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Early-adolescent male C57BL/6J and DBA/2J mice display reduced sensitivity to acute nicotine administration

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Abstract

Background: The initial response to nicotine is an important predictor of subsequent use. Multiple factors may alter this response including genetics and age of first use. Here we investigated the influence of age, genetics, and their interaction on nicotine sensitivity. We then examined whether these factors influence the relationship between initial behavioral responses and voluntary nicotine consumption in adulthood.

Methods: We measured initial nicotine responses, including nicotine-induced locomotor depression and hypothermia following an acute intraperitoneal injection (0, 0.5, or 1 mg/kg), during early-adolescence, middle-adolescence, late-adolescence, or adulthood. Thirty-five days after the initial testing, mice were assessed for voluntary oral nicotine consumption.

Results: Early-adolescent mice were more resistant to nicotine-induced hypothermia and locomotor depression than later ages, further hypothermia was influenced by genetics. In the DBA/2J strain, early-adolescent mice were insensitive to nicotine-induced hypothermia, but this response developed at later ages. In contrast, C57BL/6J mice were sensitive at all ages, but sensitivity increased across developmental age. There was little evidence of a relationship between initial behavioral response and choice nicotine consumption.

Conclusion: By understanding how age of exposure and genetics influence initial nicotine behavioral responses, we have a greater understanding of factors that make adolescents differentially sensitive to the effects of this drug.

Keywords

Nicotine; Adolescence; Locomotor stimulation; Nicotine consumption; Hypothermia

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1. Introduction

An individual's initial response to cigarettes is a key predictor of subsequent nicotine use [1]. Importantly, 9 out of 10 smokers try their first cigarette by age 18 [2]. Relative to adults, adolescents are more sensitive to reinforcing properties [3,4] and less sensitive to aversive properties of nicotine [5]. Developmental changes in the adolescent brain, including maturation of the nicotinic acetylcholine and dopamine receptor systems, likely contribute to these age-dependent differences in nicotine sensitivity [6,7]. Genetic variation also modulates nicotine sensitivity. Accordingly, human studies suggest that genetics could account for as much as 44% of the variation in smoking initiation [8]. Human genetics studies have consistently found nicotinic receptor gene variants (e.g. in the CHRNA5-CHRNA3-CHRNA4 gene cluster) to be associated with several smoking-related traits [9]. Preclinical models, which evaluate the impact of genetic variation using inbred mouse strains, have demonstrated strain differences in sensitivity to nicotine's behavioral and physiological effects. For example, adult male C57BL/6J mice have increased nicotine sensitivity and preference relative to DBA/2J mice, however this is yet to be explored in adolescent animals [10–12]. Although both age and genetics influence nicotine sensitivity, thus shaping one's propensity for continued nicotine use, little is known about the interaction between these factors.

Long-lasting behavioral and physiological changes can result from adolescent nicotine exposure, which may prime the brain for future nicotine use. In fact, individuals who tried smoking a single cigarette during early adolescence were more likely to smoke later in life compared to individuals who never experimented with cigarettes during this time [13]. Additionally, an adolescent's rating of the initial pleasant experience with first nicotine use predicted DSM-IV dependence syndrome later in life [14]. Interestingly, preclinical studies have demonstrated physiological adaptations that may contribute to these findings. A single nicotine exposure (0.4 mg/kg) during adolescence, but not adulthood, results in a long-lasting synaptic potentiation of midbrain dopamine neurons [15]. This sort of plasticity may be a key adaptation underlying the acquisition of later drug self-administration. However, few studies have investigated whether age or genetics influence the effects of a single nicotine exposure on later nicotine intake. Therefore, the goal of the current study was to examine whether age, genetics, and/or the interaction of these factors influence acute nicotine responses. Second, we sought to determine whether differences in sensitivity to acute nicotine predicted later nicotine consumption.

