Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 78 (2004) 13–25
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Sex differences in voluntary oral nicotine consumption by adolescent mice: a dose-response experiment

Laura Cousino Klein*, Michele M. Stine, David J. Vandenbergh, Courtney A. Whetzel, Helen M. Kamens

Department of Biobehavioral Health, Center for Developmental and Health Genetics, 315 East Health and Human Development Building, The Pennsylvania State University, University Park, PA 16802, USA

Received 3 September 2003; received in revised form 11 January 2004; accepted 20 January 2004

Abstract

Recent studies with adolescent rodents offer valuable information regarding the neurochemical and behavioral effects of adolescent nicotine exposure. One hundred twenty-one male and 125 female adolescent (35 days of age) C57BL/6J mice were tested for voluntary nicotine consumption by providing 24-h access to both saccharin-only (SAC) and one of six nicotine-containing solutions [10, 25, 50, 75, 100, 200 ug (-)-freebase nicotine/ml in 2% SAC] in the home cage for 7 days. Although males and females drank similar volumes (ml) of nicotine, the female mice consumed more nicotine adjusted for body weight (mg/kg) and as a percentage of total fluid intake than did the male mice. In contrast, there was no sex difference in overall serum cotinine levels (adjusted for liver weight). For all mice, nicotine consumption and serum cotinine levels increased in a dose-dependent manner, and the volume of nicotine intake (ml), percent nicotine intake, and nicotine dosage (mg/kg) on the last day of the experiment were positively correlated with cotinine levels. Cotinine levels were inversely related to body weight only for females. Sex differences in nicotine consumption, but not in cotinine levels, suggest sex differences in pharmacokinetic processes that may contribute to oral nicotine consumption behavior during periadolescence.

Keywords: Sex differences; Nicotine; Mice; Voluntary oral consumption; Two-bottle; Saccharin; Periadolescent; Dose-response; Pharmacokinetics; Genetics; Cotinine; Liver

1. Introduction

Cigarette smoking still is the single largest preventable cause of death and illness in the United States (Centers for Disease Control [CDC], 2003a). Nearly 90% of adult smokers initiate cigarette smoking before the age of 20 (i.e., adolescence; Gilpin et al., 1999; United States Department of Health and Human Services [USDHHS], 1994), and recent reports suggest that cigarette smoking rates did not change among middle-school children between 2000 and 2002 despite an increase in prevention efforts (CDC, 2003b; Chassin et al., 2003). Taken together, these data suggest that adolescent development may be a critical period during which the majority of cigarette smokers begin to smoke (Gilpin et al., 1999; USDHHS, 1994). The likelihood of quitting smoking in adulthood is decreased substantially when smoking initiation begins in adolescence. In fact, adolescents who start smoking today will smoke for as long as 20–30 years, on average, which means that they are more likely to experience the adverse health consequences of smoking than are those individuals who start to smoke later in life (Pierce and Gilpin, 1996). Despite these statistics, little is known about the progression from adolescent experimentation with cigarettes into smoking addiction.

Spear (2000) and Laviola et al. (1999) suggest that there are dramatic developmental changes in the brain associated with adolescence that may predispose an individual to experiment with alcohol, illicit drugs, and tobacco, the primary addictive ingredient of which is nicotine (USDHHS, 1988). Animal models of nicotine exposure developed with adult rodents may be used to understand why adolescents smoke cigarettes (Smith, 2003). Indeed, recent rodent models of adolescent nicotine exposure provide mounting biobehavioral support for this "critical period" hypothesis (Abreu-

* Corresponding author. Tel.: +1-814-865-8813; fax: +1-814-863-7525.
E-mail address: lcklein@psu.edu (L.C. Klein).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.01.005

Villaca et al., 2003; Adriani et al., 2002; Klein, 2001; Laviola et al., 2003; Miao et al., 1998; Trauth et al., 2000a, 2001). These adolescent rodent models include several rat (e.g., Wistar, Sprague–Dawley, Brown Norway, Wistar Kyoto) and mouse (e.g., CD-1, C57BL/6, NMRI) strains (e.g., Adriani et al., 2002; Faraday et al., 2001, 2003; Gäddnäs et al., 2001; Kelley and Middaugh, 1999; Klein, 2001; Klein et al., 2003; Levin et al., 2003; Lopez et al., 2001; Pekonen et al., 1993; Todte et al., 2001; Trauth et al., 2000a,b), with ages spanning preweaning, periadolescence (around postnatal age 30 days; Spear and Brake, 1983), and postadolescence (around postnatal age 60 days; e.g., Adriani et al., 2003; Hatchell and Collins, 1980; Klein et al., 2003; Petersen et al., 1984; Robinson et al., 1996). These models also incorporate various nicotine administration methods previously used with adults including osmotic minipump and continuous infusion methods (e.g., Bhat et al., 1994; Faraday et al., 2001; Klein, 2001; Trauth et al., 2000a), repeated injections (e.g., Hatchell and Collins, 1980; Miao et al., 1998), oral consumption (e.g., Adriani et al., 2002; Flynn et al., 1989; Gäddnäs et al., 2001; Klein et al., 2003; Pekonen et al., 1993; Todte et al., 2001), and, most recently, intravenous self-administration in rats (Levin et al., 2003). These studies offer important data for understanding the biobehavioral effects of nicotine exposure in adolescence. For example, recent findings suggest that adolescent male rats exposed to nicotine are at risk for increased opioid (Wistar; Klein, 2001) and nicotine consumption in adulthood (Sprague–Dawley; Adriani et al., 2003). There also are reports of dramatic, neurobiological effects of adolescent nicotine exposure in rats, which include immediate and long-term changes in the central nervous system dopaminergic and catecholaminergic functioning (Trauth et al., 2001), and long-lasting cellular and neuronal damage in the midbrain, hippocampus, and cerebral cortex (Abreu-Villaca et al., 2003). As this field of adolescent nicotine research continues to develop, however, new studies are needed to test the validity and parameters of adult models adapted for use with adolescent animals, particularly because the biological and behavioral characteristics of adolescence appear to be quite different than those observed in adulthood (e.g., Laviola et al., 2003).

With regard to nicotine consumption models, there is a growing interest in adapting the oral nicotine consumption method for use with adolescent rodents to examine nicotine's biobehavioral effects given the ease of administration, opportunity for continuous nicotine exposure, and production of acceptable levels of nicotine bioavailability through this administration route (Le Houezec et al., 1989). The mouse is an important model for examining the development of nicotine consumption in periadolescence. Adult mouse models of nicotine exposure have been used effectively to demonstrate nicotine's effects in reward-relevant regions of the brain (e.g., Collins et al., 1989; Mansvelder et al., 2002; Marks and Collins, 1982; Pauly et al., 1996), as well as the behavioral effects of nicotine (e.g., Hatchell and Collins, 1977, 1980). Adult and adolescent mice will readily consume nicotine in drinking water (e.g., Klein et al., 2003; Meliska et al., 1995; Robinson et al., 1996; Rowell et al., 1983; Sparks and Pauly, 1999), in amounts that yield serum nicotine, serum cotinine (nicotine's primary active metabolite), and brain tissue distribution levels comparable with the human smoker. In light of the recent mapping of the mouse genome, mouse models provide a unique opportunity to reveal genetic contributions to drug abuse. The importance of outlining the parameters of voluntary nicotine consumption in adolescent mice is highlighted by reports that genetic variability accounts for the biobehavioral differences in responses to nicotine across mouse strains (Hatchell and Collins, 1977; Smolen et al., 1994; Meliska et al., 1995), as well as in sex differences in response to nicotine (Hatchell and Collins, 1980; Rosecrans, 1972). For example, the alcohol-preferring mouse strain, C57BL/6, also prefers nicotine, relative to mice that demonstrate a low preference for alcohol (DBA/2; Meliska et al., 1995). C57BL/6 mice also prefer amphetamine more than DBA/2 mice do, suggesting that genetic factors may contribute to the use of a wide range of psychoactive drugs including nicotine. The handful of studies that have tested voluntary nicotine consumption in periadolescent mice (e.g., Adriani et al., 2002; Klein et al., 2003; Robinson et al., 1996) suggest that this method can be valuable for understanding adolescent nicotine intake behavior, and that additional studies are needed to clarify this paradigm in male and female mice. The present experiment was designed to examine potential sex differences in nicotine consumption based on earlier reports of differential sensitivity to nicotine in periadolescent male and female rodents (e.g., Faraday et al., 2001; Hatchell and Collins, 1980). In addition, we sought to determine the parameters of oral nicotine consumption by testing voluntary nicotine intake in periadolescent C57BL/6 mice across six different nicotine concentrations.

2. Methods

2.1. Animals

One hundred twenty-one male and 125 female periadolescent C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were individually housed under standard housing conditions to allow for accurate measurement of liquid and food intake. Mice were 32 days old and weighed approximately 15.81 ± 0.10 g (standard error of the mean) at the beginning of the experiment (males: 16.80 ± 0.15 g; females: 15.79 ± 0.10 g). This age is defined as early adolescence by Spear (2000), Spear and Brake (1983), and Laviola et al. (2003), and is analogous to the human developmental stage of early adolescence, a period associated with the onset of puberty and behavioral transition (Spear and Brake, 1983; reviewed by Laviola et al., 2003).

