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Delivery of nicotine aerosol to mice via a modified electronic cigarette device

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Abstract

Background—Although both men and women use e-cigarettes, most preclinical nicotine research has focused on its effects in male rodents following injection. The goals of the present study were to develop an effective e-cigarette nicotine delivery system, to compare results to those obtained after subcutaneous (s.c.) injection, and to examine sex differences in the model.

Methods—Hypothermia and locomotor suppression were assessed following aerosol exposure or s.c. injection with nicotine in female and male mice. Subsequently, plasma and brain concentrations of nicotine and cotinine were measured.

Results—Passive exposure to nicotine aerosol produced concentration-dependent and mecamylamine reversible hypothermic and locomotor suppressant effects in female and male mice, as did s.c. nicotine injection. In plasma and brain, nicotine and cotinine concentrations showed dose/concentration-dependent increases in both sexes following each route of administration. Sex differences in nicotine-induced hypothermia were dependent upon route of administration, with females showing greater hypothermia following aerosol exposure and males showing greater hypothermia following injection. In contrast, when they occurred, sex differences in nicotine and cotinine levels in brain and plasma consistently showed greater concentrations in females than males, regardless of route of administration.

Discussion—In summary, the e-cigarette exposure device described herein was used successfully to deliver pharmacologically active doses of nicotine to female and male mice. Further, plasma nicotine concentrations following exposure were similar to those after s.c.

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injection with nicotine and within the range observed in human smokers. Future research on vaped products can be strengthened by inclusion of translationally relevant routes of administration.

Keywords

cotinine; electronic cigarette; metabolism; nicotine; sex differences

1.0 Introduction

Since their introduction to the U.S. market, use of electronic cigarettes (e-cigarettes) has risen dramatically, particularly in youth in grades 6–12 (Bunnell et al., 2015; McMillen et al., 2015). For example, recent data from national surveys conducted by the Centers for Disease Control show that over 10% of male youth reported e-cigarette use in the last 30 days compared to less than 5% of adult males (Table 1). While significantly fewer female youth reported past 30-day use (~8%) compared to male youth, their recent use still remains twice that of adult females. Yet, examination of prevalence figures for more frequent use (i.e., some days or every day) reveals that the percentage of users of both sexes is higher for adults than for youth. Hence, the overall percentages across frequency suggest that youth are more likely to try e-cigarettes whereas adults are more consistent in their use, with similar percentages of men and women reporting regular use. This interpretation is consistent with previous literature reporting that adults use e-cigarettes primarily for smoking cessation (Dawkins et al., 2013) whereas adolescents use primarily for experimentation (Hughes et al., 2015), although longitudinal analysis suggests increasing adolescent use over time (Lippert, 2016).

The use of e-cigarettes by women and men argues for inclusion of both sexes in research on biological mechanisms and consequences associated with their use. To date, however, most preclinical research on tobacco and the nascent research on e-cigarettes have focused on examination of nicotine effects in male rodents following injection. Recently, several laboratories have reported on the development of methods to expose rodents to nicotine and/or tobacco via inhalation (George et al., 2010; Ponzoni et al., 2015; Smith et al., 2015). While many of these studies concentrated primarily on examination of the effects of inhaled nicotine on the pulmonary system or on developmental or toxicological effects (McGrath-Morrow et al., 2015; Misra et al., 2014; Smith et al., 2015; Sussan et al., 2015), a few studies have investigated behavioral effects of inhaled tobacco smoke (Bruijnzeel et al., 2011; de la Pena et al., 2014; de la Pena et al., 2015; Harris et al., 2010; Yamada et al., 2010) or nicotine vapor generated by bubbling air through a nicotine solution (George et al., 2010; Gilpin et al., 2014) and one lab compared the effects of chronic exposure to cigarette smoke or e-cigarette vapor (Ponzoni et al., 2015). However, none of these studies examined sex differences and only the latter study focused on a model of e-cigarette exposure. Further, most of these studies were conducted in rats. The primary metabolic enzyme for nicotine in rats is in the CYP2B family (Nakayama et al., 1993), whereas the primary enzyme in mice is CYP2A5 (Murphy et al., 2005; Siu et al., 2006), which is more closely related (84% sequence homology) to CYP2A6 (Murphy et al., 2005), the predominant liver enzyme in humans that metabolizes nicotine to cotinine (Messina et al., 1997). Hence, mice may

represent a better animal model for studies with a pharmacokinetics component (Matta et al., 2007; Siu et al., 2006).

In the present study, a commercially available tank-based e-cigarette (Brown and Cheng, 2014) was modified to permit rodent exposure to aerosolized e-liquids (i.e., solutions containing a vehicle of propylene glycol and/or vegetable glycerin with nicotine and added flavors). Hypothermia and locomotor suppression, characteristic effects of nicotine in mice (Damaj, 2001), were assessed following inhalational exposure to nicotine aerosol or after subcutaneous (s.c.) injection with nicotine in female and male mice. As a preliminary step towards verifying similar mechanisms, reversal of these effects following injection of the noncompetitive nicotine receptor antagonist mecamylamine was also assessed. Subsequently, plasma and brain concentrations of nicotine and its major metabolite cotinine (Benowitz et al., 1983; Petersen et al., 1984) were measured. Results reported here serve as proof-of-principle for a novel device capable of translationally relevant delivery of nicotine aerosol for use in mechanistic studies of behavioral and biological effects of e-cigarettes. This apparatus has also been used to deliver aerosolized stimulants to rodents (Marusich et al., 2016).

