Original Investigation

Sex differences in response to nicotine in C57Bl/6:129SvEv mice

Carolina Isiegas, Stephen D. Mague, & Julie A. Blendy

Abstract

Introduction: Human studies suggest that smoking behavior in men may depend more on the pharmacological effects of nicotine, whereas in women, this behavior may rely more on non-pharmacological factors associated with smoking. Investigation of these parameters in mice may also reveal sex differences.

Methods: Male and female C57Bl/6:129SvEv hybrid mice were exposed to increasing concentrations of nicotine in a voluntary oral nicotine consumption paradigm in which we measured fluid consumption over time and dose. Separate cohorts of mice were exposed to nicotine in a place-conditioning paradigm, and preference was determined. These behavioral models were used to examine sex differences in mice as they rely on pharmacological as well as nonpharmacological factors. Mice exposed to nicotine or saline also were tested for sex differences in locomotor-activating effects of nicotine and in analgesia using standard activity monitoring and hot-plate tests.

Results: Females responded more to the conditioned rewarding effects of nicotine compared with males. Males, however, seemed more responsive to the pharmacological aspects of nicotine by reducing nicotine drinking at higher concentrations; females maintained consistent levels of intake over several doses of nicotine. Male and female mice demonstrated similar locomotor and antinociceptive responses to nicotine.

Discussion: These data suggest that place-conditioning and two-bottle choice paradigms may be more sensitive measures of sexual dimorphism in the C57Bl/6:129SvEv hybrid mouse than are locomotor or nociception assays.

Introduction

Several studies suggest that women and men differ in the extent to which the pharmacological effects of nicotine contribute to

their smoking behavior. For example, in women, the subjective and reinforcing effects of smoking are less dependent on the amount of nicotine present in cigarettes (Perkins, Jacobs, Sanders, & Caggiula, 2002). Relative to men, women demonstrate reduced sensitivity to changes in nicotine dose via nasal spray (Perkins, 1999). By contrast, manipulations of nonpharmacological aspects of smoking, such as blocking the visual and olfactory stimuli from smoking, may reduce smoking behavior to a greater extent in women (Perkins et al., 2001). Taken together, these studies suggest a reduced influence of nicotine and a greater influence of conditioned cues on smoking behaviors in women compared with men.

Consistent with human studies, several animal studies evaluating sex differences have found that male and female rats have different sensitivities to the pharmacological effects of nicotine (Elliott, Faraday, & Grunberg, 2003; Elliott, Faraday, Phillips, & Grunberg, 2004; Faraday, Blakeman, & Grunberg, 2005; Faraday, O'Donoghue, & Grunberg, 1999, 2003). Studies in mice also have observed sex differences. For example, female mice of different inbred strains are less sensitive than male mice to the depressant effects of nicotine on spontaneous motor activity (Hatchell & Collins, 1980; Lopez, Simpson, White, & Randall, 2003), although no sex differences were observed between male and female mice of the outbred strain ICR (Damaj, 2001). Differences in nicotine-induced antinociception also have been observed. In both acute and chronic pain models, female rats are more sensitive than male rats to the analgesic effects of nicotine (Chiari, Tobin, Pan, Hood, & Eisenach, 1999; Craft & Milholland, 1998; Lavand'homme & Eisenach, 1999). In contrast, male mice of the outbred strain ICR show greater sensitivity to nicotine-induced antinociception compared with female mice (Damaj, 2001). These conflicting reports highlight the importance of considering both animal species and genetic background when examining sex differences in response to nicotine.

Nicotine can produce both rewarding and aversive effects in animals and humans depending on the experimental conditions

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and the subject's history (Goldberg & Spealman, 1982, 1983; Henningfield & Goldberg, 1983a, 1983b). The extent to which nicotine is rewarding or aversive may underlie the differences observed between men and women in smoking behavior. A mouse model for exploring the rewarding effects of nicotine is the place-conditioning procedure in which a distinct environment is repeatedly paired with administration of nicotine, resulting in the ability of that environment to elicit approach behavior and increase the time spent by the mouse in the environment associated with nicotine effects. The acquisition of nicotine place preference is likely to be correlated with the rewarding effects of nicotine, whereas its expression reflects the influence on behavior of environmental stimuli previously associated with nicotine effects (Le Foll & Goldberg, 2006).