2. Materials/Methods

2.1 Animals

Male C57BL/6J and DBA/2J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and arrived on postnatal day (PND) 25, 35, 45, or 65 [16]. Mice of the same strain and age were housed 2 per standard cage with *ad libitum* rodent chow and water. Animals were acclimated to a 12-h light/dark cycle (lights on at 0700) for 6 days prior to testing. The experimental timeline is detailed in Figure 1. All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

2.2 Drugs

(–)-Nicotine freebase, saccharin sodium salt, and quinine hemi-sulfate salt were purchased from Sigma-Aldrich. For intraperitoneal (i.p.) injections, nicotine was mixed in physiological saline (0.9% NaCl) to appropriate concentrations. For drinking solutions, nicotine and tastants were diluted in tap water.

2.3 Locomotor responses

The acute effect of nicotine on locomotor activity was examined in a symmetrical Y-maze [10] during early-adolescence (PND 31), middle-adolescence (PND 41), late-adolescence (PND 51), or adulthood (PND 71). Testing occurred between 0930 and 1430. The maze consists of 3 red covered Plexiglas arms (27 L × 8 W × 10 H; cm) [17]. The test room was brightly lit (~600 lux), but light intensity was low inside the Y-maze arms (~3 lux). Overhead cameras recorded the trials, which were analyzed with an automated video-tracking system (Anymaze® v.4.60, Stoelting, Wood Dale, IL).

Nicotine-induced locomotor activity followed a three day paradigm. Each animal received an i.p. injection of saline on days 1 and 2 and nicotine (0.5 or 1 mg/kg) or saline on day 3 immediately before placement in the Y-maze. Nicotine doses were based on prior literature [18–20]. Locomotor activity was monitored for 10 minutes (in 5 min epochs). The dependent variable was change in locomotor activity (cm; Day 3 nicotine response - Day 2 habituated baseline).

2.4 Body temperature responses

Mice were placed in individual holding cages after Y-maze testing until their rectal body temperature was taken 15 min after their injection [10] using a TH-5 Thermalert Monitoring Thermometer and RET-3 mouse rectal probe (Physitemp Instruments Inc.). The dependent variable was nicotine-induced change in body temperature (°C; Day 3 nicotine response – Day 2 habituated baseline).

2.5 Two-bottle choice nicotine drinking

Following acute nicotine testing, mice remained undisturbed for 35 days. All mice were then single housed and nicotine consumption was measured using a standard protocol [20,21]. Mice were supplied two tap water filled 25mL tubes with drinking spouts for a 2-day habituation. Mice were then given access to one tube with water and the other bottle with increasing concentrations of nicotine (25, 50, 100, or 200 µg/ml). Mice were weighed every 4 days when the concentration of nicotine was increased. Tube positions were altered every two days to avoid the development of a side bias. To account for leakage/evaporation, two cages without animals were handled the same as experimental cages and the average volume lost from these tubes was subtracted from experimental drinking values. Nicotine consumption was calculated as the average of total nicotine intake on second and fourth day of exposure for each concentration [22]. Dependent variables included nicotine consumption (mg/kg), nicotine preference (ml nicotine/ml total fluid), and total fluid consumption (ml).

2.6 Two-bottle choice tastant drinking

To ensure group differences in nicotine consumption were specific for nicotine's pharmacological effects and not related to altered taste sensitivity, mice were monitored for consumption of two non-caloric tastants (saccharin-sweet and quinine-bitter) utilizing a published procedure that mimicked nicotine drinking [20,23]. Mice were tested 1 week after nicotine consumption ended. Each tastant was presented in increasing concentrations (saccharin: 0.033 and 0.066%; quinine: 0.015 and 0.03 mM). Dependent variables included tastant consumption (mg/kg), tastant preference (ml tastant/ml total fluid) and total fluid consumption (nicotine and water (ml)).

2.7 Statistical analysis

Statistical analyses were performed in SPSS. Results were analyzed with either a repeated measures or factorial ANOVA with age of exposure, strain, nicotine dose, nicotine concentration, tastant concentration, or time as factors. Outliers (2.5 S.D. from mean) were removed. For all statistical calculations, the number of outliers and corrected sample sizes are provided in the Supplementary Tables (S1-S2). Post hoc comparisons were performed with Tukey's HSD. $\alpha < 0.05$ was considered significant.