To allow time for data collection, with minimal disruption of animals in the housing room, mice were tested in

four separate cohorts (at least 60 mice per cohort); each cohort consisted of at least 10 animals from each nicotine treatment group that included at least 5 males and 5 females. Mice were maintained on a 12-h light–dark cycle [lights on at 0900 h (three cohorts) or 0700 h (one cohort)], in a climate-controlled room with a temperature of 21 ± 2 °C and 60% relative humidity. Animals had continuous access to food (Lab Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) throughout the experiment. During baseline, mice had continuous access to tap water; during testing, mice had continuous access to two bottles, one with tap water that contained 2% saccharin and another that contained 2% saccharin solution with (-)-nicotine freebase (see below). Mice were periadolescent (35–42 days of age; Adriani et al., 1998; Klein et al., 2003; Laviola et al., 2001, 2003; Spear, 2000) during the nicotine testing phase of the experiment, a developmental period that includes the onset of puberty and a few days afterwards (Spear and Brake, 1983; reviewed by Laviola et al., 2003). The Pennsylvania State University Institutional Animal Care and Use Committee reviewed and approved all animal use procedures, and principles of laboratory animal care (National Research Council, 1985) were followed.

2.2. Drugs

(-)-Nicotine freebase and saccharin sodium hydrate were purchased from Sigma (St. Louis, MO). Freebase nicotine (NIC) was provided to the mice in one of six concentrations: 10 ug/ml (20 males; 23 females), 25 ug/ml (20 males; 21 females), 50 ug/ml (20 males; 20 females), 75 ug/ml (20 males; 20 females), 100 ug/ml (21 males; 20 females), or 200 ug/ml (20 males; 21 females) dissolved in a 2% saccharin solution to mask the taste of the nicotine (Klein et al., 2003; Sparks and Pauly, 1999). The control solution was a 2% saccharin solution (SAC; Sparks and Pauly, 1999) that did not contain nicotine. Tap water was used as the water source for all treatment groups. These NIC and SAC concentrations were selected based on previous research, which indicates that adult and adolescent C57BL/6 mice will consume freebase nicotine across these concentrations (Klein et al., 2003; Meliska et al., 1995; Pekonen et al., 1993; Robinson et al., 1996; Sparks and Pauly, 1999) and that oral consumption of nicotine will yield acceptable levels of nicotine bioavailability (Le Houezec et al., 1989). The NIC and SAC solutions were prepared frequently (every 2–3 days), based on published reports, which determined that there is no appreciable nicotine loss in solution within this time frame (Pekonen et al., 1993; Rowell et al., 1983).

2.3. Experimental procedure

2.3.1. Baseline (3 days)

Following arrival, the mice in each cohort were left undisturbed for 5 days to allow for acclimation to the animal facility prior to the start of the experiment. Next, 32-day-old mice were weighed, and food and water measurements were taken daily for three consecutive days; measurements were taken immediately after the lights were turned on in the housing room. Food consumption, body weight, and water consumption on the last day of the 3-day baseline period were used to assign the mice to one of the six nicotine-treatment groups to ensure that treatment groups did not differ significantly in any of these dependent measures, prior to the start of voluntary nicotine-consumption testing.

2.3.2. Voluntary nicotine consumption testing (7 days)

Following the last baseline day, periadolescent mice (35 days old) were given 24-h access to SAC and to one of the six NIC concentrations described above, using published methods with mice and rats (e.g., Klein et al., 2003; Meliska et al., 1995; Robinson et al., 1996; Rowell et al., 1983; Smith and Roberts, 1995; Todte et al., 2001). Prior reports demonstrate that C57BL/6 adult and periadolescent mice will consume nicotine in concentrations ranging from 50 to 200 ug/ml, and that these concentrations yield detectable serum levels of cotinine, the primary metabolite of nicotine (Klein et al., 2003; Meliska et al., 1995; Robinson et al., 1996; Sparks and Pauly, 1999). This large range of nicotine concentrations was selected to delineate a dose-response curve for voluntary oral nicotine consumption, and included several higher nicotine concentrations that mice will self-administer without acute toxic side-effects of nicotine (e.g., difficulty breathing, death). Mice had access to the two solutions for 24 h/day for 7 days; the bottles were switched between the sides daily to prevent conditioned place preference to either side of the cage (Klein, 2001; Shaham et al., 1992; Todte et al., 2001). Body weight, food consumption, and fluid intake were measured daily.

At the end of the experiment, mice were sacrificed by cervical dislocation at the beginning of the light cycle, and serum was collected and frozen at −80 °C for later cotinine assessment. Livers also were removed, weighed, and frozen on dry ice immediately following sacrifice. The order of sacrifice each morning was counterbalanced across sex, nicotine-treatment group, and experimental cohort. This sacrifice time was selected because it is the beginning of the light portion of the light cycle, and mice consume most of their fluid during the dark portion of the cycle (e.g., Kotlus and Blizard, 1998; Pietilä et al., 1995). Because nicotine has a rapid half-life in C57BL/6 mice (around 6 min) and cotinine has a longer half-life (about 20 min; Petersen et al., 1984; USDHHS, 1988) than does nicotine, cotinine was selected as a more stable index of nicotine intake and should reflect recent nicotine consumption in periadolescent mice.

2.3.3. Serum cotinine assessment

Serum cotinine levels were measured to validate nicotine exposure through oral consumption (e.g., Klein et al., 2003; Trauth et al., 2000a). Cotinine was determined by enzyme immunosorbant assay (EIA; Orasure Technology, Bethle-

hem, PA) by Salimetrics (State College, PA). Samples from each mouse were tested in duplicate in a single assay batch. The values used in the data analyses are the averages of duplicate tests. The assay had a lower limit of sensitivity of 3 ng/ml, with an average inter- and intraassay covariance of less than 10% and 5%, respectively.

2.4. Treatment of data

One female mouse in the 10 ug/ml nicotine-treatment group died during the nicotine testing phase as a result of a maxillofacial occlusion, which frequently occurs among developing C57BL/6J mice (M. Potter, personal communication); the data from this mouse are excluded from the nicotine testing phase analyses. Whole blood samples from two other mice (one male in 100 ug/ml nicotine-treatment group and one female in 10 ug/ml nicotine-treatment group) were provided to the animal facility for sentinel monitoring at the end of the experiment. The blood samples were not available for cotinine examination and, therefore, are not included in the cotinine analyses. Because of sex differences in liver weight (reported below) and the potential impact on rate of nicotine metabolism, serum cotinine levels were adjusted for liver weight to make comparisons between males and females. Natural logarithmic transformations were applied to the liver weight-adjusted cotinine data because they were not normally distributed; this transformation resulted in a normal distribution of the data. Thus, all reported analyses are based on log-transformed values, and raw cotinine values adjusted for liver weight ($\pm$ S.E.M.) were used to graph data for clarity.

To make nicotine consumption comparisons between males and females, nicotine consumption was adjusted for body weight (mg nicotine/kg body weight) for each animal on each choice day. Nicotine consumption amounts also were calculated as the volume of nicotine intake (ml) and as a percentage of total fluid intake (%) on each nicotine-consumption test day.

2.5. Statistical analyses

A 2 (Sex) $\times$ 6 (Nicotine-Treatment Group) between-subjects design was used to examine the effects of sex and nicotine treatment on (a) body weight changes during periadolescence, (b) total fluid intake (NIC and SAC consumption combined), (c) voluntary nicotine consumption amounts (mg/kg), (d) voluntary nicotine volume intake (% total fluid intake and ml nicotine intake), and (e) serum cotinine levels. The mice arrived at the animal facility and were tested in four separate cohorts (at least 60 mice per cohort) to allow time for data collection with minimal disruption of animals in the housing room. Therefore, all statistical analyses included cohort as a covariate to control for potential cohort differences in body weight, food consumption, fluid intake, and serum cotinine levels. Specifically, cohort was dummy coded into three new variables that were entered into separate analysis of covariance (ANCOVA) models. Separate repeated-measures, 2-way ANCOVAs, with sex (two levels) and nicotine treatment (six levels) as the independent measures, time as the within-subject variable, and cohort as the covariate, were conducted to examine group differences in body weight, food consumption, and fluid intake during the baseline and nicotine testing phases of the experiment. When appropriate, statistical interactions were examined using separate one-way ANCOVAs, Tukey's honestly significant difference (HSD), and Bonferroni post hoc analyses (α=.05). All significance tests were two-tailed and evaluated at α=.05. ANCOVA estimated marginal means adjusted for the cohort covariate ($\pm$ S.E.M.) are reported in the results section, tables, and figures, unless otherwise noted.