2.0 Materials and Methods

2.1 Subjects

Adult male and female ICR mice (25–35 g) [Harlan/Envigo Laboratories, Frederick, MD] were singly housed in polycarbonate cages with hardwood bedding in a temperature-controlled environment (20–24°C) with a 12 h light-dark cycle (lights on at 0600). All mice had *ad libitum* access to food and water while in their home cages. The studies were carried out in accordance with federal and state regulatory guidelines and were IACUC-approved.

2.2 Drugs and Chemicals

Mecamylamine HCl and (-)-nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO) were dissolved in physiological saline (Patterson Veterinary, Devens, MA), and the pH was adjusted to approximately neutral (pH ~ 7), as necessary. (-)-Nicotine free base (Sigma-Aldrich) was mixed with a 50:50 propylene glycol and glycerin solution (Sigma-Aldrich). Doses of nicotine for injection are expressed as mg/kg of the base. Nicotine and mecamylamine were injected subcutaneously (s.c.) at a volume of 10 ml/kg. Concentrations for aerosol administration are expressed as mg/ml in the e-cigarette tank, and may not be representative of the actual amount of nicotine inhaled.

Chemicals and reagents for the analysis of biological samples were purchased commercially and included nicotine (Sigma-Aldrich), cotinine (Toronto Research Chemicals, Toronto, ON), nicotine-d3 (Cambridge Isotope Laboratories, Tewksbury, MA), cotinine-d3 (Santa Cruz Biotechnology, Dallas, TX), ammonium acetate (Sigma-Aldrich), and formic acid and acetonitrile (Fisher Scientific, Fair Lawn, NJ). An internal standard solution was prepared in methanol (Fisher Scientific) containing 48 μg/mL nicotine-d3 and 38 μg/mL cotinine-d3. Working solutions containing both nicotine and cotinine were prepared in methanol at concentrations of 10,000 and 100 ng/mL.

2.3 Apparatus

Aerosol was generated using a modified commercially available electronic cigarette (Figure S1[1]). An iStick 30W variable wattage (eLeaf, Irvine, CA) supplied power (7W) to a CE5-S tank/clearomizer with bottom dual coil atomizer (1.8Ω) (Aspire, Kent, WA). Air/aerosol was pumped (1L/min) through the bottom of the tank and into an EZ-177 Sure-Seal 1L mouse induction anesthesia chamber (10 cm × 10 cm × 10 cm) [EZ-Anesthesia, Palmer, PA] via Tygon tubing (Fisher Scientific, Pittsburgh, PA) and controlled by 3-way stopcocks (Grainger, Raleigh, NC). The aerosol generation system was placed in a hood to avoid exposure of laboratory technicians to aerosol. Mouse locomotor activity was assessed in separate clear Plexiglas activity chambers (47 cm × 25.5 cm × 22 cm). Each chamber was surrounded by two arrays of 4 × 8 infrared photocell beams, interfaced with software for automated data collection (San Diego Instruments, San Diego, CA). Temperature readings were taken using a BAT-12 Microprobe Thermometer with RET-3 Rectal Probe (PhysiTemp Instruments Inc., Clifton, NJ). Analgesia was measured by a Tail Flick Analgesia Meter (IITC Inc. Life Science, Woodland Hills, CA).

2.4 In Vivo Pharmacology Procedure

In Experiment 1, pharmacological effects of nicotine were evaluated following nicotine exposure via aerosol (0, 12, 24, or 30 mg/ml) or subcutaneous (s.c.) injection (0, 0.5, 1.0, or 1.5 mg/kg). Mice (n=8/sex/group) were brought into the test room and weighed. After a minimum of 30-min acclimation, baseline temperature and tail flick latency were taken, as described previously (Wiley et al., 2015). The mice were then exposed to nicotine via aerosol (see below) or s.c. injection and placed back into their home cage. For aerosol exposure, mice were placed into the anesthesia chambers, where they were allowed to move freely. Subsequently, aerosol was generated for 10 seconds and held in the chamber for 1 minute. Mice were then placed back in their home cage for 2 minutes before being exposed to aerosol again for 1 minute. This process was repeated five times, such that each mouse was exposed to aerosol for 5 minutes over a 13- minute period. This procedure for aerosol exposure was based upon an initial pilot experiment showing that staggered nicotine exposure for 5 minutes over a period of time resulted in higher brain nicotine levels in male mice than did continuous exposure for 5 minutes. Ten minutes after the final aerosol exposure (or s.c. injection), temperature and tail flick latency were measured again and the mice were immediately placed into locomotor chambers for 10 minutes. Thirty-five minutes after the final exposure/s.c. injection, temperature was taken a third time. After a one-week washout, vehicle- or high dose nicotine-treated mice were treated with a single 1 mg/kg s.c. injection of mecamylamine 10 min before exposure to vehicle or high dose nicotine, respectively, aerosol or injection and were tested again as described above. Because antinociception was not observed following either route of administration at the time point measured, these data have been omitted from the results and discussion.

[1]Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:...

