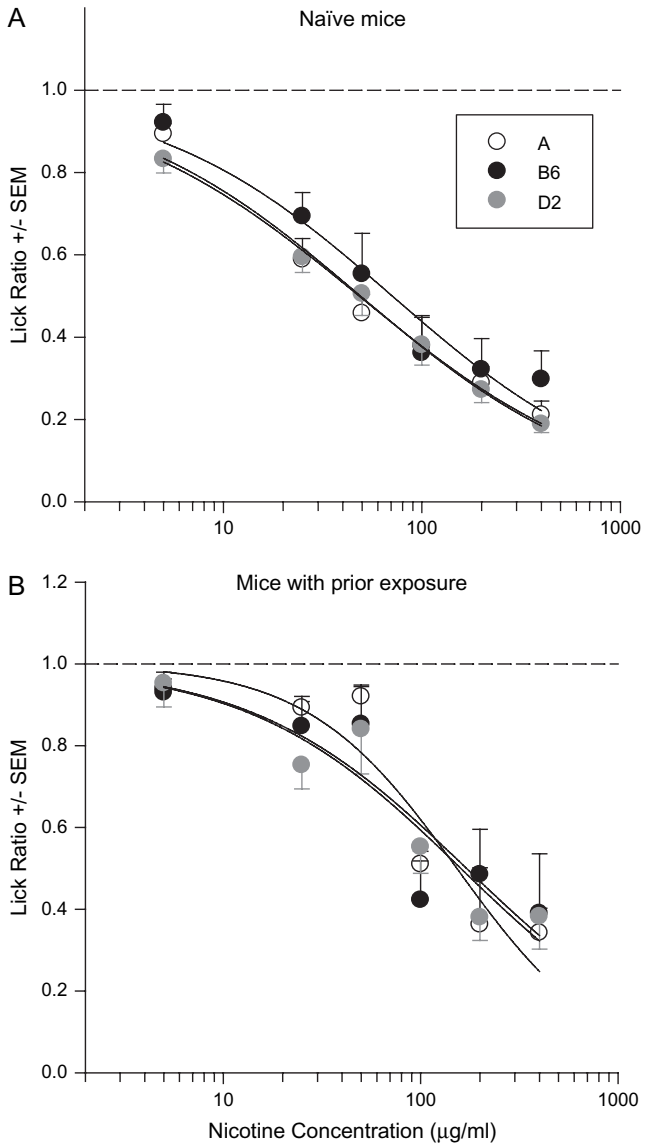

Figure 3 LRs (mean ± standard error) for A, B6, and D2 mice for concentration series of L-nicotine. Mice were either naive at the time of testing **(A)** or previously used in intake tests (**B**; e.g., Figure 1). The dashed line represents a PR of 1.0, which indicates a lick rate equal to that of water. For either condition, LRs to nicotine decreased with increasing concentration. The strains did not differ significantly in LR.

We next investigated the possible effects of prior exposure to nicotine on brief-access behavior (Figure 3B). A subset of mice ($n = 4$/strain) from the intake test were tested with the same concentration series of nicotine as the naive mice over a period of 5 consecutive days. As in the naive group, mice from all 3 strains reduced licking in a concentration-dependent fashion [$F(5,45) = 50.44$, $P < 0.00001$]. There was not a significant effect of strain [$F(2,9) = 0.03$, $P > 0.50$]. However, there was evidence for a shift in sensitivity between the inexperienced and experienced groups: The experienced mice tended to possess higher mean LRs at 25 and 50 μg/ml as compared with the inexperienced mice, reflective of a modest decrease in orosensory-based aversion.

In order to assess the stability of brief-access responses to nicotine over the 5 test days in individual mice, we examined concentration–response functions for each test day (data not shown). These data were fitted with 2-parameter logistic functions, so that the concentrations evoking half-maximal avoidance (c) could be determined and compared across days and strains. There were no significant effects of either test day [$F(4,48) = 1.43$, $P > 0.20$] or strain [$F(2,12) = 0.03$, $P > 0.5$], supporting the conclusion that the strains do not differ in level of aversion and indicating that orosensory-based aversion of nicotine remained fairly stable across the 5-day test period.