3. Results

3.1. Baseline body weight, food consumption, and water intake

There were no significant nicotine group differences in body weight, food consumption, or water intake on the last day of the baseline period, prior to nicotine-treatment group randomization (i.e., 10, 25, 50, 75, 100, or 200 μg nicotine/ml treatment group). However, males weighed more [17.9 ± 0.1 vs. 15.6 ± 0.1 g, respectively; $F(1,232) = 220.8$, $P < .0001$], ate more food [4.3 ± 0.1 vs. 4.1 ± 0.1 g, respectively; $F(1,231) = 6.98$, $P < .01$], and drank more water [7.1 ± 0.1 vs. 6.5 ± 0.1 ml, respectively; $F(1,230) = 39.16$, $P < .0001$] than did the female mice. There were no significant Sex $\times$ Nicotine-Treatment Group interactions for these three dependent variables on the last baseline day prior to the start of nicotine consumption testing. Repeated-measures ANCOVA indicated that male mice gained weight more rapidly than did the female mice [Time $\times$ Sex interaction: $F(2,462) = 19.02$, $P < .0001$] across the 3-day baseline period, but the rate of food or water intake did not differ between males and females. Importantly, body-weight gain and food or water intake did not differ among the mice later assigned to the six nicotine-treatment groups across this time period. There also were no statistically significant Time $\times$ Sex $\times$ Nicotine-Treatment Group interactions during this 3-day period, thus ensuring that mice were appropriately assigned to one of the six nicotine-treatment groups prior to the start of the nicotine consumption phase of the experiment.

3.2. Nicotine consumption testing

3.2.1. Body weight

All animals gained weight across the 7-day voluntary nicotine testing period [time effect: $F(6,1380) = 92.79$, $P <$ .0001], and males gained more weight than did the females [Time $\times$ Sex interaction: $F(6,1380) = 50.36$, $P <$

.0001]. Males also weighed more than did the females during this test period [18.5 ± 0.1 vs. 15.8 ± 0.1 g, respectively; $F(1,230) = 434.25$, $P < .0001$]. Separate one-way ANCOVAs confirmed this sex difference on each day of nicotine testing [Fs(1,230)>251.85, Ps < .0001]. With respect to nicotine treatment, groups did not differ in mean body weight during this time period.

3.2.2. Food consumption

Consistent with the body weight data, food consumption increased over the 7-day testing period [time effect: $F(6,1350) = 10.85$, $P < .0001$]. Males ate more food than did the females [3.93 ± 0.02 vs. 3.70 ± 0.02 g, respectively; $F(1,225) = 65.59$, $P < .0001$], but there was no statistically significant Time × Sex interaction, which suggests that food consumption increased similarly for males and females during this time period. With respect to nicotine treatment, groups did not differ in food consumption amounts.

3.2.3. Total fluid intake (NIC and SAC consumption combined)

Total fluid intake increased across the 7-day voluntary nicotine testing period for all mice [time effect: $F(6,1356) = 31.34$, $P < .0001$] such that overall fluid intake followed a U-shaped curve across the 7-day testing period, a finding consistent with Rowell et al. (1983). Specifically, total fluid intake was highest on Days 1 (9.2 ± 0.1 ml) and 4–7 (range: 9.0–9.7 ± 0.1 ml) of nicotine access, and lowest on Days 2 and 3 (8.6 ± 0.1 ml and 8.7 ± 0.1 ml, respectively) of nicotine consumption testing (Days 2 and 3 are significantly different from all other days; Bonferroni post hoc). Males consumed more fluid than did the females during this test period [9.6 ± 0.1 vs. 8.8 ± 0.1 ml, respectively; $F(1,226) = 54.50$, $P < .0001$]. With respect to nicotine treatment, groups did not differ in total fluid intake during testing. Importantly, there were no Time × Nicotine-Treatment Group, Time × Sex, or Time × Group × Sex interactions. Thus, underlying group differences in overall fluid intake did not influence the nicotine consumption findings.

3.2.4. Voluntary nicotine volume consumption (ml)

The volume of nicotine intake (ml) was examined to determine group differences in nicotine consumption. Consistent with overall fluid intake, there was a main effect for time [time effect: $F(6,1362) = 8.61$, $P < .0001$] such that nicotine consumption (ml) among mice followed a U-shaped curve across the 7-day testing period. Specifically, the volume of nicotine consumption was highest on the 1st (3.5 ± 0.1 ml), 6th (3.6 ± 0.1 ml), and 7th (3.6 ± 0.1 ml) days of nicotine access, and lowest on the 2nd (3.0 ± 0.1 ml) and 3rd (2.9 ± 0.1 ml) days of nicotine consumption testing (Days 2 and 3 significantly different from Days 1, 6, and 7; Bonferroni post hoc). This 2-day dip in nicotine intake and overall fluid consumption is consistent with other reports (e.g., Rowell et al., 1983) and may be the result of a nicotine-stimulated antidiuretic hormone release (e.g., USDHHS, 1988), which leads to a retention of fluid and a decrease in urine output. Because small rodents are very sensitive to changes in fluid balance, they will adjust their fluid intake to compensate for reduced urine output (Gäddnäs et al., 2001). It is probable that the rapid growth of adolescent mice during this time period resulted in a quick recovery of fluid intake to maintain growth.

There also was a main effect for the nicotine-treatment group [$F(5,227) = 7.09$, $P < .0001$; see Fig. 1]. Specifically, mice in the 10 and 25 ug/ml NIC groups also drank more nicotine than did the mice in the 100 ug/ml NIC group, and mice in the four lowest nicotine concentration groups (i.e., 10, 25, 50 and 75 ug/ml groups) drank significantly more nicotine (ml) than did the mice in the highest nicotine concentration groups (Bonferroni post hoc). There was no statistically significant Time × Nicotine-Treatment Group interaction, which suggests that this group difference was consistent across the 7-day test period. Male and female mice voluntarily consumed similar amounts of nicotine (ml); there were no other statistically significant group or time interactions.

3.2.5. Voluntary nicotine intake as percent total fluid consumption (%)

Nicotine intake also was evaluated by calculating the amount of nicotine consumed as a percentage of total fluid intake on each test day (%). Consistent with the volume of nicotine intake data, there was a main effect for nicotine-treatment group [$F(5,226) = 4.94$, $P < .0001$; see Fig. 2]. The mice in the two lowest nicotine concentration groups (i.e., 10, 25) and in the 75 ug/ml groups drank significantly more nicotine as a percentage of total fluid intake (%) than did the mice in the highest nicotine-concentration group

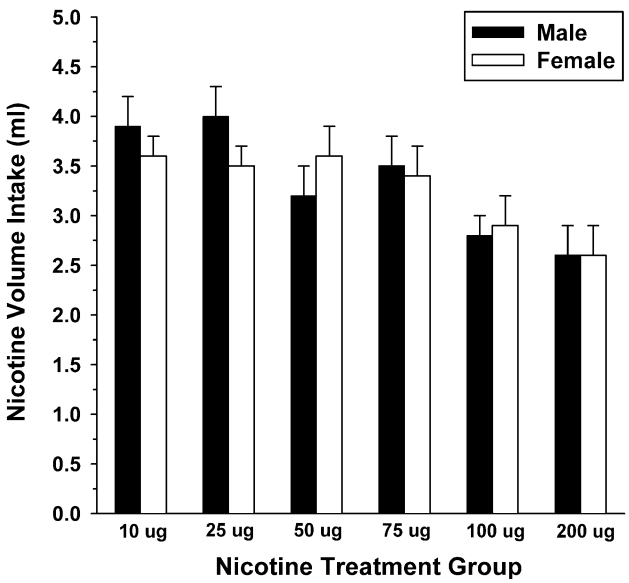

Fig. 1. Mean voluntary nicotine volume intake (ml) averaged across the 7-day test period by male (■) and female (□) periadolescent mice in each of the six nicotine treatment groups (± S.E.M.).

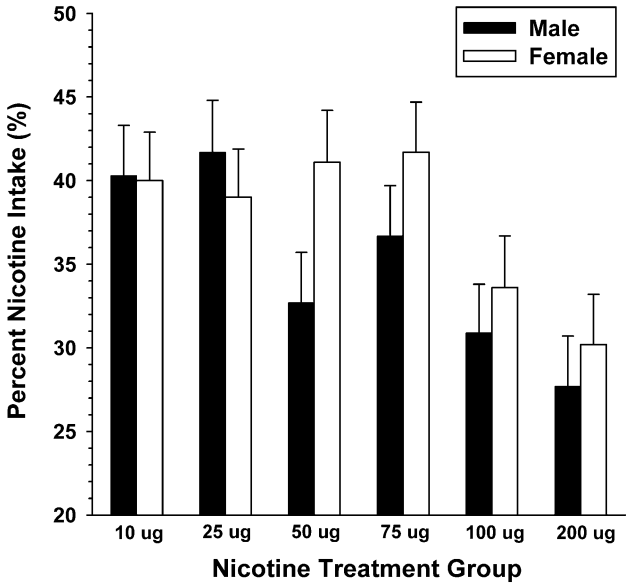

Fig. 2. Mean nicotine consumption as a percentage of total fluid intake (%) averaged across the 7-day test period by male (■) and female (□) periadolescent mice in each of the six nicotine treatment groups (± S.E.M.).

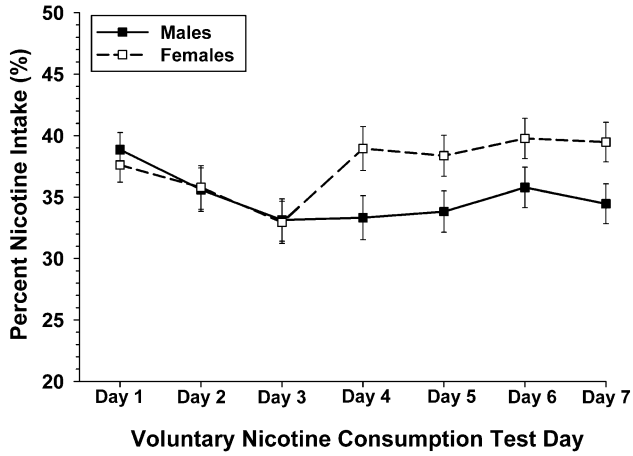

Fig. 3. Voluntary nicotine consumption as a percentage of total fluid intake (%) across the 7-day test period by male (■) and female (□) periadolescent mice (± S.E.M.).