The two-bottle choice paradigm is an alternative behavior used in rodents to address the active self-administration of nicotine that parallels the human condition. This procedure measures directly the amount of nicotine consumed and enables investigators to distinguish direct nicotine effects from nicotine-associated cues, a prominent feature of the place-conditioning paradigm. Two-bottle choice has been reported to demonstrate both aversion and preference for nicotine in mice depending on nicotine concentration (Adriani, Macri, Pacifici, & Laviola, 2002; Agatsuma et al., 2006; Klein, Stine, Pfaff, & Vandenberg, 2003; Klein, Stine, Vandenberg, Whetzel, & Kamens, 2004; Lee, Chen, Shih, & Hiroi, 2004; Meliska, Bartke, McGlacken, & Jensen, 1995; Robinson, Marks, & Collins, 1996; Zhu et al., 2005). In a two-bottle choice paradigm, mice are exposed to two bottles: one containing water and the other nicotine, the locations of which are switched every few days to prevent conditioned place associations from masking drug effects (Klein, 2001; Klein et al., 2004; Shaham, Alvares, Nespor, & Grunberg, 1992; Todte et al., 2001).

Modeling sex differences in laboratory animals is critical to understanding the underlying mechanisms associated with nicotine dependence. A major advance in the investigation of the molecular and genetic mechanisms contributing to complex diseases, such as nicotine dependence, has been the utility of homologous recombination and gene targeting in mice. The two prominent strains used in this approach are C57BL/6 and 129SvEv (and various substrains of the latter), with a majority of knockout mice being generated in a hybrid strain of these two. Thus, the objective of the present study was twofold. First, we wanted to compare conditioned effects of nicotine with voluntary self-administration of nicotine. Second, we wanted to assess sex differences in nicotine responses in C57BL/6:129SvEv F1 hybrid mice. Information derived from these studies will help establish baseline measurements with respect to place conditioning and voluntary oral nicotine consumption, as well as locomotor activity and antinociception, with the goal of informing future studies aimed at investigating the molecular underpinnings of sex differences.

Methods

Subjects

Male and female F1 hybrids of C57BL/6NTac:129S6SvEvTac mice aged 3–5 months were used for all experiments. Animals were housed in a 21 °C humidity-controlled animal care facility approved by the Association for Assessment and Accreditation

of Laboratory Animal Care; food (Laboratory Autoclavable Rodent Diet 5010; LabDiet, Richmond, IN) and water were available *ad libitum*. The rooms were on a 12-hr light–dark cycle with lights on at 7 a.m. and off at 7 p.m. Separate cohorts of mice were used for all behavioral assays.

Drugs

For the two-bottle choice paradigm, a solution of (–)-nicotine-free base (1.01 g/ml unit; Sigma, St. Louis, MO) in tap water was used at final concentrations of 3, 6, 12, and 24 µg/ml. The nicotine solution was prepared every 3 days based on a published report that determined that no appreciable loss of nicotine in solution occurs within this time frame (Rowell, Hurst, Marlowe, & Bennett, 1983). For place conditioning, locomotor activity, and hot-plate tests, (–)-nicotine hydrogen tartrate salt (Sigma) in 0.9% saline was used. Nicotine was injected intraperitoneally at final concentrations of 0.16, 0.32, or 0.65 mg/kg nicotine-free base.