2.5 Biological Sample Preparation

Biological samples (plasma and brain) were obtained from experimentally naïve mice administered nicotine via s.c. injection or aerosol exposure, as described above. Sacrifice and collection of blood and tissue occurred 10 minutes after the final aerosol exposure or s.c. injection.

2.5.1 Plasma Samples—Plasma calibration standards and quality control samples were prepared using pooled plasma from remaining control samples. Standards, quality controls, and samples were prepared by spiking 200 μL of plasma with 10 μL of internal standard solution, and an appropriate volume of calibration solution. Calibration standards were created at 1, 2, 5, 10, 100, 500, and 1000 ng/mL for both analytes, and quality control samples were made at 5 and 500 ng/mL for both analytes. Sample extraction was achieved by diluting to a final volume of 1 mL with methanol, vortex mixing for 2 minutes followed by centrifugation at 12,000 RCF for 10 minutes. The supernatants were analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

2.5.2 Brain Samples—Whole brains were placed into a 15 mL falcon tube along with 20 2.3-mm stainless steel beads (BioSpec Products, Bartlesville, OK) and 4 μL of WFI quality water (Corning, Manassas, VA) for every mg of brain tissue. Samples were homogenized in a SPEX Sample Prep Geno-Grinder (Metuchen, NJ) at 1750 RPM for 2 minutes, then centrifuged in a Beckman Coulter Allegra X-15R Centrifuge (Pasadena, CA) for 2 minutes at 1750 RCF to remove any tissue from the lid. Since two layers were observed, the samples were lightly vortexed to assure a homogenous solution. Pooled homogenate used for calibration standards and quality control samples was created using 500 μL from each control sample. Standards, quality controls and samples were prepared by spiking 200 μL of brain homogenate with 10 μL of internal standard solution, and an appropriate volume of calibration solution. The calibration range in homogenate was the same as for plasma, described above. Sample extraction was achieved by diluting to a final volume of 1 mL with methanol, vortex mixing for 2 minutes followed by centrifugation at 12,000 RCF for 10 minutes. The supernatants were analyzed by HPLC-MS/MS.

2.6 Analysis of Biological Samples

The HPLC-MS/MS system consisted of an Agilent 1100 (Santa Clara, CA) coupled to an API-4000 with a TurboIonSpray source (Sciex, Framingham, MA). The auto sampler was maintained at 35°C for the brain tissues and 10°C for plasma. Chromatographic analysis was performed using a Phenomenex (Torrance, CA) Luna Phenyl-Hexyl column (150 × 4.60 mm i.d., 3-μm particle size) and a Phenomenex SecurityGuard AQ C18 4 × 2.0 mm column filter. Five microliters of sample were injected onto the column and elution of the analytes and internal standard was achieved at 35°C using a binary gradient and a flow rate of 0.8 mL/min. The mobile phases consisted of 5 mM ammonium acetate in water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The gradient was 5% mobile phase B for 2 minutes, 5 to 10% B from 2 to 5 minutes, then to 95% B in 2 minutes and held for 0.5 minutes. MS detection was performed with the electrospray ionization source operated in positive ion mode with an ion source temperature of 500 °C and an ion spray voltage of 5000V. Transitions monitored were *m/z* 163.2 → 84.0

for nicotine, 177.1 → 80.1 for cotinine, 166.1 → 89.1 for nicotine-d3, and 180.2 → 101.0 for cotinine-d3. The retention times were 2.4 and 3.0 min for nicotine and cotinine, respectively. Analyst software version 1.6.2 was used for data acquisition and analysis.

2.7 Statistical Analysis

Temperature readings were analyzed as change in temperature from pre-dosing baseline and locomotor activity was measured as total beam breaks during the 10-min session. Mean (±SEM) values for these in vivo measures and for plasma and brain concentrations of nicotine and cotinine were calculated separately for each sex and administered nicotine dose/concentration. For each route of administration, separate factorial ANOVAs (sex X concentration/dose) were performed. Tukey-Kramer post-hoc tests (α=0.05) were used to specify individual differences in the means for all significant ANOVAs.

3.0 Results

Figure 1 shows the effects of nicotine delivered via aerosol (top panels) and s.c. injection (bottom panels) on change in temperature 10 min after exposure (left panels), change in temperature 35 min after exposure (middle panels), and locomotor activity (right panels) in male and female mice. Regardless of route of administration, nicotine significantly decreased temperature at both time points and suppressed locomotor activity in both sexes. Significant decreases in temperature (at both time points) and locomotor activity were observed following aerosol delivery of nicotine [Fig. 1, panel A, main effects of concentration for 10-min temp: $F(3,56)=56.93$, $p<0.05$; Fig. 1, panel B, 35-min temp: $F(3,56)=11.25$, $p<0.05$; and Fig. 1, panel C, locomotor activity: $F(3,56)=24.15$, $p<0.05$] and following s.c. nicotine injection [Fig. 1, panel D, main effects of dose for 10-min temp: $F(3,56)=48.92$, $p<0.05$; Fig. 1, panel E, 35-min temp: $F(3,56)=27.79$, $p<0.05$; and Fig. 1, panel F, locomotor activity: $F(3,56)=11.66$, $p<0.05$]. At 10 and 35 minutes post-exposure, the magnitude of temperature decrease produced by nicotine aerosol in females exceeded that seen with males [Fig. 1, panel A, main effect of sex: $F(1,56)=10.80$, $p<0.05$ and Fig. 1, panel B, main effect of sex: $F(1,56)=4.82$, $p<0.05$, respectively]. Concentration-dependent decreases in locomotor activity were similar across both sexes (Fig. 1, panel C). Temperature decreases were also observed at 10 minutes following s.c. nicotine injection; however, sex differences were not apparent (Fig. 1, panel D). Further, temperatures were decreased to a similar magnitude across all s.c. nicotine doses, suggesting that these doses were at the maximal asymptotic end of the dose-effect curve at this time-point. By 35 minutes post-injection, females, but not males, that received lower nicotine doses had recovered baseline body temperature (Fig. 1, panel E). S.c. nicotine decreased locomotor activity in both sexes to a similar extent (Fig. 1, panel F). Nicotine-induced decreases in body temperature and locomotor activity were reversed by pre-treatment with mecamylamine (1 mg/kg, s.c.) following both routes of administration (left side of each panel). While female and male rats significantly differed in the degree of temperature change at the 10 minutes time-point following exposure to nicotine + mecamylamine [Fig. 1, panel A; $F(3,56)=3.34$, $p<0.05$], this difference may have been driven by the fact that the 24 mg/ml concentration of nicotine alone produced significant sex differences in hypothermia.