(i.e., 200 ug/ml; Bonferroni post hoc). There was no Nicotine-Treatment Group × Sex interaction.

In contrast to the nicotine volume consumption data, however, there was a Sex × Time interaction [$F(6,1356) = 2.76$, $P = .01$] and two-way ANCOVAs were conducted separately for each test day, with sex and group as the independent variables, to examine this interaction. Female mice consumed more nicotine as a percentage of total fluid intake than did the males on Test Days 4–7 (see Fig. 3).

This sex difference was statistically significant on Days 4 [39.2 ± 1.8% vs. 33.7 ± 1.8%, respectively; $F(1,230) = 4.76$, $P < .05$], 5 [38.3 ± 1.7% vs. 33.7 ± 1.7%, respectively; $F(1,230) = 3.87$, $P = .05$], and 7 [39.2 ± 1.6% vs. 34.3 ± 1.6%, respectively; $F(1,229) = 4.81$, $P < .05$] of testing, and approached statistical significance on Day 6 of testing [39.7 ± 1.6% vs. 36.0 ± 1.6%, respectively; $F(1,230) = 2.56$, $P = .10$]. There were no other statistically significant interactions for this dependent measure.

3.2.6. Nicotine dosage (mg/kg)

To make adjustments for body weight differences between the male and female mice, nicotine intake on each test day was adjusted for body weight on the corresponding test day to derive a consumed nicotine dosage (mg/kg) per mouse. This body weight adjustment resulted in a main effect for sex, where females consumed significantly more nicotine (mg/kg) than did the male mice, regardless of nicotine-treatment group [14.7 ± 0.5 vs. 12.2 ± 0.5 mg/kg, respectively; sex effect: $F(1,5) = 12.68$, $P < .0001$; see Fig. 4 and Table 1].

Repeated-measures ANCOVA also indicated a marginal Sex × Time interaction [$F(6,1362) = 2.05$, $P = .056$]. Two-way ANCOVAs were conducted separately for each test day, with sex and group as the independent variables, to examine this interaction and for consistency with the nicotine fluid consumption analyses reported above. Regardless of nicotine-treatment group, female mice consumed more nicotine on a milligram per kilogram basis than did the male mice starting on the 3rd test day, continuing through the end of the experiment [see Fig. 4; Day 3: $F(1,229) = 7.15$, $P < .01$; Days 4–7: Fs$(1,230) > 5.33$, Ps $< .05$].

Consistent with the percent nicotine consumption results, there also was a main effect for nicotine-treatment group [$F(1,5) = 148.86$, $P < .0001$; see Table 1 and Fig. 5]. Nicotine dosage consumption (mg/kg) differed significantly across the nicotine-treatment groups in the expected direction (10 ug/ml: 2.15 ± 0.8 mg/kg; 25 ug/ml: 5.53 ± 0.8 mg/kg; 50 ug/ml: 10.18 ± 0.8 mg/kg; 75 ug/ml: 15.35 ± 0.8 mg/kg; 100 ug/ml: 16.9 ± 0.8 mg/kg; 200 ug/ml: 30.6 ± 0.8 mg/kg; Bonferroni post hoc) with two caveats: (1) Mice in

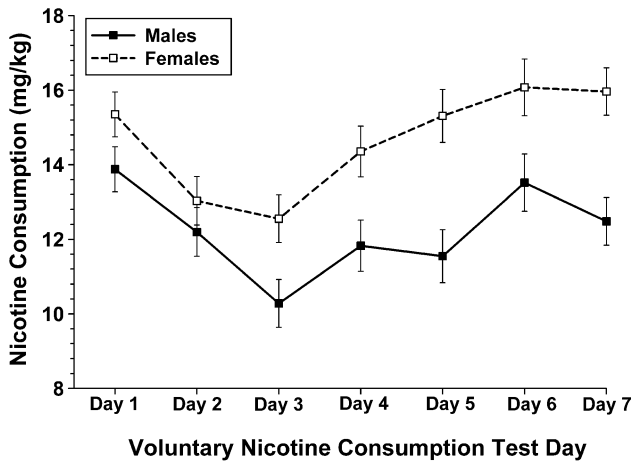

Fig. 4. Voluntary nicotine consumption adjusted for body weight (mg/kg) across the 7-day test period by male (■) and female (□) periadolescent mice (± S.E.M.).

Table 1
Adjusted nicotine consumption dosage (mg/kg) for male and female periadolescent mice across 7-day voluntary nicotine consumption testing (± S.E.M.)

Nicotine treatment group	Voluntary nicotine dosage consumption (mg/kg)						
	Day 1	Day 2	Day 3 *	Day 4 *	Day 5 *	Day 6 *	Day 7 *
10 ug/ml	2.3 ± 1.0[a,b,c]	2.1 ± 1.1[a,b,c]	1.8 ± 1.1[a,b,c]	2.0 ± 1.2[a,b,c]	2.2 ± 1.2[a,b,c]	2.3 ± 1.3[a,b,c]	2.2 ± 1.1[a,b,c]
Male (n = 20)	2.4 ± 1.5	2.0 ± 1.6	2.0 ± 1.6	2.1 ± 1.7	2.3 ± 1.7	2.0 ± 1.9	2.0 ± 1.6
Female (n = 22)	2.2 ± 1.4	2.1 ± 1.5	1.7 ± 1.5	2.0 ± 1.6	2.1 ± 1.7	2.7 ± 1.8	2.4 ± 1.5
25 ug/ml	6.2 ± 1.1[a,b,c]	4.8 ± 1.1[a,b,c]	5.2 ± 1.1[b,c]	5.0 ± 1.2[a,b,c]	5.9 ± 1.2[b,c]	5.4 ± 1.3[a,b,c]	6.2 ± 1.1[b,c]
Male (n = 19)	6.3 ± 1.5	4.8 ± 1.6	5.7 ± 1.6	4.9 ± 1.7	6.1 ± 1.8	5.1 ± 1.9	6.1 ± 1.6
Female (n = 21)	6.0 ± 1.4	4.9 ± 1.6	4.7 ± 1.5	5.1 ± 1.6	5.7 ± 1.7	5.7 ± 1.8	6.3 ± 1.5
50 ug/ml	11.8 ± 1.1[c,d,e]	10.1 ± 1.1[c,d,e]	8.6 ± 1.1[b,c,d]	10.2 ± 1.2[c,d,e]	9.2 ± 1.2[b,c,d]	11.0 ± 1.3[b,c,d,e]	10.4 ± 1.1[b,c,d]
Male (n = 20)	12.1 ± 1.5	9.0 ± 1.6	7.3 ± 1.6	8.0 ± 1.7	7.7 ± 1.7	9.0 ± 1.9	8.0 ± 1.6
Female (n = 19)	11.4 ± 1.5	11.1 ± 1.6	9.9 ± 1.6	12.3 ± 1.7	10.8 ± 1.8 **	12.9 ± 1.9	12.9 ± 1.6
75 ug/ml	15.7 ± 1.0[d,e]	14.1 ± 1.1[d,e]	14.0 ± 1.1[a,d,e]	15.3 ± 1.2[d,e]	15.8 ± 1.2[a,d,e]	17.3 ± 1.3[a,d,e]	15.3 ± 1.1[a,d,e]
Male (n = 20)	15.1 ± 1.5	14.1 ± 1.6	13.2 ± 1.6	14.1 ± 1.7	14.3 ± 1.7	15.7 ± 1.9	13.9 ± 1.6
Female (n = 20)	16.3 ± 1.5	14.2 ± 1.6	14.8 ± 1.6	16.5 ± 1.7	17.3 ± 1.7	18.9 ± 1.9	16.6 ± 1.6
100 ug/ml	18.5 ± 1.0[a,d,e]	16.2 ± 1.1[a,d,e]	14.0 ± 1.11[a,d,e]	17.2 ± 1.2[a,d,e]	16.6 ± 1.2[a,d,e]	18.6 ± 1.3[a,d,e]	17.5 ± 1.1[a,d,e]
Male (n = 21)	16.9 ± 1.4	16.5 ± 1.6	12.1 ± 1.5	15.6 ± 1.6	12.5 ± 1.7	18.1 ± 1.8	14.7 ± 1.5
Female (n = 20)	20.0 ± 1.5	16.0 ± 1.6	16.0 ± 1.6	18.7 ± 1.7	20.7 ± 1.7 **	19.0 ± 1.9	20.3 ± 1.6
200 ug/ml	33.3 ± 1.0[a,b,c,d,e]	28.4 ± 1.1[a,b,c,d,e]	24.8 ± 1.11[a,b,c,d,e]	29.0 ± 1.2[a,b,c,d,e]	30.8 ± 1.2[a,b,c,d,e]	34.2 ± 1.3[a,b,c,d,e]	33.7 ± 1.1[a,b,c,d,e]
Male (n = 20)	30.5 ± 1.5	26.9 ± 1.6	21.5 ± 1.6	26.3 ± 1.7	26.2 ± 1.7	31.2 ± 1.9	30.1 ± 1.6
Female (n = 20)	36.1 ± 1.5	30.0 ± 1.6	28.2 ± 1.6	31.6 ± 1.7	35.3 ± 1.7	37.3 ± 1.9	37.2 ± 1.6

[a] Bonferroni post hoc tests: differs from 50 ug/ml.
[b] Bonferroni post hoc tests: differs from 75 ug/ml.
[c] Bonferroni post hoc tests: differs from 100 ug/ml.
[d] Bonferroni post hoc tests: differs from 10 ug/ml.
[e] Bonferroni post hoc tests: differs from 25 ug/ml.
* Overall main effect for sex: Females>Males; $P < .05$.
** Within-group main effect for sex: Females>Males, $P < .05$.

the 75 and 100 ug/ml treatment groups consumed similar nicotine dosage amounts (mg/kg), and (2) nicotine consumption by mice in the 10 and 25 ug/ml nicotine-treatment groups was marginally different (P=.06).