3. Results

3.1 Nicotine-induced hypothermia

Early-adolescent mice were more resistant to nicotine-induced hypothermia than adult animals (Fig. 2). Overall, DBA/2J mice were less sensitive to nicotine-induced hypothermia than C57BL/6J mice (main effect of strain: $F_{1,236}=46.5$, $p<0.001$). Additional analyses were conducted within each strain due to a significant strain X treatment interaction ($F_{2,236}=20.8$, $p<0.001$). In C57BL/6J mice, nicotine resulted in a dose-dependent decrease in body temperature (main effect of treatment: $F_{2,118}=69.7$, $p<0.001$; post-hoc all $p<0.05$) and early-adolescent animals were less sensitive to nicotine's hypothermic effects than adults (Fig. 2A; main effect of age: $F_{3,118}=2.8$, $p<0.05$; post-hoc $p<0.05$). The age X treatment interaction was not significant. In DBA/2J mice, age interacted with treatment (Fig. 2B; $F_{6,118}=2.5$, $p<0.05$) such that sensitivity to nicotine developed as age increased. The 1 mg/kg dose resulted in greater hypothermia than saline for middle-adolescent and late-adolescent, but not early-adolescent, DBA/2J mice (main effect of treatment: middle-adolescent mice $F_{2,30}=4.2$, $p<0.05$; late-adolescent mice $F_{2,29}=3.8$, $p<0.05$, post hoc all $p<0.05$). In adulthood, 1 mg/kg nicotine resulted in greater hypothermia compared to both saline and 0.5 mg/kg nicotine (main effect of treatment: $F_{2,30}=10.8$, $p<0.001$; post-hoc all $p<0.05$).

3.2 Locomotor response to acute nicotine

Overall, early-adolescent mice were less sensitive to nicotine-induced locomotor depression than older animals, but no strain difference was observed (Fig. 2). When locomotor activity was examined across 5 min epochs there was a significant main effect of time ($F_{1,207}=34.4$, $p<0.001$), thus further analyses were conducted for each 5 min period. In the first 5 min, early-adolescent mice were less sensitive to nicotine-induced locomotor depression than middle-adolescent, late-adolescent, and adult animals (Fig. 2C-D; main effect of age:

$F_{3,233}=5.2$, $p<0.01$; post hoc all $p<0.05$). The 1 mg/kg dose resulted in greater locomotor depression than saline (main effect of treatment: $F_{2,233}=6.0$, $p<0.01$; post hoc $p<0.01$). During the second 5 min, both the 0.5 and 1 mg/kg nicotine caused a dose-dependent decrease in locomotor activity relative to saline (Fig. 2E-F; main effect of treatment: $F_{2,232}=53.7$, $p<0.001$, post hoc all $p<0.001$). There were no other significant main effects or interactions.