Concomitant with the observed pharmacological effects of nicotine, concentrations of nicotine and cotinine in the plasma (Figure 2) and brain (Figure 3) showed dose/concentration-dependent increases in mice of both sexes following each route of administration. Significant concentrations of nicotine were absorbed into the plasma [Figure 2, panels A and B; $F(3,40)=30.21$, $p<0.05$ and $F(3,40)=186.79$, $p<0.05$ for dose/concentration main effects for aerosol and injection, respectively] and subsequently metabolized to cotinine [Figure 2, panels C and D; $F(3,40)=16.34$, $p<0.05$ and $F(3,40)=89.90$, $p<0.05$ for dose/concentration main effects for aerosol and injection, respectively]. Similarly, nicotine and cotinine were distributed to the brain, with significantly increased concentrations of both compounds at higher doses, regardless of route of administration [Figure 3; $F(3,40)=36.60$, $p<0.05$ and $F(3,40)=90.80$, $p<0.05$ for dose/concentration main effects for brain nicotine level after aerosol exposure or injection, respectively and $F(3,40)=23.58$, $p<0.05$ and $F(3,40)=47.85$, $p<0.05$ for dose/concentration main effects for brain cotinine levels after aerosol exposure or injection, respectively]. Direct comparisons of plasma and brain levels across route of administration were precluded because of differences in concentrations/doses administered and in the amount of time required to deliver aerosol (~ 13 minutes) vs. injection (< 1 minute), as well as differences in experimental time points: i.e., mice received initial exposure to aerosol 25 min, whereas injections were administered 10 min, prior to biological sample collection. For example, consistent with the longer exposure time before sample collection, the ratios of cotinine: nicotine in the plasma and in the brain were reliably higher for inhalation than for s.c. injection across all concentrations (Table 2), suggesting that the longer time since exposure initiation in the aerosol exposed mice may have allowed greater metabolism of nicotine to cotinine. Sex differences in nicotine concentrations in plasma and brain occurred only in plasma at the 24 mg/ml aerosolized nicotine concentration, with females exhibiting significantly greater concentration of nicotine than males [Figure 2, panel A; sex X dose interaction: $F(3,40)=2.94$, $p<0.05$]. For s.c. injections, females also showed higher levels of cotinine in plasma [Figure 2, panel D; sex X dose interaction: $F(3,40)=7.41$, $p<0.05$]. Sex differences in brain cotinine concentrations occurred at higher concentrations/doses for both routes of administration [Figure 3, panels C and D; sex X dose interactions: $F(3,40)=2.98$, $p<0.05$ and $F(3,40)=10.72$, $p<0.05$ for aerosol and s.c. injection, respectively]. Again, concentrations were significantly higher in females than in males.

4.0 Discussion

The results of the present study offer proof-of-principle evidence that characteristic concentration-dependent nicotine-induced pharmacological effects can be elicited following exposure to nicotine aerosol delivered via an e-cigarette device in female and male mice. Maximal effects in female mice were similar to those observed after s.c. injection with nicotine, a more traditional route of administration for exposure of rodents to nicotine. In male mice, similarity of the maximal effects was time- and task-dependent, with similar between-route maximal hypothermic effect at 10 minutes, but less pronounced locomotor activity suppression and hypothermia at 35 minutes following aerosol exposure. In both sexes, nicotine concentrations in the plasma after aerosol administration were within the range (10–50 ng/ml) of those observed in human cigarette smokers (Matta et al., 2007) and

similar to or higher than those reported after human e-cigarette use in an experimental setting (Lopez et al., 2016; Ramoa et al., 2016; Velez de Mendizabal et al., 2015). Brain nicotine levels following exposure to aerosol concentrations of 24 and 30 mg/ml nicotine reached those observed with pharmacologically active s.c. nicotine doses of 0.5–1 mg/kg. Since nicotine discrimination studies in mice typically employ approximate parenteral doses of 0.3–1 mg/kg (Caine et al., 2014; Cunningham and McMahon, 2013; Varvel et al., 1999), these results suggest that the e-cigarette apparatus described here exposed mice to the interoceptive cues of nicotine, which may play a role in maintaining nicotine addiction. Previous studies have also demonstrated that repeated exposure to nicotine aerosol/vapor can induce dependence in rats (George et al., 2010; Gilpin et al., 2014) and mice (Ponzoni et al., 2015). In all groups, the noncompetitive nicotinic receptor antagonist mecamylamine attenuated the acute hypothermic and locomotor suppressant effects of nicotine.

