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Adolescent Rats Self-Administer Less Nicotine Than Adults at Low Doses

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Abstract

Introduction: Although nearly 90% of current smokers initiated tobacco use during adolescence, little is known about reinforcement by nicotine in adolescents. Researchers are currently investigating whether a potential public health policy setting a tobacco product standard with very low nicotine levels would improve public health, and it is essential to understand whether data generated in adults translates to adolescents, particularly as it relates to the threshold dose of nicotine required to support smoking. The present study compared self-administration of low doses of nicotine between adolescent and adult rats.

Methods: Adolescent (postnatal day [P] 30) and adult (P90) male and female rats were allowed to nosepoke to receive intravenous infusions of nicotine (3–100 μg/kg/infusion) during 16 daily 1-hour sessions.

Results: At 10 μg/kg/infusion nicotine, adolescent rats earned significantly fewer infusions than adults. When responding for 30 μg/kg/infusion nicotine, rats of both ages earned a similar number of infusions; however, there were subtle differences in the distribution of infusions across the 1-hour session. No sex differences were apparent in either age group at any dose.

Conclusions: These results demonstrate that adolescent rats are less sensitive than adults to the primary reinforcing effects of nicotine. However, at nicotine doses that support self-administration in both age groups, adolescent and adult rats do not differ in acquisition or number of infusions earned. These results suggest that reducing nicotine levels in cigarettes to a level that does not support smoking in adults may be sufficient to reduce the acquisition of smoking in adolescents.

Implications: The results of the present studies demonstrate that adolescent rats are less sensitive than adults to the primary reinforcing effects of nicotine. These results suggest that reducing nicotine levels in cigarettes to a level that does not support smoking in adults will be sufficient to reduce the acquisition of smoking in adolescents.

Introduction

Approximately 90% of current daily smokers in the United States initiated tobacco use prior to the age of 18[1] and recent national surveys find that nearly a quarter of high school students reported current use of tobacco products.[2] Preclinical studies examining self-administration of nicotine, the primary reinforcing and addictive chemical in tobacco, in adolescent rats have also lent support to the

notion that adolescence may be a period of particular vulnerability to the rewarding effects of nicotine.[3–8]

The Family Smoking Prevention and Tobacco Control Act (Congress 1256 §907(d) (3) (B), 2009) gives the Food and Drug Administration (FDA) authority to establish tobacco product standards to improve public health by reducing the use of tobacco products. One potential strategy is to establish maximal allowable nicotine levels for cigarettes below the threshold required to maintain smoking. Given the importance of the adolescent period for the initiation of smoking behavior and the likelihood that the majority of data relevant to a nicotine reduction policy will be generated in adults, understanding how data from adults relates to adolescents is essential. This is particularly important regarding the initiation of smoking, as such data for adolescents would become available only after nicotine product standards are in place.

Several previous studies have compared nicotine self-administration between adolescent and adult rats,[4–6,9–12] and the data are inconsistent. Furthermore, the majority of these studies used prior training for the operant behavior and/or delivered potentially rewarding cues along with nicotine, which confounds the assessment of acquisition of nicotine self-administration. Therefore, the present studies compared nicotine self-administration between adolescent and adult rats, especially at low doses of nicotine, in a manner that would allow the assessment of acquisition of nicotine self-administration based on the primary reinforcing properties of the drug. Furthermore, because evidence suggests a sex-difference in the susceptibility for initiation and continued tobacco product use,[13] both males and females were studied at each age group.

Methods

Subjects

Male and female Sprague-Dawley rats (Harlan Farms, Indianapolis, IN) were used in these studies. Adolescent rats were weaned and shipped on postnatal day (P) 21, and arrived on P21 or 22. Adult rats arrived on P82. Studies were run in a series of cohorts, with subsets of each group spread across multiple cohorts. All rats were housed individually in hanging wire mesh cages or in isolated tub cages on a ventilated rack, in a temperature and humidity controlled colony room. Rats were maintained on a reversed 12-hour light/dark cycle (lights off 7 AM), and all experimental procedures were carried out during the dark phase of the cycle. All animals had *ad libitum* access to food (Purina LabDiet 5000 or 5001) and water in their home cages throughout the course of the study, except during 1-hour self-administration sessions, when animals were removed from their home cage and put in operant chambers. Male and female adolescent rats weighed ~80 grams (g) at the start of self-administration (P30). Over the course of the 16 self-administration sessions, body weight in adolescent rats approximately doubled; adult rats gained approximately 10% of their body weight. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Apparatus

All experimental sessions were conducted in 24 × 31 × 21 cm^3 (w × l × h) commercial operant chambers (Med Associates, St Albans, VT). Chambers were enclosed in sound-attenuating cubicles with a ventilation fan. Operant chambers were outfitted with two nosepoke holes (2.5 cm in diameter), spaced 14 cm apart. White cue lights (3.5 cm in diameter) were located 6.25 cm above the top of each nosepoke portal. A red houselight was located 1 cm below the ceiling of the chamber in the center of the wall containing both nosepoke portals and stimulus lights. Intravenous (i.v.) infusions were delivered via an infusion pump through tubing connected to each animal's catheter. Tubing was protected by a metal casing connected to a swivel system that allowed for virtually unrestricted movement.