3.3 Choice nicotine consumption

The influence of age of first nicotine exposure on later drug consumption was more important in C57BL/6J than DBA/2J mice (Fig. 3). Overall, C57BL/6J mice consumed more nicotine than DBA/2J mice (main effect of treatment: $F_{1,198}=48.1$, $p<0.001$). Further analyses were conducted in each strain separately due to significant strain X concentration ($F_{3,594}=25.7$, $p<0.001$) and concentration X treatment ($F_{6,594}=2.4$, $p<0.05$) interactions. Within C57BL/6J mice, there was a significant concentration X age X treatment interaction ($F_{18,303}=1.7$, $p<0.05$). To decompose this interaction, we performed additional analyses at each nicotine concentration. At 50 $\mu\text{g/ml}$ nicotine, a significant main effect of age was observed ($F_{3,119}=4.8$, $p<0.01$), mice with acute exposure during early-adolescence (3.92 ± 0.29) drank significantly more nicotine than mice exposed in late-adolescence (2.64 ± 0.35) and adulthood (2.19 ± 0.22) (all $p<0.05$). At the highest nicotine concentration (200 $\mu\text{g/ml}$), a significant treatment X age interaction was detected ($F_{6,114}=2.5$, $p<0.05$). Mice exposed to 0.5 mg/kg nicotine in early-adolescence consumed significantly less nicotine compared to animals that received saline (main effect of treatment: Fig. 3A; $F_{2,29}=3.7$, $p<0.05$, post hoc all $p<0.05$). Mice exposed to 0.5 mg/kg nicotine in late-adolescence consumed more nicotine than the animals who exposed to 1 mg/kg nicotine (main effect of treatment: $F_{2,27}=4.5$, $p<0.05$, post hoc all $p<0.05$). No significant differences were observed in either middle-adolescent or adult animals. In contrast, when DBA/2J mice were examined there were no significant main effects or interactions with age of acute nicotine exposure. There was a significant concentration X treatment interaction ($F_{6,291}=6.1$, $p<0.001$) where follow-up analysis suggested that mice exposed to 1 mg/kg nicotine consumed more 100 $\mu\text{g/ml}$ nicotine than saline treated animals (Fig. 3B, 3D, 3F, 3H; $p<0.05$).

There was no impact of a single prior exposure to nicotine on later nicotine preference (Fig. 4). Overall, C57BL/6J mice preferred more nicotine than DBA/2J mice (main effect of strain: $F_{1,205}=42.9$, $p<0.001$). Similar to nicotine consumption, each strain was subsequently examined independently due to a significant strain X concentration X treatment interaction ($F_{6,615}=2.3$, $p<0.05$). In C57BL/6J mice, preference decreased as nicotine concentration increased (main effect of concentration: $F_{3,318}=63.8$, $p<0.001$; post hoc all $p<0.05$). No other significant effects were detected. In DBA/2J mice, we observed a significant concentration X treatment interaction ($F_{6,297}=3.1$, $p<0.01$). At low nicotine concentrations (25 and 50 $\mu\text{g/ml}$) we found no significant change in nicotine preference as a result to prior nicotine administration, but at higher nicotine concentrations differences emerged. At 100 $\mu\text{g/ml}$, mice pre-treated with 1 mg/kg nicotine preferred significantly more nicotine than saline treated animals (Fig. 4F; main effect of treatment: $F_{2,116}=4.1$, $p<0.05$; post hoc all $p<0.05$). At 200 $\mu\text{g/ml}$ nicotine, mice exposed to 0.5 mg/kg nicotine preferred more nicotine

than did mice exposed to saline or 1 mg/kg nicotine (Fig. 4B, 4D, 4F, 4H; $F_{2,115}=5.2$, $p<0.01$; post hoc all $p<0.05$).

There were minor changes in total fluid consumption that were driven by the concentration of nicotine available (Fig. 5). DBA/2J mice consumed more fluid than C57BL/6J mice (main effect of strain: $F_{1,208}=74.1$, $p<0.001$). Further analyses were conducted for each strain separately due to this significant strain X concentration interaction ($F_{3,624}=6.1$, $p<0.001$). In C57BL/6J mice, total fluid consumption increased as nicotine concentration increased (main effect of concentration: $F_{3,312}=9.2$, $p<0.001$, post hoc all $p<0.05$). In DBA/2J mice, age of initial exposure interacted with concentration ($F_{9,312}=2.4$, $p<0.05$). Within each treatment age group there was a significant main effect of concentration (early-adolescence: $F_{3,81}=6.9$, $p<0.001$; middle-adolescence: $F_{3,84}=16.7$, $p<0.001$; late-adolescence: $F_{3,84}=9.8$, $p<0.001$; adult: $F_{3,87}=5.7$, $p<0.01$). Mice exposed to nicotine in early-adolescence consumed more total fluid when 25 $\mu\text{g/ml}$ nicotine was available compared to any other nicotine concentration (all $p<0.01$). A similar pattern emerged in middle-adolescent mice, except fluid intake decreased above 50 $\mu\text{g/ml}$ nicotine (all $p<0.01$). Adult and late-adolescent mice consumed less fluid as nicotine concentration increased, but this leveled off between 100 and 200 $\mu\text{g/ml}$ nicotine (late-adolescent: all $p<0.01$; adult: all $p<0.05$).