Despite the similarity in mecamylamine’s effects, sex differences in nicotine's effects on temperature were observed. Interestingly, however, the direction of the differences was dependent upon route of administration, with females showing greater sensitivity to nicotine's hypothermic effects via aerosol exposure and males showing greater sensitivity following s.c. injection. These differences in the in vivo pharmacological effects were not associated with sex differences in brain nicotine concentrations, although plasma nicotine levels were elevated at 24 mg/ml nicotine aerosol in female (vs. male) mice. Since aerosol nicotine concentration was not adjusted for bodyweight, this difference may have resulted from exposure to different nicotine doses. However, sex differences were not observed in nicotine's suppressant effects on locomotion for either route of administration. Although concentration-dependent sex differences in the metabolism of nicotine to cotinine have been reported in mice (Siu et al., 2006), “response specificity” in nicotine’s effects across sex has been noted previously in ICR mice (Damaj, 2001), suggesting that sex differences in nicotine-induced pharmacological effects in mice are not likely to be mediated solely by pharmacokinetic factors. In humans, nicotine is more reinforcing in men than women (Perkins et al., 2009), whereas cigarette smoking is associated with greater conditioned reinforcement and sensory effects in women than men (Perkins et al., 1999; Perkins et al., 2001); and while both men and women show cigarette smoking-related cue reactivity, they have different physiological responses to these cues (Pogun and Yararbas, 2009). Results from preliminary studies have suggested that sensory cues (e.g., flavors) associated with e-cigarettes may also differ between women and men (Dawkins et al., 2013). In the absence of aerosol exposure techniques such as the one described herein, investigation of a potential modulatory role for flavors in animal models of nicotine dependence may be difficult.

Direct comparison of results in mice to topography and use profiles in humans is complicated somewhat by between-species differences in respiratory physiology. Unlike adult humans, rodents are obligate nose breathers. Further, their respiratory rate is much higher than the rate for humans (~ 163 vs. 12–20 breaths/min, respectively). In this study, mouse exposure to nicotine aerosol was, by necessity, passive and duration was controlled by the experimenter. In contrast, human exposure is active. In the natural environment, puff duration and other puff parameters (e.g., number of puffs, inter-puff interval, flow rate, volume) are controlled by the user (Robinson et al., 2015; Robinson et al., 2016), although manipulation of these parameters through controlled vaping bouts has occurred in laboratory

settings (Spindle et al., 2016). In humans, average puff duration for e-cigarettes (“cigalikes” and tank-based systems) appeared dependent upon nicotine concentration (Lopez et al., 2016; Ramoa et al., 2016; Spindle et al., 2016) and showed considerable variability, ranging from 1.8 to 6.1 s (Cunningham et al., 2016; Ramoa et al., 2016; Robinson et al., 2015; Spindle et al., 2016). In the present study, duration of aerosol generation was 10 seconds, with subsequent 1-minute hold in the chamber. Exposure occurred for a total of 5 minutes over the course of a 13-minute period. By comparison, Spindle et al. (2016) reported that average puff durations of 4.5 seconds occurred in experienced e-cigarette users during a controlled vaping bout of 10 puffs with 30 seconds inter-puff intervals. Hence, human users received 45 seconds active exposure to nicotine aerosol over a ~ 5-minute period versus 50 seconds active aerosol generation and a total 5-minute exposure period over a 13-minute time span for the mice. Under conditions of ad libitum access, humans averaged puff durations of 5.3 seconds and puff numbers of 62.55 for a total exposure duration of ~ 332 seconds (or 5 minutes) over a 90-minute session. While caveats apply with regard to the impact of species differences, total exposure periods in the mice were similar to those reported in the Spindle et al. (2016) study in humans.

In summary, the e-cigarette exposure device described herein was used successfully to deliver pharmacologically active doses of nicotine to female and male mice. Further, plasma nicotine concentrations following exposure were similar to those observed after s.c. injection with a low dose of nicotine as well as within the range observed in human smokers. Consequently, this mouse model of exposure to actual e-cigarette emissions has high translational relevance, although issues related to control of exposure dose still need to be solved through additional modification of the system. In May 2016, the U.S. Food and Drug Administration (FDA) extended their regulatory purview to e-cigarettes in a process known as “deeming.” Deeming placed emphasis on data-driven conclusions regarding the potential harms of e-cigarettes and emphasized the need for additional research, including preclinical investigation of underlying mechanisms. The present results suggest that route of administration should be an integral consideration of this research and may play a crucial role in examination of the contribution of flavors to nicotine dependence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- E-cigarette use is prevalent in youth and in adult men and women.
- This study compared effects of nicotine aerosol and s.c. nicotine in mouse model.
- Female mice were more sensitive to the hypothermic effects of aerosolized nicotine.
- Male mice were more sensitive to the hypothermic effects of s.c. nicotine.
- Feasibility of aerosol nicotine delivery via e-cigarette device is demonstrated.