Drugs

Nicotine hydrogen tartrate salt (Sigma, St Louis, MO) was dissolved in 0.9% saline. The doses of nicotine available for self-administration were 3, 10, 30, or 100 µg/kg/infusion (reported as free base). All solutions were sterilized by being passed through a 0.22 µm filter. The infusion volume for the present studies was 0.4 mL/kg delivered over 3–4 seconds. This was changed from our standard infusion volume of 0.1 mL/kg delivered over approximately 1 second,[14–16] to ensure that the infusion volume for the adolescent rats was sufficient to fill the catheter. Drug infusion volume and duration were dependent on the body weight of each rat; increases in body weight were accompanied by increases in drug infusion duration and, by extension, volume of infusion.

Procedures

Surgery, Catheter Construction, Catheter Maintenance and Patency Tests

Rats were anesthetized with isoflurane (2%–3% in 100% O2) and implanted with chronic indwelling catheters into the right external jugular vein on P24-25 (adolescent) or P84-85 (adult). Differences between adolescent and adult catheters included the length of the pedestal bolt (14 mm for adolescents and 20 mm for adults; Plastics One), as well as the length of silastic tubing (0.3 mm internal diameter, 0.63 mm external diameter) inserted into the jugular vein, ending in the right atrium of the heart. In adolescent animals, the length was adjusted based on the body weight of each animal: between 1.1 to 1.6 cm for animals weighing between 34 to 62 g, starting with 1.1 cm for rats up to 40 g and then adding 0.1 cm in 5-g intervals. In adults, the tubing was inserted 3.6 cm into the vein, as in previous reports.[14–16]

After surgery, animals recovered in their home cage for 5–6 days. For the first 5 days following surgery, catheters were flushed once daily with 0.1 mL sterile saline containing heparin (3 U), timentin (6.67 mg), and streptokinase (833 U) to prevent infection and maintain catheter patency. After this postsurgical period, catheters were flushed daily with saline solution containing heparin and timentin. Catheter patency was tested following the final self-administration session; a solution of methohexital (Brevital; 5 mg/kg) was infused into the catheter. Animals that did not show signs of ataxia almost immediately upon administration of Brevital were considered to have failed the patency test and were excluded from data analyses. Of the initial 68 adolescent rats, 51 passed the patency test, and of the initial 44 adult rats, 42 passed the patency test.

Self-Administration

Self-administration began on P30 for adolescents and P90 for adults. Animals were assigned to self-administer a single dose of nicotine: 3, 10, 30, or 100 (adolescents only) µg/kg/infusion. As the goal of the study was to compare the threshold dose supporting acquisition, we tested doses lower than the standard dose of 30 µg/kg/infusion, as well as a dose higher than this dose in adolescents, due to uncertainty regarding the threshold dose for self-administration in adolescent

rats. The number of rats included in each group is noted in Table 1. A saline control group was not included because it was expected that the lowest dose tested would not support self-administration, as indicated by a lack of a significant difference in responding on the active versus inactive nosepoke hole. Furthermore, in previous studies using adult male rats, responding for 3 µg/kg/infusion did not differ from responding for vehicle.[16]

All animals were tested on a fixed ratio 2 schedule of reinforcement. Completing two nosepoke responses in the randomly assigned active nosepoke portal resulted in a nicotine infusion paired with a cue-light presentation, which was composed of a 15-second stimulus light presentation above the active portal. In prior studies we have documented that this cue by itself is not reinforcing,[17] unlike the complex visual stimulus we have used in studies of reinforcement enhancing effects of nicotine.[18–23] A 60-second timeout period followed each infusion during which nosepokes had no consequence. Inactive responses were recorded but had no scheduled consequences. Self-administration sessions were 1 hour in length and were conducted daily for 16 sessions. Thus, adolescent rats were P45 and adults P105 at the final session.

For this study, a period of 16 days was chosen to examine the acquisition of nicotine self-administration in both adolescents and adults. Prototypical developmental changes associated with adolescence, including hormonal changes, occur approximately during P28-42 in rats.[24] This age range is approximate and relatively conservative as it certainly differs slightly between males and females, beginning earlier in females, and likely extending later in males.[6,24,25] Based on this age range proposed by Spear,[24] as well as other studies examining adolescent self-administration,[6,11,12] we selected initiation ages to ensure that the period during which rats had access to nicotine fell during adolescence (P30-45) or adulthood (P90-105).

Data Analysis

Data analyses assessed (1) which doses supported targeted reinforced behavior (active responding) in each group, (2) the impact of age, sex, and dose on the number of infusions earned, (3) the impact of age, sex, and dose on the proportion of animals to acquire self-administration and on the rate of acquisition, and (4) the impact of age and sex on responding within the session.