As was the case with the inexperienced mice, repeated-measures ANOVA on half-maximal avoidance (c) did not indicate a significant effect of test day [$F(4,16) = 2.1$, $P > 0.12$] or strain [$F(2,4) = 0.36$, $P > 0.5$]. Comparisons of c parameters from the experienced versus inexperienced groups

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(collapsed across strains; c derived from individual mean functions) indicated a significant shift in sensitivity: the average concentration evoking half-maximal avoidance in experienced mice was 155.63 mg/ml, whereas it was 52.48 mg/ml for the inexperienced mice (group comparison with Student t; $t = 4.67$, $P < 0.0001$).

We estimated nicotine intake during the brief-access tests, in order to assess whether 24-h nicotine dose was comparable to that during the 2-bottle intake tests. Because the volume licked from any one of the stimulus bottles during the daily session was minute, making accurate measurement difficult, we instead counted licks in each trial and multiplied these by a factor (0.0012) representing fluid per lick (milliliter). We have previously measured licking and intake simultaneously in large samples of inbred mice over consecutive days; tested in this way, mice consume an average of ~1.2 µl per lick (Boughter J, unpublished data). These measurements were collected using the same sipper tube orifice size as in the present experiment and agree with previous studies (Dotson and Spector 2005; Boughter et al. 2006). In the current study, data from the single training day with water (20-min session) generally agree (A = 1.24, B6 = 1.07, and D2 = 1.14 µl per lick), but the factor (1.2) from the more robust data set was used.

Mean 24-h dose across the 5 brief-access sessions for all strains is displayed in Figure 4. As expected, strains did not differ significantly from one another in either the naive or the experienced condition (1-way ANOVA, P values >0.39). Naive versus experienced mice were compared within each strain by way of t-test; only the experienced A mice displayed significantly greater dose than their naive counterparts ($t = 3.84$, $P < 0.005$). For all A and D2 mice, the 24-h dose of nicotine that mice received during brief-access testing (mean = 1.92 mg/kg for A and 2.07 mg/kg for D2) was comparable to that received during extended 2-bottle testing with 75-µg/ml nicotine (calculated from mean 48-h data; mean = 1.48 mg/kg for A and 1.91 mg/kg for D2). Even though the total volume of nicotine consumed during the brief-access test is much smaller than the volume consumed in the intake test, the mice are sampling concentrations stronger than 75 µg/ml. For B6 mice, the dose received in the intake test (mean = 4.36 mg/kg) was substantially higher than that in the brief-access test (mean = 1.98 mg/kg). As noted earlier, this difference is due to the increase in nicotine consumption that developed among B6 mice after 5–6 days of exposure.

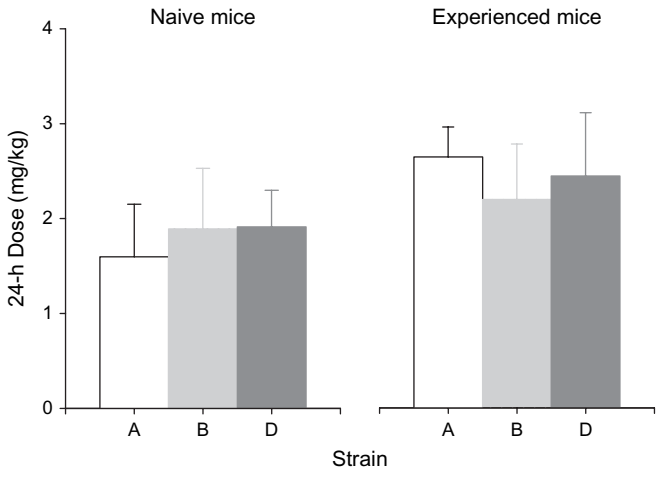

Figure 4 Average 24-h dose in the brief-access experiment, for both the naive and experienced groups of A, B6, and D2 mice. Data are estimated from lick data across 5 days of testing.