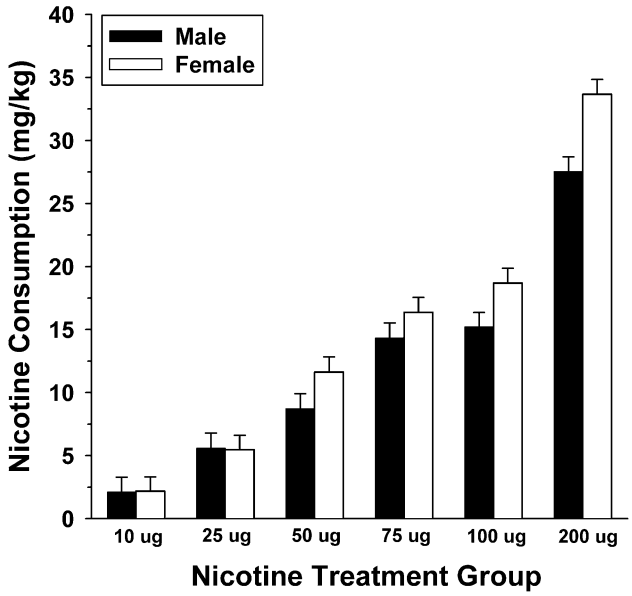

Fig. 5. Mean voluntary nicotine consumption adjusted for body weight (mg/kg) averaged across the 7-day test period by male (■) and female (□) periadolescent mice in each of the six nicotine concentrations (± S.E.M.).

This main effect for nicotine-treatment group persisted over time [Nicotine-Treatment Group × Time interaction: $F(30,1362) = 2.07$, P=.001; see Table 2]. Two-way ANCOVAs were conducted separately for each test day, with sex and group as the independent variables, to examine this interaction. The main effects for sex were reported above. Analyses revealed that nicotine dosage consumption (mg/kg) differed significantly across the nicotine-treatment groups, on each test day and in the expected directions [Days 1–3: Fs(1,229)>56.29, Ps < .0001; Days 4–7: Fs(1,230)>66.35, Ps < .0001; see Table 2]. There also was a significant Sex × Nicotine-Treatment Group interaction on

Table 2
Mean serum cotinine levels adjusted for liver weight (ng/ml/g) among male and female mice following 7-day voluntary oral nicotine consumption (± S.E.M.)

Nicotine treatment group	Serum cotinine levels (ng/ml/g)
10 ug/ml (n = 41)	15.15 ± 9.35[†,‡,§]
25 ug/ml (n = 41)	30.13 ± 6.54 *[,§]
50 ug/ml (n = 39)	50.07 ± 14.50 *
75 ug/ml (n = 39)	62.57 ± 7.83 *[,†]
100 ug/ml (n = 39)	81.49 ± 14.38 *[,†]
200 ug/ml (n = 41)	108.90 ± 23.41 *[,†]

* Significantly different from 10 ug/ml group (Bonferroni post hoc).
† Significantly different from 25 ug/ml group (Bonferroni post hoc).
‡ Significantly different from 50 ug/ml group (Bonferroni post hoc).
§ Significantly different from 75, 100, and 200 ug/ml group (Bonferroni post hoc).

the 5th test day [$F(5,230) = 2.37$, $P < .05$]. One-way ANCOVAs were conducted separately by nicotine-treatment group, with sex as the independent variable, to examine this interaction on Day 5 of testing. Results revealed that females in the 50 and 100 ug/ml nicotine-treatment groups consumed significantly more nicotine (mg/kg) than did the male mice in the respective treatment groups [$Fs(1,35) > 6.28$, $Ps < .05$] on the 5th day of voluntary nicotine-consumption testing (see Table 2).

3.3. Serum cotinine levels and liver weight

Livers were collected and weighed immediately following sacrifice, and cotinine was measured in serum collected on the last day of nicotine consumption testing. Male mice had larger livers than did females [1.66 ± 0.01 g vs. 1.42 ± 0.01 g, respectively; $F(1,225) = 352.48$, $P < .0001$], but nicotine treatment did not alter liver weights and there was no Sex × Nicotine-Treatment Group interaction. Because this sex difference in liver weight could affect nicotine and cotinine pharmacokinetics, the serum cotinine levels were adjusted for liver weight to make appropriate comparisons between males and females in subsequent analyses (R. Tyndale, personal communication). The raw, unadjusted data are presented in Fig. 6.

A two-way ANCOVA, with sex and nicotine-treatment group as the independent variables and cohort as a covariate, was conducted on log-transformed, liver weight-adjusted serum cotinine levels. Consistent with nicotine consumption results, the nicotine-treatment groups differed from one another in that serum cotinine levels increased as nicotine concentration increased [nicotine-treatment group effect: $F(5,225) = 17.34$, $P < .0001$; see Table 2]. The sex difference in nicotine consumption (mg/kg) was not confirmed by cotinine analysis; females and males displayed similar cotinine levels [3.20 ± 0.12 vs. 2.86 ± 0.13 ng/ml/g, respectively; $F(1,225) = 3.76$, n.s.].

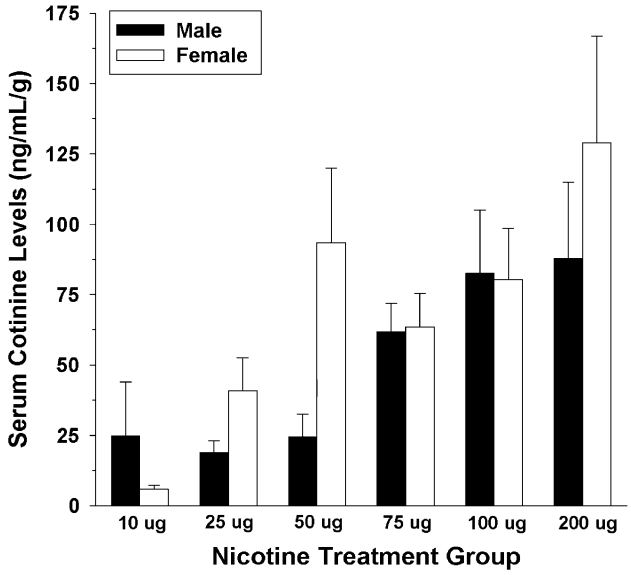

Fig. 6. Serum levels of cotinine adjusted for liver weight (ng/ml/g) displayed by male (■) and female (□) periadolescent mice on the last day of the experiment following 7 days of voluntary consumption of one of the six nicotine solutions (± S.E.M.).

In contrast, there was an unexpected statistically significant Sex × Nicotine-Treatment Group interaction [$F(5,225) = 2.50$, $P < .05$; see Fig. 6]. One-way ANCOVAs, conducted separately by nicotine-treatment group, with sex as the independent variable, revealed that females displayed higher cotinine levels (adjusted for liver weight) than did the males only in the 50 ug/ml nicotine-treatment group [3.86 ± 0.33 vs. 2.23 ± 0.33 ng/ml/g, respectively; $F(1,34) = 12.21$, $P = .001$; see Fig. 6]. There were no sex differences in serum cotinine levels among mice in any of the other nicotine-treatment groups.

3.4. Serum cotinine levels, nicotine consumption, and body weight

Separate correlation coefficients were calculated to examine the relationship between (1) serum cotinine levels and nicotine consumption, and (2) serum cotinine levels and body weight on the last day of the experiment. Partial correlations were used to control for cohort in the analyses. Nicotine consumption (ml), percent nicotine intake (%), and nicotine dosage (mg/kg) on the last day of the 7-day test period were selected for assessment because of the temporal proximity of consumption with cotinine assessment. As expected, liver weight was positively correlated with body weight on the last day of the experiment ($n = 235$; $r = .91$, $P < .0001$). Therefore, log-transformed cotinine levels (ng/ml), unadjusted for liver weight, were used to examine the relationship between cotinine and nicotine dosage (mg/kg) because body weight is part of the dosage calculation. Importantly, cotinine levels were positively correlated with average milliliters of nicotine intake ($n = 235$; $r = .35$, $P < .0001$), percent nicotine intake ($n = 234$; $r = .39$, $P < .0001$), and nicotine dosage ($n = 237$; $r = .62$, $P < .0001$), confirming nicotine administration through the oral administration route.