Two-bottle choice

Two-bottle choice was used as a paradigm to test voluntary oral nicotine consumption. Mice (10 males and 10 females) were individually housed in hanging wire cages with food available *ad libitum*. Mice were subjected to a 6-day acclimation period, during which they had access to two graduated cylinders containing water. During the entire testing period (24 days), mice had access to two graduated cylinders: one containing water and the other nicotine solution. Mice were exposed to increasing concentrations of nicotine (3, 6, 12, and 24 µg/ml for 6 days each), and the location of the graduated cylinders was switched every 3 days to prevent development of location bias. Fluid intake was measured daily at 10 a.m. Body weight was measured, and fresh nicotine solution was made every 3 days. Nicotine preference or aversion was expressed as a ratio of fluid intake from the nicotine solution divided by the total fluid intake. Nicotine and water consumption were expressed as total volume of nicotine or water (in milliliters) consumed during the 6 days that animals had access to a given nicotine concentration divided by body weight (in grams). Nicotine intake was expressed as nicotine (in milligrams) consumed during the 6 days that animals had access to a given nicotine concentration divided by body weight (in grams).

Place conditioning

Place-conditioning boxes consisted of two distinct plastic sides (20 × 20 × 20 cm): one with stripes on the walls and a metal grid over cage bedding and the other with gray walls and smooth flooring. A partition separated the two sides with an opening (5 × 5 cm) that allowed access to either side of the chamber during preconditioning and test days. This partition was closed off during pairing days. Behavior of animals was videotaped and scored by a blinded observer.

Preconditioning phase. On Day 1, mice were allowed to explore both sides of the place-conditioning boxes for 900 s. The animal was scored as being on a particular side if the head and front paws were in that chamber. This predrug preference baseline was used to separate mice into groups with approximately equal biases for each side. None of the mice exhibited a significant preference for one side over the other on the preconditioning day.

Conditioning phase. Days 2–9 were conditioning days. A solid partition separated the two sides so that animals were

confined to one side or the other of the conditioning box. The saline-paired group ($n = 6$ males and $n = 6$ females) received saline injections (0.9% sodium chloride) on both sides of the boxes, while the drug-paired group ($n = 8$ males and $n = 8$ females) received nicotine (0.32 mg/kg) on one side and saline on the other side. On a given day, mice received either saline or nicotine followed by placement into either the striped or the solid gray compartment for 30 min. On the following day, mice received the alternate treatment and were placed in the alternate environment. The conditioning procedure was counterbalanced within each group for injection order (saline or nicotine) and side of the compartment that was paired with the drug.

Testing phase. On Day 10, all mice were given a saline injection and allowed to roam freely between the two sides for 900 s. Time spent on each side was recorded, and data were expressed as time spent on the drug-paired side minus time spent on the saline-paired side.

Locomotor activity

Locomotor activity was assessed in a "home cage" activity monitoring system (Med Associates, St. Albans, VT). The home cage (28.9 × 17.8 × 12 cm) was placed in a photobeam frame (30 × 24 × 8 cm) with sensors arranged in an eight-beam array strip. Mice were injected with nicotine (0.16 or 0.65 mg/kg) or saline and immediately after were individually placed in the cages. Beam break data were read into Med Associates personal computer-designed software and monitored for 30 min.

Hot-plate test

Mice were injected with saline or nicotine (0.16 or 0.65 mg/kg) and after 5 min were placed into a glass square on a hot plate (Columbus Instruments, Columbus, OH) maintained at 55 °C. Two nociceptive thresholds were evaluated: the latency (in seconds) to lick the paws and the first jump observed. A 120-s cutoff was used to prevent tissue damage.

Data analyses

Repeated measures analyses of variance (ANOVAs) were used to analyze the two-bottle choice data using SigmaStat with Student–Newman–Keuls or Tukey post-hoc tests. One- and two-way ANOVAs were used to analyze place-conditioning, locomotor activity, and hot-plate data using StatView with Bonferroni/Dunn or Student–Newman–Keuls post-hoc tests. Significance was established at a p level of less than .05.