Discussion

Previous studies measuring nicotine consumption in mice using single or multiple bottle paradigms have fostered genetic approaches to nicotine addiction (e.g., Butt et al. 2005; Li et al. 2005; Agatsuma et al. 2006). However, it is uncertain if variation in sensitivity to the taste or trigeminal properties of nicotine plays a role in differential strain responses. We conducted and compared the results from a 2-bottle intake assay with sensory-based, brief-access tests. We found that mice exhibiting greater consumption of nicotine in intake tests (B6 mice) did not differ from other strains in orosensory response, supporting the assertion that differential consumption is due to the postingestive properties of nicotine (Butt et al. 2005; Li et al. 2006).

In a 2-bottle intake test, we confirmed that B6 mice (especially female B6 mice) tend to consume more nicotine than D2 or A mice. Because previous studies have characterized this strain difference in consumption over an extended range, we focused on a single concentration (75 µm/ml) deemed likely to result in strain differences (Meliska et al. 1995; Robinson et al. 1996). Both B6 males and females increased consumption relative to D2 and A mice of either sex, although this effect was more pronounced in the B6 female mice. Increased consumption of nicotine by B6 females relative to B6 males has been noted previously (Meliska et al. 1995; Klein et al. 2004; Li et al. 2005). However, in the study of Robinson et al. (1996), only male mice were used and B6 males demonstrated greater consumption of nicotine than males of 5 other strains, including D2 and A. In the study of Meliska et al. (1995), greater consumption of 63 and 100 µm/ml nicotine by B6 males relative to D2 males was noted, although females of these strains showed a broader difference. The nature of this sex difference is not well understood. Klein et al. (2004) found that whereas B6 females voluntarily consumed more nicotine than males (measured every 24 h for 7 days), mice of both sexes did not differ in serum cotinine levels (measured after 7 days of testing was complete). Cotinine is a metabolite of nicotine; this finding suggests a sex difference in nicotine pharmacokinetics.

For some bitter-tasting stimuli such as quinine or the acetylated sugar sucrose octaacetate (SOA), brief-access tests and preference tests yield essentially concentration-by-concentration similarity, and strain differences show a high degree of similarity between tests (Boughter et al.

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2002, 2005). The results of these 2 previously published studies indicate that strain variation in preference is mediated by orosensory cues. Indeed, differential aversion of these compounds is strongly linked to polymorphisms in *Tas2r* bitter taste receptor genes (Chandrashekar et al. 2000; Bachmanov et al. 2001; Nelson et al. 2005). For other bitter compounds such as phenylthiocarbamide, brief-access and preference tests give differential results, arguing for the involvement of postingestive factors such as toxicity or learned aversions in the intake response (Whitney and Harder 1986; Nelson et al. 2003; St John et al. 2005). For nicotine, the dissimilarity of strain differences between brief-access and preference functions also points to a postingestive effect.

In brief-access tests, strain differences in regard to a broad range of nicotine concentrations were not evident. We did not measure significant effects of sex or experience (test day). Because the brief-access test measures an immediate sensory-based response, we conclude that the greater preference of B6 mice (and especially B6 females) for nicotine in the 2-bottle choice test is probably not mediated by an attenuation in sensory response to this stimulus. This conclusion is consistent with that of a previous study where mice consumed more D-nicotine than L-nicotine in 4-bottle tests (Butt et al. 2005), possibly due to the fact that L-nicotine is a more potent enantiomer in terms of drug effect than D-nicotine (Marks et al. 1996). Presumably, however, D-nicotine may also be a less effective taste or trigeminal stimulus. Indeed, in brief-access tests, B6 and A mice expressed less aversion (more licks) to D-nicotine than L-nicotine (100 or 200 mg/ml; Boughter J, Glatt A, unpublished data).