Next, cotinine levels, unadjusted for liver weight, were used to examine the relationship between nicotine exposure and body weight on the last day of nicotine testing. Overall, cotinine levels and body weight were not related ($n = 238$, $r = -.09$, $P = .19$). However, because of sex differences in body weight, correlations were conducted separately for males and females. Again, there was no correlation between cotinine (ng/ml) and body weight among male mice ($n = 115$; $r = .03$, $P = .80$). In contrast, cotinine levels were negatively correlated with body weight among female mice ($n = 118$; $r = -.18$, $P = .05$), a finding consistent with previous reports of adult rats exposed to nicotine. This effect is particularly evident with bland food availability and females as in the current experiment (e.g., Grunberg, 1982; Grunberg et al., 1986).

4. Discussion

The present experiment examined voluntary nicotine consumption by male and female periadolescent mice across six different nicotine concentrations. Consistent with adult studies of male mice, results suggest that both male and female periadolescent C57BL/6J mice will voluntarily consume nicotine across a wide range of nicotine concentrations (Robinson et al., 1996), and that this consumption results in a measurable increase in serum cotinine levels. With regard to adolescent versus adult voluntary nicotine intake, the available data suggest that adolescent C57BL/6 mice may be vulnerable to increased nicotine consumption at higher nicotine concentrations. Specifically, Robinson et al. (1996) report nicotine consumption amounts by C57BL/6 male mice provided access to base nicotine in concentrations similar to the present study and in 0.2% SAC (Robinson et al., 1996). Although the saccharin concentration provided to adolescent mice was higher than that provided to adult mice (2% vs. 0.2%, respectively), a comparison of our data with that of Robinson et al. (1996) indicates that adolescent and adult mice provided with access to 25 ug/ml of nicotine consumed similar amounts of nicotine (6.2 mg/kg vs. approximately 4.5 mg/kg, respectively). Then, as nicotine concentration increased, nicotine consumption by the adolescent male and female mice exceeded that of the adult male mice in a nonlinear fashion, such that at the highest concentration (200 ug/ml), adolescent mice consumed three times as much nicotine as did the adult mice (33.7 mg/kg vs. approximately 11 mg/kg, respectively). These data also are consistent with a report by Meliska et al. (1995). Our adolescent male and female mice consumed 2 to 10 times more base nicotine (depending on nicotine concentration) than did the adult male and female C57BL/6 mice that were provided access to L-nicotine hemisulfate concentrations ranging from 1 to 100 ug/ml (dissolved in tap water; Meliska et al., 1995). Only at the lowest nicotine concentration (10 ug/ml) did adolescent mice consume similar amounts of nicotine (2.2 mg/kg/day) compared with the maximum amount of nicotine consumed by the adult mice (3.0 mg/kg/day; Meliska et al., 1995). Comparisons with this second study should be approached with caution, however, because nicotine salt dissolved in tap water was used in the Meliska et al. (1995) study, making it more bitter than the base nicotine dissolved in saccharin that was used in the present study. Future studies that include male and female adolescent and adult mice are needed to make more accurate comparisons of potential age-related differences in voluntary nicotine-consumption behavior. Studies that follow nicotine consumption by mice from adolescence into adulthood also would provide invaluable data on the transition from the initiation of nicotine consumption to the maintenance of the behavior. Taken together, the nicotine consumption and cotinine data from the present report suggest that the voluntary oral nicotine consumption model is a valid method for examining the effects of nicotine consumption in periadolescent C57BL/6J mice.

The finding that adolescent female mice consume more nicotine, adjusted for body weight (mg/kg) and as a percentage of total fluid intake than do the male mice, is consistent with studies of adult rats and illicit drugs (e.g., morphine, heroin, fentanyl, cocaine; Alexander et al., 1978; Campbell et al., 2002; Klein et al., 1997; Lynch and Carroll, 1999), particularly the finding that this sex difference emerges within a few days of drug access (e.g., Klein et al., 1997). These consumption data also parallel sex differences in oral nicotine consumption in adult mice (Meliska et al., 1995), intravenous self-administration in rats (Donny et al., 2000), and newer reports of differential biological and behavioral sensitivity to nicotine in male and female adolescent rats (e.g., Faraday et al., 2001; Klein, 2001; Trauth et al., 2000a).

This sex difference in nicotine consumption suggests that female periadolescent mice may be more or less sensitive to the biobehavioral effects of nicotine. The single behavioral outcome measurement of voluntary nicotine intake in the present study makes it difficult to determine where males and females fall on the dose-response curve with respect to one another. The serum cotinine data may provide some insight into the direction of this effect. Specifically, despite the overwhelming sex difference in nicotine consumption, there was no observable sex difference in serum cotinine levels. These cotinine data indicate that female mice may metabolize nicotine more quickly or eliminate cotinine more slowly than do the male mice, which would result in increased nicotine consumption by female mice to maintain steady plasma nicotine levels. This sex difference in nicotine pharmacokinetics was suggested earlier by Hatchell and Collins (1980), who reported that postadolescent (postnatal day 56) female C57BL/6 mice eliminate nicotine faster than do male mice, but they display similar brain nicotine levels. Interestingly, Hatchell and Collins (1980) also reported that C57BL/6 female mice were less behaviorally sensitive (as indexed by locomotion behavior) to injected nicotine than were male mice, a finding consistent with other studies of locomotor responses in adolescent rats infused with nicotine (osmotic minipump; e.g., Faraday et al., 2001). Whether sex differences in nicotine pharmacodynamics contributed to the present findings cannot be determined, and we were unable to find published reports of sex differences in pharmacodynamic effects of nicotine in adolescent mice, suggesting that this is an important area of future research investigations. It is possible that the pharmacodynamic profile is not different between males and females, and that sex differences in nicotine pharmacokinetics accounted for the observed differences in nicotine consumption. Taken together, the cotinine and nicotine consumption data indicate that females may display decreased sensitivity to nicotine's biobehavioral effects as a result of differential nicotine metabolism compared with males.

The present findings appear to be the first report of voluntary nicotine intake by periadolescent mice across varying nicotine concentrations and the resultant cotinine levels. Importantly, they suggest that studies of nicotine elimination pharmacokinetics in adolescent animals are needed to develop an understanding of sex differences in nicotine consumption and subsequent pharmacodynamic actions of nicotine. Additional studies also are needed to determine whether these self-administered nicotine amounts are rewarding and whether they are similarly rewarding for both sexes. For example, providing mice oral access to multiple concentrations of nicotine simultaneously (e.g., 3- and 4-bottle choice tests) would allow the determination of sex differences in the reinforcing efficacy of nicotine. Intravenous nicotine self-administration studies also could be used for this purpose, although it might be difficult to establish stable intravenous self-administration in adolescent mice within the short developmental window of adolescence. Changes in oral nicotine consumption following the administration of centrally acting nicotinic cholinergic receptor antagonists, and the observation of concomitant withdrawal behaviors, also could provide insight into sex differences in the rewarding effects of nicotine.

The nicotine preference data might shed light on the question of whether mice in the present study consumed nicotine for its rewarding properties. It was an initial concern that average nicotine intake did not surpass 50% in any of the six nicotine-treatment groups, a finding that would suggest nicotine avoidance for adult animals. One potential explanation for this result is that the 7-day test window is too short for nicotine preference behavior to emerge. Although a recent study in our laboratory suggests that periadolescent male C57BL/6J mice develop a preference for a 50 ug/ml nicotine solution within a 7-day test period, this behavior only was observed in mice following prenatal nicotine exposure (Klein et al., 2003). Compared with male mice exposed to nicotine during prenatal development, male and female mice not exposed to nicotine during prenatal development did not display nicotine preference, a finding consistent with the present results. These mice also consumed significantly less nicotine (mg/kg) than did the male mice following prenatal nicotine exposure. Interestingly, an earlier study of voluntary oral nicotine consumption in male Sprague–Dawley rats reported that the preference behavior emerged only after an initial 8 days of testing, across a wide range of nicotine concentrations (Flynn et al., 1989). These data also are consistent with a report by Adriani et al. (2002) that middle adolescent (postnatal days 37–47) outbred male CD-1 mice do not develop a preference for nicotine during a 12-day test period. Taken together, these findings suggest that additional studies are needed that extend the 7-day voluntary test period and that include female animals to gain a better understanding of the development of nicotine-preference behavior during adolescence in males and females. Several concentrations of nicotine also need to be made available to animals in light of earlier reports with humans that nicotine intake by females via cigarette smoking may be particularly dependent on nicotine levels (e.g., Silverstein et al., 1980, 1982).