Results

Sex differences in the two-bottle choice paradigm

To examine sex differences in voluntary oral nicotine consumption, males and females were evaluated in the two-bottle choice paradigm across a range of nicotine concentrations for which reward or aversion has been reported (Agatsuma et al., 2006; Lee et al., 2004; Zhu et al., 2005). Nicotine preference ratios revealed a reduced preference for nicotine (aversion) in males as nicotine concentration increased, whereas females consumed nicotine and water equally regardless of the nicotine concentration (Figure 1). A significant interaction of sex by nicotine dose indicated that increasing nicotine concentration differentially

affected male and female nicotine preference ratios; two-way ANOVA with repeated measures, $F(3, 54) = 3.076$, $p = .035$. Specifically, at higher concentrations, males preferred plain water over water containing nicotine, as shown by the significant decrease in the nicotine preference ratio; one-way ANOVA with repeated measures, $F(3, 27) = 6.575$, $p = .002$. This decrease, however, was not observed in females; one-way ANOVA with repeated measures, $F(3, 27) = 0.159$, $p = .923$. Student–Newman–Keuls post-hoc tests revealed a significant decrease in the nicotine preference ratio in males at 24 µg/ml compared with 3 µg/ml ($p < .001$) and 6 µg/ml ($p = .006$) and at 12 µg/ml compared with 3 µg/ml ($p = .018$).

Voluntary oral nicotine consumption also was assessed by the amount of nicotine consumed after adjusting for body weight (Supplementary Figure 1). A two-way ANOVA with repeated measures revealed an effect of sex, $F(1, 54) = 6.888$, $p = .017$, in which females consumed significantly more nicotine than males. Consistent with the nicotine preference ratios, there was also an interaction between sex and nicotine dose, $F(3, 54) = 6.067$, $p = .001$, further corroborating the differential effect of increases in nicotine concentration on voluntary oral nicotine consumption in males and females. Specifically, Student–Newman–Keuls post-hoc tests showed that nicotine consumption was significantly higher in females than in males at the highest nicotine dose (24 µg/ml; $p < .001$).

The total volume of nicotine intake normalized to body weight was examined to determine sex differences in the amount of nicotine solution consumed (Supplementary Figure 2). At low nicotine concentrations, males drank equal amounts of nicotine and water. However, males consumed less nicotine at the highest nicotine concentrations; one-way ANOVA with repeated measures, $F(3, 27) = 7.279$, $p < .001$. Student–Newman–Keuls

Figure 1. Male mice display aversion to nicotine solution as nicotine concentration increases. Increasing nicotine concentration differentially affected the nicotine consumption ratio in males ($n = 10$) and females ($n = 10$) as revealed by a significant interaction of sex by nicotine dose. Data are expressed as the average of nicotine ratios, which are calculated by dividing the volume of nicotine fluid consumption (in milliliters) by the total fluid consumption (in milliliters) across the 6-day period during which animals were exposed to a given nicotine concentration. Values are mean with SEM. * $p < .05$ from preference ratio at 3 µg/ml. ** $p < .01$ from preference ratio at 6 µg/ml. † $p < .001$ from preference ratio at 3 µg/ml.

post-hoc tests revealed a significant decrease in the volume of nicotine consumed at 12 $\mu\text{g}/\text{ml}$ ($p = 0.028$) and 24 $\mu\text{g}/\text{ml}$ ($p < .001$), compared with 3 $\mu\text{g}/\text{ml}$, and at 24 $\mu\text{g}/\text{ml}$, compared with 6 $\mu\text{g}/\text{ml}$ ($p = .030$). In contrast, females drank similar amounts of nicotine solution across all nicotine doses; one-way ANOVA with repeated measures, $F(3, 27) = 0.662$, $p = .583$. Further analysis revealed a marginal sex by nicotine dose interaction; two-way ANOVA with repeated measures, $F(3, 54) = 2.527$, $p = .067$, indicating that the volume of nicotine consumed by males and females was affected differentially by the increase in nicotine concentration.