Even though the brief-access functions of individual mice did not change significantly with repeated testing, it was interesting that mice with prior experience in the 2-bottle tests had right-shifted functions (less sensitive) when compared with naive mice. However, it is important to note that this effect was primarily at intermediate concentrations; mice of all strains were still indifferent to 5 mg/ml while displaying strong aversion to 200 and 400 mg/ml. In any case, the strains did not differ. The curve shift is in agreement with the results of Flynn et al. (1989), who reported an increase in taste palatability in rats following chronic nicotine exposure in a taste reactivity test. In a previous study, mice given chronic forced exposure to SOA showed less aversion when given a 2-bottle test with this bitter compound. It is also possible, although not likely, that increased age in the experienced mice contributes to the curve shift (Tordoff 2007). In the current study, experienced mice were administered the brief-access test 1 week after the cessation of the intake test, so the ages of each group were similar.

In the brief-access tests, there were no effects of sex on nicotine avoidance. There exists no evidence that B6 males and females differ in level of aversion to bitter stimuli such as quinine, SOA, or propylthiouracil, either in preference tests or in brief-access tests (Lush 1984; Glendinning et al. 2002; Boughter et al. 2005).

If variation in orosensory ability does not contribute to strain differences in intake, then what does? The search for genetic factors underlying nicotine administration has yielded several possibilities. It is possible that variation in metabolism of nicotine may play a role in overall strain differences in intake. For example, genetic polymorphisms associated with nicotine metabolism in humans may contribute to nicotine use and addiction (Tutka et al. 2005). However, Hatchell and Collins (1980) did not report significant overall differences between B6 and D2 mice in liver nicotine elimination rates. Petersen et al. (1984) did not find strain differences between B6, D2, or C3H males in the time course of blood nicotine concentration following injection. Other studies on mechanisms of nicotine action in these strains do not provide a clear explanation for differential intake. Marks et al. (1983) did not find strain differences (including B6 and D2) in terms of nicotine binding in several brain regions. A recent report indicated that nicotine consumption was correlated with variation of a polymorphic nicotinic receptor, *Chrna4* (Butt et al. 2005). However, a functional relationship between this receptor and nicotine intake has yet to be demonstrated, and the relationship of sex differences in nicotine intake to *Chrna4* is unknown.

Another possibility, however, is that B6 mice possess a more general affinity for orally ingested drugs or hedonically positive substances. It has long been appreciated that B6 mice consume more ethanol relative to several other strains, including D2 (McClearn and Rodgers 1959; Belknap et al. 1993). B6 mice display a stronger preference for saccharin and other sweet-tasting stimuli relative to D2 and A mice, although this is thought to be primarily due to possession of the "preferrer" haplotype of the sweet taste receptor gene *Tas1r3*, whereas D2 and A mice possess the "non-preferrer" haplotype (e.g., Reed et al. 2004). Interestingly, B6 mice also show elevated consumption of amphetamine (Meliska et al. 1995) and morphine solutions (Horowitz et al. 1977; Berrettini, Alexander, et al. 1994), suggesting the possibility of a common intake mechanism in these mice related to reward or addiction circuitry. B6 females tend to consume more ethanol than males (e.g., Meliska et al. 1995; Li et al. 2005), but this is not the case for amphetamine (Meliska et al. 1995) or morphine (Berrettini, Ferraro, et al. 1994), suggestive of a common mechanism perhaps for ethanol and nicotine. Furthermore, there is evidence in crosses of B6 and C3H/HeJ mice of a strong correlation between nicotine and ethanol intake (Li et al. 2005), suggestive of genetic overlap between consumption of these drugs. Additionally, B6 mice have been shown to have altered dopamine receptor activity relative to D2 mice, which may play a role in global differences in addiction behavior (Ng et al. 1994). Still another possibility is that the strain difference reflects a postingestive, but not drug-related, effect, such as tolerance to toxicity or differences in taste aversion learning (St John et al. 2005).

In conclusion, the results of this study demonstrate that strain differences in preference and consumption of nicotine

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cannot be predicted by variation in orosensory response to this bitter-tasting compound. The results validate the use of the 2-bottle test to gage variation in nicotine consumption, although the question of why B6 mice (and B6 females in particular) display elevated consumption relative to other strains remains unanswered. Future genetic approaches in mice concerning nicotine intake/addiction may focus on applying agonists or antagonists of nicotine-specific mechanisms, or more general drug mechanisms, directly to particular brain areas and measuring effects of these manipulations on nicotine consumption.

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