To examine the preference data more closely, we evaluated preference behavior on an individual level and determined whether any mice displayed preference for the nicotine solution. We found that 18% of mice in the present experiment ($n=44$) displayed preference for the nicotine solution, with a significant proportion of these mice coming from the four lowest concentration groups [$\chi^2(5)=12.80$, $P<.05$; 10 ug/ml (8 mice), 25 ug/ml (11 mice), 50 ug/ml (7 mice), and 75 ug/ml (12 mice) nicotine-treatment groups vs. 100 ug/ml (4 mice) and 200 ug/ml (2 mice) nicotine-treatment groups]. More specifically, nearly 25% of the total mice in the lower four nicotine concentration groups displayed nicotine preference, whereas only 7% of the total mice in the highest two groups displayed nicotine preference. Among the mice that displayed preference for the nicotine solution, this behavior was evident on Day 1, regardless of nicotine-treatment group, and remained stable across the 7-day test period. Similarly, the rest of the mice displayed avoidance on the 1st day of testing (i.e., less than 40% total fluid consumption; Tordoff and Bachmanov, 2003), and this behavior also remained stable across the 7-day test period. These data suggest that a small percentage of periadolescent mice rapidly develop and maintain nicotine preference across a short period of nicotine access, and that the concentration of nicotine affects the percentage of animals that will develop this preference. Further studies that include additional nicotine concentrations and longer test periods are needed to determine the effect of nicotine concentration on the development of nicotine preference during adolescence. These studies also should include adult animals to evaluate age-related differences in preference development. Because mice in the current study were genetically identical (i.e., inbred), it is surprising that they displayed such distinct variability in the development of preference behavior and nicotine consumption, suggesting important environmental contributions (e.g., nicotine concentration) to voluntary nicotine intake. Additional rodent studies that include inbred strain panels of mice are needed to separate environmental and genetic factors that contribute to the development of voluntary nicotine consumption during adolescence. Perhaps, more importantly, selective breeding studies with mice that develop high nicotine preference during the initial days of nicotine access would provide valuable insight into the genetic and environmental contributors to individual differences in the vulnerability to consume nicotine during adolescence. Taken a step further, these analyses highlight the importance of examining drug consumption behavior at both the group and individual levels.

One factor that may have influenced nicotine consumption by periadolescent mice is the use of saccharin to mask the freebase nicotine. Earlier studies with mice have shown that the addition of even a small amount of saccharin (e.g., 2

uM) to a nicotine solution can increase total fluid intake (e.g., Rowell et al., 1983). However, Robinson et al. (1996) have shown that 0.2% saccharin does not significantly enhance the dosage of freebase nicotine consumed by adult male C57BL/6J mice, at concentrations similar with those used in the present experiment. Indeed, the present experiment used a saccharin concentration 10 times higher than that used by Robinson et al. (1996), a difference that could have a significant impact on the amount of nicotine consumed by the adolescent mice. While saccharin could have affected fluid intake in the present experiment, it is important to note that there were no nicotine group differences in total fluid intake, which suggests that group differences in nicotine consumption were driven by nicotine concentration and not by saccharin. As with the preference data, there were individual differences in nicotine consumption, which suggest important environmental and genetic contributions to this behavior. Inbred mouse strains differ in saccharin preference, and a genetic basis for differences in sweet solution preferences has been suggested for several years (e.g., Belknap et al., 1992; Capeless and Whitney, 1995; Lush, 1989). Additional studies that include adolescent and adult mice of different inbred mouse strains and are provided access to ascending concentrations of nicotine and saccharin are needed to determine the impact of sweetened solutions on voluntary oral nicotine consumption.

With respect to body weight, there was no effect of nicotine consumption on body weight gains in the present study; that is, repeated-measures within-subject analyses did not reveal a body weight effect of nicotine concentration group over the 7-day treatment period. These data are in contrast to many reports on the inverse relationship between nicotine and body weight gain in adult rats and humans (e.g., Grunberg, 1982; Grunberg et al., 1986, 1988), and in adolescent rats (Klein, 2001). Interestingly, Faraday et al. (2001) found this nicotine–body weight relationship among adolescent male, but not female, rats, suggesting that age and sex are important variables that contribute to this relationship.

A separate, more critical point to consider is that the nicotine–body weight relationship may not occur in rodents that consume nicotine in drinking water. In addition to the present data, several other oral nicotine consumption studies with male and/or female mice (e.g., CD-1, C57BL/6, NMRI, Swiss-Webster) fail to find this inverse relationship (Adriani et al., 2002; Kelley and Middaugh, 1999; Pekonen et al., 1993; Rowell et al., 1983; Sparks and Pauly, 1999), as do studies with adult male rats (Flynn et al., 1989; Liu et al., 2003). These studies, across a wide range of nicotine concentrations and self-administered dosages, suggest that an oral nicotine consumption paradigm in mice may not produce (1) the body weight changes observed in earlier animal studies using different administration methods or (2) requisite nicotine levels associated with this body weight effect. One conclusion from these data is that the body weight effect of nicotine in mice operates on a mechanism that is different from that of adult rats and humans. With regard to the adolescent human literature, these data with mice appear to be consistent with new reports that adolescent smoking actually may be associated with increased body mass index (Cooper et al., 2003; Klesges et al., 1998a), and that there is a minimal effect of cigarette smoking on body weight in young adults (Klesges et al., 1998b).

Although the within-subjects analyses did not suggest a nicotine–body weight relationship, individual cotinine levels were inversely correlated with body weight amounts at the end of the experiment among females, but not among males. That is, when serum cotinine levels, a pharmacologically relevant proxy for nicotine self-administration, were allowed to vary on an individual basis, the body weight–nicotine exposure relationship was revealed. Thus, the nicotine-treatment group analyses masked the more pronounced contribution of individual voluntary nicotine intake—regardless of concentration of the consumed nicotine—to body weight. Consistent with the cotinine and nicotine preference data, these correlational analyses highlight the importance of examining individual contributions to observed behavioral effects. Additional studies are needed that include other inbred mouse strains to deconstruct the individual variability in nicotine's effects and, subsequently, Genetic × Environmental influences on the biobehavioral actions of nicotine in adolescence.

5. Summary

The present findings suggest important sex differences in nicotine self-administration, nicotine pharmacokinetics, and the effects of nicotine on body weight in adolescent C57BL/6J mice across six nicotine concentrations, using a voluntary oral nicotine consumption paradigm. To the extent that this model predicts nicotine consumption by adolescent humans, it could be used to understand why adolescent humans begin to smoke, and how smoking might increase the propensity to consume other addictive drugs (Klein, 2001). Mouse models of drug addiction provide a unique and important opportunity to advance our understanding of genetic and environmental influences on the development of nicotine addiction. The extension of this voluntary nicotine intake model into other mouse strains, additional developmental stages (e.g., post adolescence, young adulthood, late adulthood), and longer periods of nicotine access will yield exciting and new insights into smoking behavior by adolescents and, perhaps, innovative treatments for nicotine dependence.

Acknowledgements

This research was conducted at The Pennsylvania State University and was supported by NIDA grant 1RO3DA015114-01 (LCK). HMK now is in the Department of Behavioral Neuroscience at Oregon Health & Science

University. Portions of this work were presented at the annual meetings of the Society for Research on Nicotine and Tobacco, Savannah, GA, February 2002, the Society of Biological Psychiatry, Philadelphia, PA, May 2002, and the American Psychological Association, Toronto, Ontario, Canada, August 2003. We thank R. Ceballos, C. DeNucci, J. Heaton, J. Mericle, I. Rodrigues, and B. Saltsman for their dedicated assistance with data collection, Dr. John Graham for guidance with statistical analyses, and Dr. Sheila West for editorial comments.

References

Abreu-Villaca Y, Seidler FJ, Tate CA, Slotkin TA. Nicotine is a neurotoxin in the adolescent brain: critical periods, patterns of exposure, regional selectivity, and dose thresholds for macromolecular alterations. Brain Res 2003;979:114–28.

Adriani W, Chiarotti F, Laviola G. Elevated novelty seeking and peculiar D-amphetamine sensitization in periadolescent mice compared with adult mice. Behav Neurosci 1998;5:1152–66.

Adriani W, Macri S, Pacifici R, Laviola G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology 2002;27:212–24.

Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moel M, Smit AB, et al. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. J Neurosci 2003;23:4712–6.

Alexander BK, Coambs RB, Hadaway PF. The effects of housing and gender on morphine self-administration in rats. Psychopharmacology 1978;58:75–9.

Belknap JK, Crabbe JC, Plomin R, McClearn GE, Sampson KE, O'Toole LA, et al. Single-locus control of saccharin intake in BXD/Ty recombinant inbred (RI) mice: some methodological implications for RI strain analysis. Behav Genet 1992;22:81–100.

Bhat RV, Marks MJ, Collins AC. Effects of chronic nicotine infusion on kinetics of high-affinity nicotine binding. J Neurochem 1994;62:574–81.

Campbell UC, Morgan AD, Carroll ME. Sex differences in the effects of baclofen on the acquisition of intravenous cocaine self-administration in rats. Drug Alcohol Depend 2002;66:61–9.

Capeless CG, Whitney G. The genetic basis of preference for sweet substances among inbred strains of mice: preference ratio phenotypes and the alleles of the Sac and dpa loci. Chem Senses 1995;20:291–8.

Centers for Disease Control and Prevention G. Prevalence of current cigarette smoking among adults and changes in prevalence of current and some day smoking—United States, 1996–2001. Morb Mort Wkly Rep 2003a;52:303–7.

Centers for Disease Control and Prevention G. Tobacco use among middle and high school students—United States, 2002. Morb Mort Wkly Rep 2003b;52:1096–8.

Chassin L, Presson CC, Sherman SJ, Kim K. Historical changes in cigarette smoking and smoking-related beliefs after 2 decades in a midwestern community. Health Psychol 2003;22:347–53.

Collins AC, Marks MJ, Pauly JR. Differential effect of chronic nicotine treatment on nicotinic receptor numbers in various brain regions of mice. J Subst Abuse 1989;1:273–86.