Given that we did not include saccharin to mask the bitter taste of nicotine, the procedure left open the possibility that the males have an aversion to the taste of the nicotine solution that may increase as the nicotine concentration increases. To address this issue, we assessed the response of male and female mice to a bitter taste by presenting quinine (33 μM) in a two-bottle choice paradigm. The levels of aversion to quinine were similar in males and females (data not shown). As a result of this finding, our data from the voluntary oral nicotine consumption experiment suggest that males respond more to some other pharmacological effect of nicotine that causes the reduction in consumption.

Sex differences in place conditioning

To examine sex differences in the rewarding effects of nicotine, we subjected male and female mice to place conditioning (Figure 2). Preconditioning data revealed no significant differences between treatment groups. A three-way ANOVA calculated for the difference in time spent on the nicotine-paired side compared with the saline-paired side showed a significant interaction of conditioning and treatment, $F(1, 50) = 5.152$, $p = .028$. Bonferroni/Dunn post-hoc tests demonstrated that animals preferred nicotine-paired environments ($p < .01$ comparing

Figure 2. Nicotine administration elicits place preference to nicotine-paired environments. Following conditioning, animals preferred nicotine-paired environments. Data are expressed as time spent in drug-paired environments minus time spent in non-drug-paired environments before and after drug conditioning. Values are means with SEM; $N = 6-8$. * $p < .05$ compared with the preconditioning test. ** $p < .01$ compared with saline-conditioned males and females.

nicotine- vs. saline-treated animals following conditioning; $p < .05$ comparing postconditioned nicotine-treated animals vs. preconditioning). We found a strong trend for an increase in nicotine reward in females compared with males, but there was not a significant interaction of sex and drug treatment.

Sex differences in nicotine-induced hypolocomotion and antinociception

To evaluate sex differences in other nicotine-mediated responses, we examined locomotor activity following an acute nicotine injection. Ambulations in a locomotor activity paradigm were quantified in male and female mice. Analysis of the nicotine effect revealed a significant interaction of time and treatment; two-way ANOVA with repeated measures, $F(10, 325) = 11.405$, $p < .0001$. Bonferroni/Dunn post-hoc tests demonstrated a significant decrease in activity with the high dose of nicotine (0.65 mg/kg) compared with both the saline and the low-dose (0.16 mg/kg) groups ($p < .0001$) at 5, 10, and 15 min following nicotine administration (Figure 3).

Sex differences in nicotine-induced antinociception were assessed with a hot-plate test (Figure 4). A two-way ANOVA for the licking response revealed a significant effect of sex, $F(1, 66) = 4.064$, $p = .0479$, and of treatment, $F(2, 66) = 13.203$, $p < .0001$, but no interaction between sex and treatment, $F(2, 66) = 0.654$, $p = .5233$, indicating that acute nicotine administration (0.65 mg/kg, intraperitoneally) increased analgesia equally in males and females, as measured by latency for licking response. Overall, these data indicate that in C57Bl/6:129SvEv hybrid mice, lick latency was more sensitive than jump latency to the analgesic properties of nicotine and that sex did not contribute significantly to these effects.

Discussion

The present study examined the responses of adult male and female mice to various pharmacological effects of nicotine, including nicotine consumption, conditioned reward, hypolocomotion, and analgesia. Adult male and female mice exhibited

Figure 3. Male and female mice show similar hypolocomotor responses to nicotine administration. A high dose of nicotine (0.65 mg/kg) reduced locomotor activity significantly in both male and female mice. Data are expressed as the number of ambulations occurring during each 5-min time interval. Values are means with SEM; $N = 24$. * $p < .0001$ compared with animals treated with either saline or 0.16 mg/kg nicotine.