3.4 Choice tastant consumption

Prior nicotine exposure did not influence tastant consumption (Supplementary Tables S3-S4). Overall C57BL/6J mice consumed significantly more saccharin than DBA/2J mice (main effect of strain: $F_{1,207}=8.9$, $p<0.001$). Further analyses were performed within each strain due to a significant strain x saccharin concentration interaction ($F_{1,207}=18.3$, $p<0.001$). Mice of both strains consumed more saccharin as concentration increased (main effect of saccharin concentration: C57BL/6J - $F_{1,101}=38.8$, $p<0.001$; DBA/2J - $F_{1,106}=51.1$, $p<0.001$). In C57BL/6J mice, mice that were initially exposed in early- and middle-adolescence consumed more saccharin than those exposed in adulthood (main effect of age: $F_{3,105}=5.9$, $p<0.01$; post hoc all $p<0.05$). In DBA/2J mice, more saccharin was consumed when the higher concentration was available (main effect of concentration: $F_{1,107}=123.6$, $p<0.001$). Overall, DBA/2J mice consumed more than C57BL/6J mice (main effect of strain: $F_{1,211}=79.3$, $p<0.001$). Further analyses were performed within each strain due to a significant strain x concentration interaction ($F_{1,211}=20.5$, $p<0.001$). Mice of both strains increased consumption as quinine concentration increased (main effect of concentration: C57BL/6J - $F_{1,106}=36.1$, $p<0.001$; DBA/2J - $F_{1,105}=83.2$, $p<0.001$).

Prior nicotine exposure did not affect saccharin or quinine preference in DBA/2J or C57BL/6J mice (Supplementary Tables S3-S4). Overall C57BL/6J mice had a higher preference for saccharin than DBA/2J mice (main effect of strain: $F_{1,207}=178.9$, $p<0.001$). Further analyses were performed within each strain separately due to a significant strain x saccharin concentration interaction ($F_{1,207}=18.3$, $p<0.001$). Preference increased as saccharin concentration increased for both strains (main effect of saccharin concentration: C57BL/6J - $F_{1,101}=38.8$, $p<0.001$; DBA/2J - $F_{1,106}=51.1$, $p<0.001$), but there were no other significant effects in these analyses. Further analyses were performed within each strain separately due to a significant strain x quinine concentration interaction ($F_{1,212}=5.5$,

$p < 0.05$). In C57BL/6J mice, quinine preference decreased as concentration increased (main effect of concentration: $F_{1,107} = 10.8$, $p < 0.01$). There were no significant effects in DBA/2J mice.

Prior nicotine exposure did not affect total fluid consumption during tastant testing (Supplementary Tables S3-S4). Overall, C57BL/6J mice consumed more fluid during saccharin testing than DBA/2J mice (main effect of strain: $F_{1,212} = 5.0$, $p < 0.001$). Further analysis was conducted separately for each strain due to significant saccharin concentration X strain ($F_{1,212} = 80.3$, $p < 0.001$) and concentration X age ($F_{1,212} = 80.3$, $p < 0.001$) interactions. Total fluid intake increased as saccharin concentration increased for both strains (main effect of saccharin concentration: C57BL/6J - $F_{1,106} = 168.1$, $p < 0.001$; DBA/2J - $F_{1,106} = 9.0$, $p < 0.01$). In C57BL/6J mice, the adult exposure group (i.e., the oldest mice) consumed more total fluid during saccharin testing than the early-adolescent exposure group (i.e. the youngest mice) (main effect of age: $F_{3,106} = 3.1$, $p < 0.05$; post hoc $p < 0.05$). No other significant effects were observed in either strain. Analyses for total fluid consumption during quinine testing were conducted separately for each strain due to significant age X strain interaction ($F_{3,216} = 4.8$, $p < 0.01$). In C57BL/6J mice, total fluid intake decreased when the higher concentration of quinine was available (main effect of concentration: $F_{1,108} = 37.2$, $p < 0.001$). In DBA/2J mice, total fluid intake decreased as quinine concentration increased (main effect of concentration: $F_{1,108} = 51.3$, $p < 0.001$). The mice in the early-adolescent exposure group also had lower total fluid intake during quinine testing than those in the later age groups (main effect of age: $F_{3,108} = 3.8$, $p < 0.05$; post hoc all $p < 0.05$).