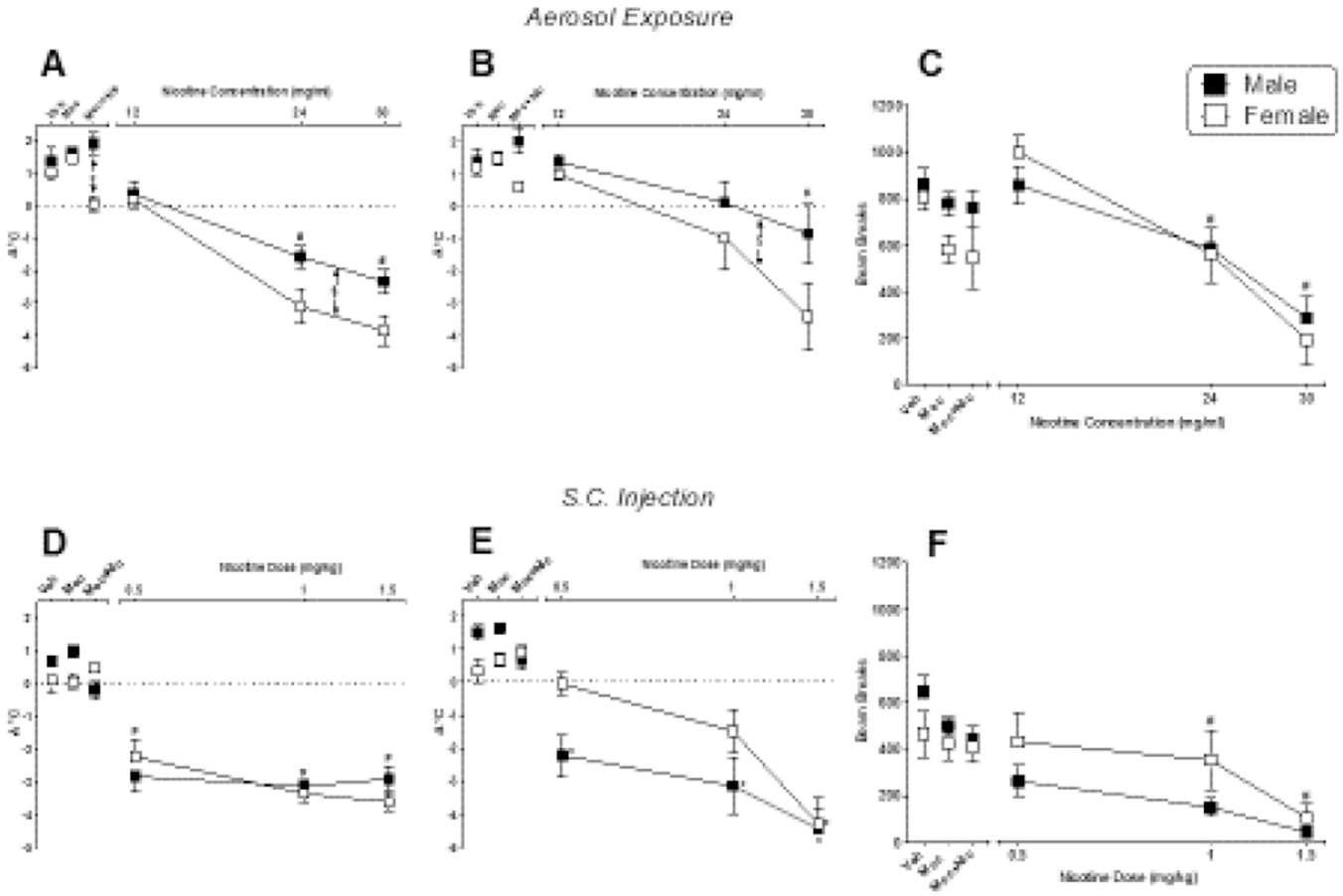

Figure 1.

Pharmacological effects of nicotine administered via whole body aerosol exposure (top panels) or s.c. injection (bottom panels) in male (filled squares) and female (unfilled squares) mice. Changes in rectal temperature 10 and 35 min after the final aerosol exposure (panels A and B, respectively) or s.c. injection (panels D and E, respectively) are shown, as are the effects of aerosolized and s.c. nicotine on locomotor activity (panels C and F, respectively). Results of tests with vehicle/saline, mecamylamine alone (1 mg/kg, s.c.), and mecamylamine (1 mg/kg, s.c.) plus nicotine (30 mg/mL aerosol exposure or 1.5 mg/kg s.c. injection) are shown at the left side of each panel. Each value represents the mean (± SEM) of 8 mice. $ indicates significant main effect for sex. # indicates significant main effect of dose/concentration (compared to vehicle). * indicates significant interaction and difference compared to vehicle for specific sex. $P<0.05$ for all post hoc comparisons.