Figure 4. Male and female mice show similar antinociceptive responses to nicotine administration. Both males and females showed significant increases in the latency to lick their paws following nicotine administration (0.65 mg/kg), whereas the jump latency was not altered significantly. Data are expressed as latency (in seconds) to lick their paws or jump. Values are means with SEM; $N = 12$. ** $p < .01$ from saline and 0.16 groups.

differential sensitivity for nicotine in the C57BL/6:129SvEv hybrid mice, though this effect depended on the behavioral paradigm being examined. Our results in the two-bottle choice paradigm indicated that males consumed less nicotine as the concentration of nicotine increased, whereas females consumed a consistent amount of nicotine across a range of concentrations. The observation that adult male mice consumed less nicotine than water, compared with female mice, after adjusting for body weight, is consistent with a study in which female adolescent mice consumed more nicotine, compared with males, after adjusting for body weight and as a percentage of total fluid intake (Klein et al., 2004).

Nicotine aversion ratio data and the consumption data are in agreement with the sex differences observed in adult mice in a two-bottle choice paradigm (Meliska et al., 1995). However, these studies were conducted in C57BL/6 mice and did not address the possibility that the differences observed were due to potential sex differences in response to the bitter taste of nicotine. Our quinine two-bottle choice data showing similar levels of quinine avoidance in male and female mice help to rule out that confounding possibility. At concentrations of nicotine used in the present study, neither male nor female mice demonstrated a preference for nicotine over water. Higher concentrations of nicotine (up to 60 $\mu\text{g}/\text{ml}$) in the C57BL/6:129SvEv hybrid mice caused some lethality in both males and females (data not shown). As a result, we were not able to evaluate preference for nicotine consumption, as has been demonstrated in a number of other mouse strains (Klein et al., 2004; Li, Karadsheh, Jenkins, & Stitzel, 2005; Meliska et al., 1995; Robinson et al., 1996). Indeed, other studies have used considerably higher concentrations of nicotine in two-bottle choice paradigms, up to 200 $\mu\text{g}/\text{ml}$; however, none of these studies included the 129SvEv strain alone or as part of an F1 hybrid line. It would be interesting to determine the characteristics of the 129SvEv strain that make it so sensitive to nicotine in a voluntary consumption paradigm.

Previous studies in our laboratory with C57BL/6:129SvEv hybrid mice have used both males and females in nicotine-

conditioned place preference, but these studies were not powered sufficiently to separate sex differences. In the present study, we also found a significant effect of nicotine reward. Although there was no interaction between sex and nicotine, females appeared to spend more time in nicotine-paired environments than males.

Studies examining nicotine-conditioned place preference in mice have been performed almost exclusively in males (Agatsuma et al., 2006; Balerio, Aso, & Maldonado, 2005; Grabus, Martin, Brown, & Damaj, 2006; McGeehan & Olive, 2003; Nolley & Kelley, 2007; Rauhut, Hawrylak, & Mardekian, 2008; Sahraei et al., 2004) or in both males and females (Blendy et al., 2005; Walters, Brown, Changeux, Martin, & Damaj, 2006; Walters, Cleck, Kuo, & Blendy, 2005). Males and females of the ICR mouse strain have been characterized extensively but in separate studies (Grabus et al., 2006; Kota, Martin, & Damaj, 2008; Kota, Martin, Robinson, & Damaj, 2007). Females of this strain demonstrated preference at 1.0 mg/kg nicotine-free base (Kota et al., 2008), whereas males did not (Kota et al., 2007). However, these studies used multiple doses of nicotine and demonstrated an effect of 0.5 mg/kg nicotine-free base in male but not in female mice. Because these studies were carried out in separate cohorts, within-study analyses between sexes are precluded. Regardless, these data together with the present study indicate distinct differences in responses to conditioned rewarding properties of nicotine in males and females.