4. Discussion

We investigated the influence of age and genetics on acute nicotine sensitivity in male C57BL/6J and DBA/2J mice. We further sought to determine if this acute exposure influenced later nicotine consumption. We found that early-adolescent mice were less sensitive to nicotine's initial hypothermic and locomotor depressant effects than were mid-adolescent, late-adolescent, or adult mice. Interestingly, we observed an interaction between age of initial exposure and genetic background on nicotine-induced hypothermia. In particular, the early-adolescent C57BL/6J mice were less sensitive to nicotine-induced hypothermia than adults, but this response was completely absent in early-adolescent DBA/2J mice. Finally, we found little evidence that the age of initial exposure predicted later nicotine consumption.

Our data suggest early-adolescence is a unique time period for behavioral (locomotor activity) and physiological (body temperature) nicotine responses. There are mixed results on the influence of age of acute exposure for nicotine responses. Our results are consistent with findings in C57BL/6J mice [24], but are in contrast to prior work in ICR mice that reported no difference in nicotine-induced hypothermia or locomotor activity between early-adolescent and adult animals [3]. Similar to our locomotor findings, prior research has shown that early-adolescent mice are less sensitive to nicotine-induced locomotor depression than older mice [24], with other groups actually observing locomotor activation in early-adolescence compared to depression in adults [4]. Finally, early-adolescent mice were more sensitive to the rewarding properties of nicotine, as measured with conditioned place

preference, and displayed greater nicotine preference, as measured via oral nicotine consumption, than adult animals [3,4]. In these cases increased sensitivity occurred after repeated exposure/availability, therefore differences in sensitivity could be related to exposure duration or the type of behavior measured.

The differences in nicotine sensitivity across age could be attributed to a number of factors including metabolic differences or variation in receptor functionality. Work examining age differences in nicotine metabolism is inconclusive. Prior work examining nicotine metabolism in early-adolescent and adult C57BL/6J mice found no differences in plasma or renal cotinine levels [25]. However other work in C57BL/6J mice observed lower plasma cotinine levels in male adolescent mice following a 0.5 mg/kg nicotine injection relative to adult mice [24]. Thus, it remains possible that differences in metabolism may alter behavior across ages. Enhanced acetylcholine receptor function has been found in early-adolescent ICR mice relative to adults, and may also account for behavioral differences observed across age groups [3].

We found that C57BL/6J mice were more sensitive to nicotine-induced hypothermia and consumed significantly more nicotine compared to DBA/2J mice. These results are consistent with findings in adult mice [10,26,27], DBA/2J and C57BL/6J mice are known to exhibit different phenotypic responses to both acute and chronic nicotine [26]. No differences in nicotine metabolism were observed between these strains [28], but differences in nicotinic acetylcholine receptor function have been found [29]. Therefore, it is possible that the behavioral differences observed here may reflect genetic differences in nicotinic receptor function between these strains. Additional studies are required to test this hypothesis.

Consistent with previous work, we observed no difference in nicotine-induced locomotor depression between C57BL/6J and DBA/2J mice [26]. Interestingly, we also found that mice tested in early-adolescence had reduced locomotor depression relative to later ages during the first 5 min of testing. These results are consistent with prior work in rats demonstrating locomotor stimulation or no effect on locomotor activity during early-adolescence, but decreased activity in adults [30,31].