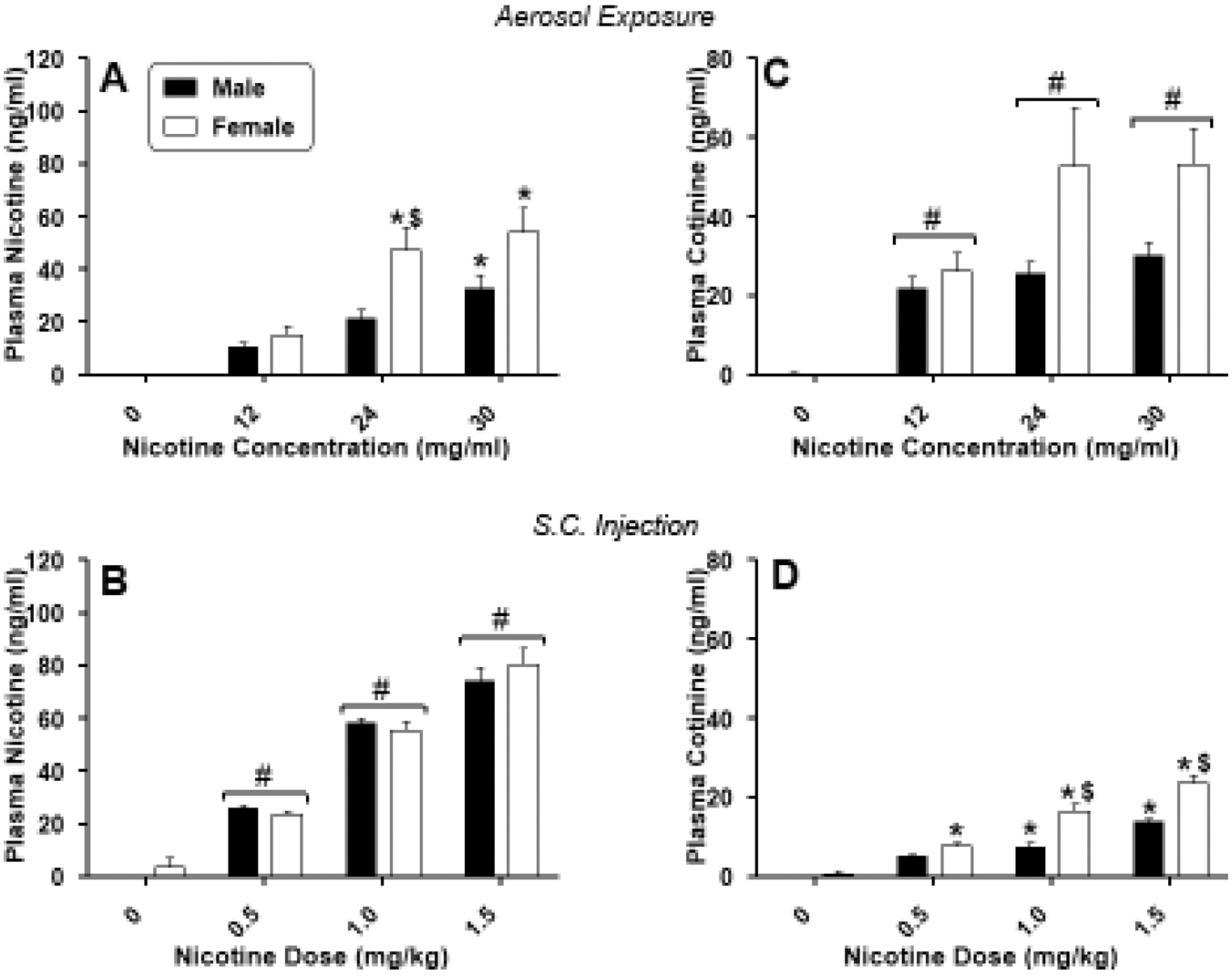

Figure 2.

Concentrations of nicotine (left panels) and cotinine (right panels) in the plasma of male (filled squares) and female (unfilled squares) mice after exposure to nicotine aerosol (top panels) or s.c. nicotine injection (bottom panels). Each value represents the mean (± SEM) of 8 mice. # indicates main effect of nicotine concentration/dose (compared to vehicle). * indicates significant interaction and difference compared to vehicle for specific sex. $ indicates significant interaction and sex difference at specified dose. $P<0.05$ for all post hoc comparisons.