Place-conditioning and two-bottle choice paradigms rely on chronic or repeated nicotine exposure. To examine sex differences following acute nicotine administration, we evaluated behaviors sensitive to acute exposure to nicotine, such as locomotor activity and antinociception. Previous studies (Hatchell & Collins, 1980; Lopez et al., 2003) in mice of inbred strains show that females are less sensitive than males to nicotine-induced hypolocomotion, in agreement with our observations. In contrast, sex differences were not observed in mice of the ICR outbred strain (Damaj, 2001). In antinociception studies, male mice of the ICR outbred strain appear more sensitive than female mice to nicotine-induced analgesia (Damaj, 2001), in contrast to our data in the C57BL/6:129SvEv F1 hybrid strain, for which no sex differences were observed. Possible differences in nicotine receptor affinity, density, or functional regulation or differences in nicotine metabolism may account for these discrepancies across strains and sexes. Indeed, *in vivo* and *in vitro* studies found evidence for sexual dimorphism of nicotine metabolism and distribution in the rat, showing that male rats metabolize nicotine faster than their female counterparts (Kyerematen, Owens, Chattopadhyay, deBethizy, & Vesell, 1988). Although such an exhaustive study has not been conducted in mice, it was recently reported that males, but not females, that are high-nicotine consumers show increased levels of the enzyme responsible for nicotine metabolism CYP2A5 and faster *in vitro* nicotine metabolism relative to low-nicotine consumers (Siu, Wildenauer, & Tyndale, 2006). These data suggest that differential nicotine consumption behaviors may correlate with metabolism in male but not in female mice.

The influence of sex in the regulation of central dopaminergic neurotransmission may be a contributing factor in modulating nicotine responses. For example, the increase in the extracellular dopamine (DA) concentration in the nucleus accumbens has been reported to be higher in female rats than in

male rats following systemic nicotine administration (Pogun, 2001), implying a greater DA response to nicotine in female rats. Furthermore, young and aged female rats have higher striatal DA and homovanillic acid (HVA) levels than male rats, and aging reduces DA and HVA in males but not in females (Dorce & Palermo-Neto, 1994). In addition, endogenous ovarian hormones, but not testicular hormones, modulate extracellular striatal DA concentrations in rats (Xiao & Becker, 1994). These differences may be related to the observations that female rats appear to be more sensitive than male rats to the reinforcing effects of psychostimulants that increase DA levels in the synaptic cleft (Becker, 1999; Dalton, Vickers, & Roberts, 1986). They also may explain our finding that nicotine (0.32 mg/kg) seems to be more rewarding in female mice than in male mice in a place-conditioning paradigm. Alternatively, this result may reflect a differential response to conditioned cues due to sexual dimorphism, similar to that reported in learning and cognitive style (Kanit, Koylu, Erdogan, & Pogun, 2005; Kanit et al., 2000).

Nicotine dependence is a complex condition that presents challenges to efforts aimed at identifying its biological underpinnings. Among the many interacting neurobiological systems implicated in nicotine dependence, emerging preclinical and clinical data support a role for sex differences in response to nicotine and the enduring nature of nicotine dependence. Mice are tractable models for investigating the molecular and genetic factors that mediate nicotine dependence. The C57BL/6 and 129SvEv strains are commonly used for the development of genetic mouse models. Although these mice are initially from a mixed genetic background rather than from a controlled F1 hybrid, information regarding nicotine response in a variety of behaviors is of value, particularly for the study of newly generated knockout mice in which extensive backcrossing has not occurred and these strain alleles are represented more equally. Our data suggest that in this hybrid, males respond more to the pharmacological effects and less to the conditioning effects of nicotine compared with females. Although additional studies and paradigms should be examined, these data correlate with human studies showing that smoking behavior in men is more dependent on nicotine's pharmacological factors, whereas in women, it relies more on nicotine-conditioned cues. In contrast, other acute effects of nicotine appear to be unaffected. Continued attention to sex differences, as well as strain differences in animal studies together with mouse models that enable genetic investigation of these effects, will help elucidate factors underlying nicotine dependence.

Supplementary material

Supplementary Figures 1 and 2 can be found at *Nicotine & Tobacco Research* online (<http://www.ntr.oxfordjournals.org/>).

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Declaration of Interests

None declared.

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