The current study is, to the best of our knowledge, the first to systematically examine whether age of initial exposure and genetic background interact to influence the lasting behavioral effects of a single nicotine exposure. In the DBA/2J strain, mice that received 1 mg/kg nicotine were more likely to consume and prefer nicotine, but only at the 100 µg/ml concentration. In contrast, no consistent effects were observed in C57BL/6J mice. Prior work by Kota and colleagues found that 14 nicotine injections in early-adolescence resulted in enhanced nicotine reward during adulthood. This enhancement was not observed following either 2 or 8 nicotine injections [32]. Thus, it is possible that we may have observed a stronger relationship between initial sensitivity and later nicotine consumption if nicotine was repeatedly administered.

Smoking cigarettes is the leading cause of preventable death worldwide and an individual's first exposure often occurs during adolescence. Our study suggests that genetics and age

shape an individual's sensitivity. Specifically, early-adolescence appears to be a unique period for nicotine exposure whereby animals are less sensitive to nicotine's hypothermic and locomotor depressant effects. Future studies should focus on identifying the mechanisms which make early-adolescents less sensitive to nicotine's behavioral and physiological effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Early-adolescent mice are less sensitive to nicotine-induced hypothermia.
- Early-adolescent mice are less sensitive to nicotine-induced locomotor depression.
- Sensitivity to nicotine-induced hypothermia develops across age in DBA/2J mice.
- Prior nicotine exposure leads to increased nicotine consumption in DBA/2J mice.

Figure 1.

Diagram illustrating timing of behavioral experiments. Ages of testing are stated as PND.

Figure 2.

Sensitivity to nicotine-induced hypothermia and locomotor depression was reduced during early-adolescence. Data (mean $\pm$ SEM) represent change in body temperature in C57BL/6J (A) and DBA/2J mice (B), locomotor activity for min 0-5 in C57BL/6J (C) and DBA/2J mice (D), and locomotor activity for min 5-10 in C57BL/6J (E) and DBA/2J mice (F). N=9-11 animals/group. *significantly different from the saline control. #significantly different between ages, ‡significantly different from 1 mg/kg. †significantly different from the saline control and 0.5 mg/kg nicotine group.

Figure 3.

DBA/2J mice that received 1 mg/kg nicotine consumed significantly more 100 μg/ml nicotine. C57BL/6J mice pre-treated during early adolescence consumed more 50 μg/ml nicotine. Data (mean ± SEM) represent nicotine consumption 35 days after a single nicotine injection (i.p.) in early adolescent C57BL/6J (A) and DBA/2J mice (B), middle adolescent C57BL/6J (C) and DBA/2J mice (D), late adolescent C57BL/6J (E) and DBA/2J mice (F), adult C57BL/6J (G) and DBA/2J mice (H). N=8-11 animals/group. *significantly different from the saline control across age groups. #significantly different from the saline control, ‡significantly different from 1 mg/kg.

Figure 4.

DBA/2J mice that received 1 mg/kg nicotine preferred significantly more 100 µg/ml nicotine. Data (mean $\pm$ SEM) represent nicotine preference 35 days after a single nicotine injection (i.p.) in early adolescent C57BL/6J (A) and DBA/2J mice (B), middle adolescent C57BL/6J (C) and DBA/2J mice (D), late adolescent C57BL/6J (E) and DBA/2J mice (F), adult C57BL/6J (G) and DBA/2J mice (H). N=8-11 animals/group. *significantly different from the saline control across age groups, †significantly different from the saline control and 1 mg/kg nicotine group across age groups.

Figure 5.

Prior nicotine exposure did not influence total fluid consumption. Data (mean $\pm$ SEM) represent total fluid intake 35 days after a single nicotine injection (i.p.) in early adolescent C57BL/6J (A) and DBA/2J mice (B), middle adolescent C57BL/6J (C) and DBA/2J mice (D), late adolescent C57BL/6J (E) and DBA/2J mice (F), adult C57BL/6J (G) and DBA/2J mice (H). N=8-11 animals/group.