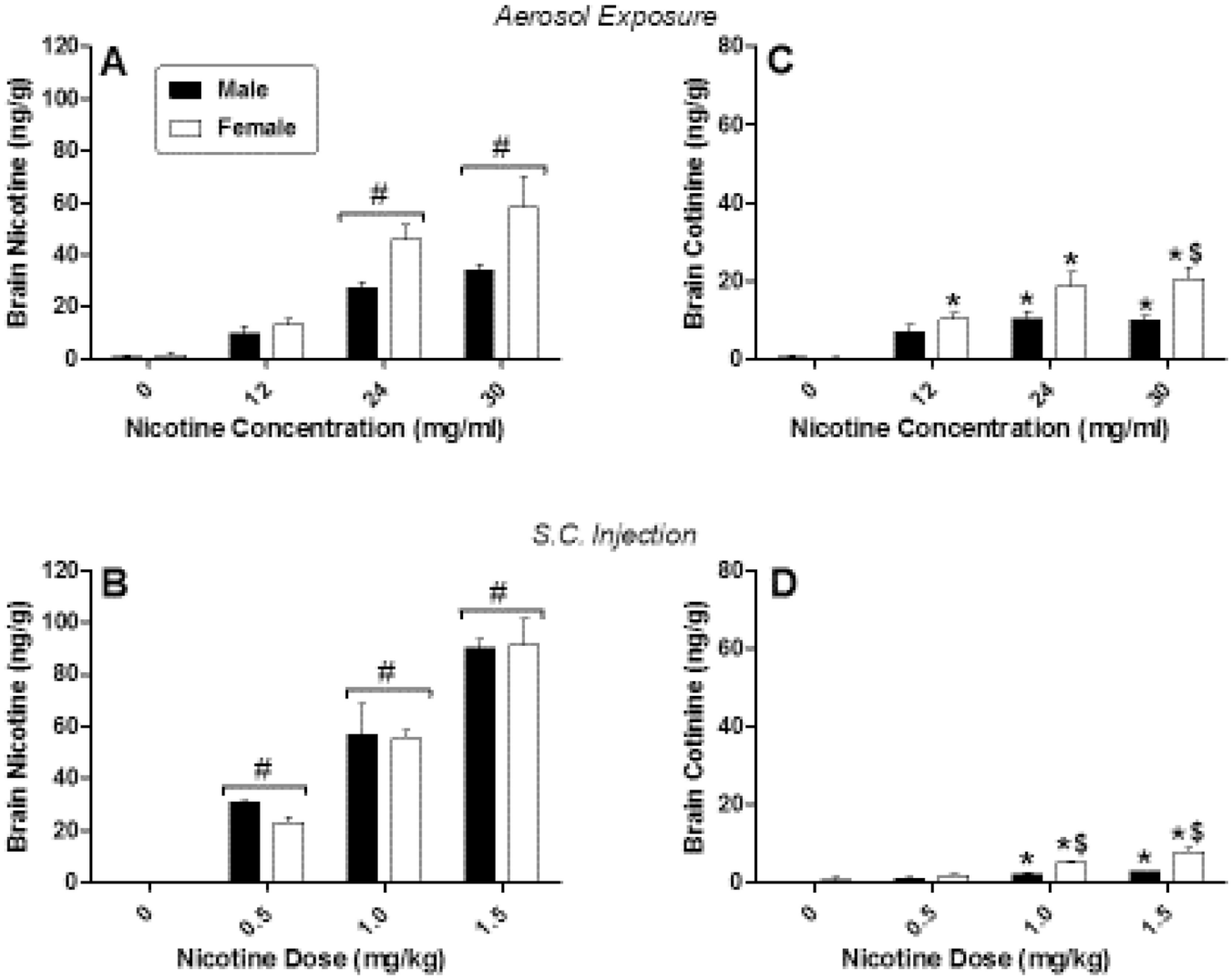

Figure 3.

Concentrations of nicotine (left panels) and cotinine (right panels) in the brains of male (filled squares) and female (unfilled squares) mice after exposure to nicotine aerosol (top panels) or s.c. nicotine injection (bottom panels). Each value represents the mean (± SEM) of 8 mice. # indicates main effect of nicotine concentration/dose (compared to vehicle). * indicates significant interaction and difference compared to vehicle for specific sex. $ indicates significant interaction and sex difference at specified dose. $P<0.05$ for all post hoc comparisons.

Table 1

Self-reported e-cigarette use among male and female youth and adults [1]

	CDC National Youth Tobacco Survey 2014		CDC National Adult Tobacco Survey 2013	
Current E-cigarette Use	**Male** ***N (weighted %)***	**Female** ***N (weighted %)***	**Male** ***N (weighted %)***	**Female** ***N (weighted %)***
Past 30-day use	1,156 (10.3%)	833 * (8.1%)	809 (4.7%)	854 * (3.6%)
Some days	1,021 (9.2%)	782 * (7.6%)	691 (23.2%)	640 (25.7%)
Every day	135 (1.2%)	51 * (0.6%)	163 (5.5%)	169 (5.1%)

[1] N = number of individuals who responded positively to the indicated question (weighted % of total number of males or females surveyed). For the youth survey, the use of e-cigarette use options were presented as “0 days”, “1 or 2 days”, “3 to 5”, “6 to 9”, “10 to 19”, “20 to 29”, and “all 30 days”. 1–29 days were recoded as “Some days”, 30 was recoded as “Everyday”.

* $P \leq 0.001$ (males vs females) based upon Wald test.

Table 2

Cotinine: nicotine ratio in plasma and brain following aerosol exposure and s.c. injection with nicotine *

Route of Administration	Concentration/Dose	Plasma		Brain	
		Males	Females	Males	Females
Aerosol Exposure	12 mg/ml	2.16 (0.31)	1.81 (0.27)	0.73 (0.14)	0.86 (0.18)
	24 mg/ml	1.27 (0.20)	1.07 (0.13)	0.39 (0.09)	0.44 (0.08)
	30 mg/ml	0.96 (0.13)	1.0 (0.07)	0.30 (0.04)	0.37 (0.04)
S.C. Injection	0.5 mg/kg	0.20 (0.01)	0.34 (0.06)	0.04 (0)	0.09 (0.01)
	1 mg/kg	0.13 (0.02)	0.31 (0.05)	0.03 (0)	0.10 (0.01)
	1.5 mg/kg	0.20 (0.02)	0.30 (0.02)	0.03 (0)	0.10 (0.02)

* Each value represents the mean (± SEM) of data from 6 mice. Plasma and brain concentrations were measured in the same mice. Values > 1 indicate greater cotinine than nicotine concentrations whereas values < 1 indicate greater concentrations of nicotine.