Cooper TV, Klesges RC, Robinson LA, Zbikowski SM. A prospective evaluation of the relationships between smoking dosage and body mass index in an adolescent, biracial cohort. Addict Behav 2003;28:501–12.

Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldovan V, Booth S, et al. Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. Psychopharmacology (Berl) 2000;151:392–405.

Faraday MM, Elliott BM, Grunberg NE. Adult vs. adolescent rats differ in biobehavioral responses to chronic nicotine administration. Pharmacol Biochem Behav 2001;70:475–89.

Faraday MM, Elliott BM, Phillips JM, Grunberg NE. Adolescent and adult male rats differ in sensitivity to nicotine's activity effects. Pharmacol Biochem Behav 2003;74:917–31.

Flynn FW, Webster M, Ksir C. Chronic voluntary nicotine drinking enhances nicotine palatability in rats. Behav Neurosci 1989;103:356–64.

Gäddnäs H, Pietilä K, Piepponen TP, Ahtee L. Enhanced motor activity and brain dopamine turnover in mice during long-term nicotine administration in the drinking water. Pharmacol Biochem Behav 2001;70:497–503.

Gilpin EA, Choi WS, Berry C, Pierce JP. How many adolescents start smoking each day in the United States? J Adolesc Health 1999;25:248–55.

Grunberg NE. The effects of nicotine and cigarette smoking on food consumption and taste preferences. Addict Behav 1982;7:317–31.

Grunberg NE, Bowen DJ, Winders SE. Effects of nicotine on body weight and food consumption in female rats. Psychopharmacology 1986;90:101–5.

Grunberg NE, Popp KA, Winders SE. Effects of nicotine on body weight in rats with access to "junk" foods. Psychopharmacology 1988;94:536–9.

Hatchell PC, Collins AC. Influences of genotype and sex on behavioral tolerance to nicotine in mice. Pharmacol Biochem Behav 1977;6:25–30.

Hatchell PC, Collins AC. The influence of genotype and sex on behavioral sensitivity to nicotine in mice. Psychopharmacology (Berl) 1980;71:45–9.

Kelley BM, Middaugh LD. Periadolescent nicotine exposure reduces cocaine reward in adult mice. J Addict Dis 1999;18:27–39.

Klein LC. Effects of adolescent nicotine exposure on opioid consumption and neuroendocrine responses in adult male and female rats. Exp Clin Psychopharmacol 2001;9:251–61.

Klein LC, Popke EJ, Grunberg NE. Sex differences in effects of predictable and unpredictable foot shock on fentanyl self-administration in rats. Exp Clin Psychopharmacol 1997;5:99–106.

Klein LC, Stine MM, Pfaff DW, Vandenbergh DJ. Maternal nicotine exposure increases nicotine preference in periadolescent male but not female mice. Nicotine Tob Res 2003;5:117–24.

Klesges RC, Robinson LA, Zbikowski SM. Is smoking associated with lower body mass in adolescents? A large-scale biracial investigation. Addict Behav 1998a;23:109–13.

Klesges RC, Zbikowski SM, Lando HA, Haddock CK, Talcott GW, Robinson LA. The relationship between smoking and body weight in a population of young military personnel. Health Psychol 1998b;17:454–8.

Kotlus BS, Blizard DA. Measuring gustatory variation in mice: a short-term fluid-intake test. Physiol Behav 1998;64:37–47.

Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev 1999;23:993–1010.

Laviola G, Pascucci T, Pieretti S. Striatal dopamine sensitization to D-amphetamine in periadolescent but not in adult rats. Pharmacol Biochem Behav 2001;68:115–24.

Laviola G, Macri S, Morley-Fletcher S, Adriani W. Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. Neurosci Biobehav Rev 2003;27:19–31.

Le Houezec J, Martin C, Cohen C, Molimard R. Failure of behavioral dependence induction and oral nicotine bioavailability in rats. Physiol Behav 1989;45:103–8.

Levin ED, Rezvani AH, Montoya D, Rose JE, Swartzwelder HS. Adolescent-onset nicotine self-administration modeled in female rats. Psychopharmacology (Berl). 2003 [May 23, Electronic publication ahead of print].

Liu RH, Mizuta M, Matsukura S. Long-term oral nicotine administration reduces insulin resistance in obese rats. Eur J Pharmacol 2003;458:227–34.

Lopez MF, White NM, Randall CL. Alcohol tolerance and nicotine cross-tolerance in adolescent mice. Addict Biol 2001;6:119–27.

Lush IE. The genetics of tasting in mice: VI. Saccharin, acesulfame, dulcin and sucrose. Genet Res 1989;53:95–9.

Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 1999;144:77–82.

Mansvelder HD, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. Neuron 2002;33: 905–19.

Marks MJ, Collins AC. Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. Mol Pharmacol 1982;22:554–64.

Meliska CJ, Bartke A, McGlacken G, Jensen RA. Ethanol, nicotine, amphetamine, and aspartame consumption and preferences in C57BL/6 and DBA/2 mice. Pharmacol Biochem Behav 1995;50:619–26.

Miao H, Liu C, Bishop K, Gong ZH, Nordberg A, Zhang X. Nicotine exposure during a critical period of development leads to persistent changes in nicotinic acetylcholine receptors of adult rat brain. J Neurochem 1998;70:752–62.

National Research Council. Guide for the care and use of laboratory animals: a report of the Institute of Laboratory Animal Resources Committee on Care and Use of Laboratory Animals. Report no. 85-23. Washington (DC): U.S. Department of Health and Human Services, 1985.

Pauly JR, Marks MJ, Robinson SF, van de Kamp JL, Collins AC. Chronic nicotine and mecamylamine treatment increase brain nicotinic receptor binding without changing α4 or β2 mRNA levels. J Pharmacol Exp Ther 1996;278:361–9.

Pekonen K, Karlsson C, Laakso I, Ahtee L. Plasma nicotine and cotinine concentrations in mice after chronic oral nicotine administration and challenge doses. Eur J Pharm Sci 1993;1:13–8.

Petersen DR, Norris KJ, Thompson JA. A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. Drug Metab Dispos 1984;12:725–31.

Pierce JP, Gilpin E. How long will today's new adolescent smoker be addicted to cigarettes? Am J Public Health 1996;86:253–6.

Pietilä K, Laakso I, Ahtee L. Chronic oral nicotine administration affects the circadian rhythm of dopamine and 5-hydroxytryptamine metabolism in the striata of mice. Naunyn-Schmiedeberg's Arch Pharmacol 1995; 353:110–5.

Robinson SF, Marks MJ, Collins AC. Inbred mouse strains vary in oral self-selection of nicotine. Psychopharmacology (Berl) 1996;124:332–9.

Rosecrans JA. Brain area nicotine levels in male and female rats with difference levels of spontaneous activity. Neuropharmacology 1972; 11:863–70.

Rowell PP, Hurst HE, Bennett BD. Oral administration of nicotine: its uptake and distribution after chronic administration to mice. J Pharmacol Methods 1983;9:249–61.

Shaham Y, Alvares K, Nespor SM, Grunberg NE. Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacol Biochem Behav 1992;41:615–9.

Silverstein B, Feld S, Kozlowski LT. The availability of low-nicotine cigarettes as a cause of cigarette smoking among teenage females. J Health Soc Behav 1980;21:383–8.

Silverstein B, Kelly E, Swan J, Kozlowski LT. Physiological predisposition toward becoming a cigarette smoker: experimental evidence for a sex difference. Addict Behav 1982;7:83–6.

Smith RF. Animal models of periadolescent substance abuse. Neurotoxicol Teratol 2003;25:291–301.

Smith A, Roberts DC. Oral self-administration of sweetened nicotine solutions by rats. Psychopharmacology (Berl) 1995;120:341–6.

Smolen A, Marks MJ, DeFries JC, Henderson ND. Individual differences in sensitivity to nicotine in mice: response to six generations of selective breeding. Pharmacol Biochem Behav 1994;23:987–93.

Sparks JA, Pauly JR. Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. Psychopharmacology 1999;141:145–53.

Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.

Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;16:83–109.

Todte K, Tselis N, Dadmarz M, Golden G, Ferraro T, Berrettini WH, et al. Effects of strain, behavior and age on the self-administration of ethanol, nicotine, cocaine and morphine by two rat strains. Neuropsychobiology 2001;44:150–5.

Tordoff MG, Bachmanov AA. Mouse taste preference tests: why only two bottles? Chem Senses 2003;28:315–24.

Trauth JA, Seidler EC, Slotkin TA. Persistent and delayed behavioral changes after nicotine treatment in adolescent rats. Brain Res 2000a; 880:167–72.

Trauth JA, Seidler FJ, Slotkin TA. An animal model of adolescent nicotine exposure: effects on gene expression and macromolecular constituents in rat brain regions. Brain Res 2000b;867:29–39.

Trauth JA, Seidler FJ, Ali SF, Slotkin TA. Adolescent nicotine exposure produces immediate and long-term changes in CNS noradrenergic and dopaminergic function. Brain Res 2001;892:269–80.

United States Department of Health and Human Services. The health consequences of smoking: nicotine addiction. A report of the Surgeon General. DHHS Pub. No. 88-8406. Washington (DC): U.S. Government Printing Office, 1988.

United States Department of Health and Human Services. Preventing tobacco use among young people: a report of the Surgeon General. Washington (DC): U.S. Government Printing Office, 1994.