Responding at the active and inactive nosepoke portals in each group during the last three sessions (sessions 14–16) was compared using paired *t* tests to assess which groups supported behavior targeted to the active nosepoke portal rather than a generalized increase in responding. For all other analyses, multi-factor analysis of variance (ANOVA) omnibus tests were conducted, and follow-up log-linear tests (proportion to acquire data) or *t* tests were conducted in the case of significant omnibus tests. Follow-up tests were collapsed across sex when the omnibus test did not reveal any main effects or interactions with sex. To assess the impact of age, sex, and dose on the likelihood of acquiring self-administration, a criterion for self-administration was established: three consecutive sessions of (1) at least five or more infusions and (2) active responding greater than inactive responding, and these two conditions had to be met on at least half of the sessions beginning with the first of the three consecutive sessions satisfying the two conditions.

When Levene's test for equality of variances was significant, the Satterthwaite approximation for degrees of freedom was used. An alpha level of $P < .05$ was used as the cutoff for statistical significance, unless noted otherwise. Statistical analyses were performed using SPSS (version 21).

Table 1. Final Sample Sizes for Each Group

	Final sample sizes			
Nicotine dose (µg/kg/infusion)	3	10	30	100
Adolescent males	3	9	11	4
Adolescent females	4	14	10	4
Adult males	6	8	8	NA
Adult females	4	8	8	NA

These numbers reflect sample sizes with animals that failed the final patency test removed from analyses. NA reflects a condition in which group(s) were not tested.

Results

Active and Inactive Responding Across Sessions

The overall patterns of responding at both the active and inactive nosepoke portals across the 16 daily 1-hour self-administration sessions for each group at each dose are presented in Supplementary Figure S1. Responding at the inactive nosepoke portal was low and did not differ between groups at any dose of nicotine tested. At 30 µg/kg/infusion nicotine, responding in the active nosepoke portal was significantly greater ($P < .05$), and at least two times higher, than responding in the inactive portal regardless of age or sex. At the 10 µg/kg/infusion dose, active responding was greater than inactive responding only for the adult groups ($P < .05$). At the 3 µg/kg/infusion dose there were no significant differences between active and inactive responding at either age or sex. For the 100 dose, which was tested only in a small group of adolescents, active responding was significantly greater than inactive responding in males ($P < .05$) but was a nonsignificant trend in females ($P < .1$; Supplementary Figure S2).

Impact of Age, Sex, and Dose on Number of Infusions Earned

To examine the impact of age and sex on self-administration across a range of doses, the number of infusions earned on the final 3 days of self-administration were analyzed (Figure 1). An omnibus three-factor ANOVA (dose × age × sex) revealed a significant effect of dose (F (2,81) = 20.54, $P < .05$) and age (F (1,81) = 4.36, $P < .05$) but not sex. There was also a significant interaction between dose and age (F (2,81) = 3.44, $P < .05$). Follow-up comparisons conducted at each dose showed that there was a difference between adolescent and adult rats in number of infusions earned only at the 10 µg/kg/infusion dose of nicotine (t (21.35) = 4.08, $P < .05$). Differences between adolescents and adults were not observed at 3 µg/kg/infusion, where self-administration was not observed, or at 30 µg/kg/infusion, a dose that elicited robust self-administration by all groups.

Adolescent rats were also tested at a higher dose of nicotine (100 µg/kg/infusion) to determine whether a larger dose of nicotine might elicit more self-administration. It is known that 30 µg/kg/infusion is at, or near, the peak of the dose-response curve for adult rats.[22,26,27] Across the last 3 days of self-administration at 100 µg/kg/infusion, adolescent male rats earned 6.7 ± 0.7 infusions (n = 4) whereas female rats earned 7.1 ± 0.6 (n = 4). Males and females did not differ, and number of earned infusions for 100 µg/kg/infusion did not differ from that observed with 30 µg/kg/infusion.

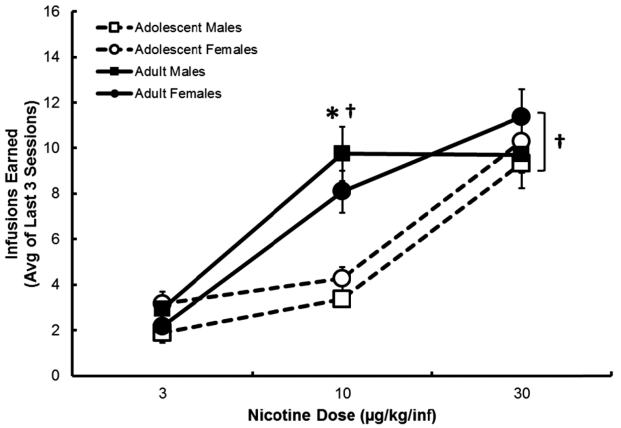

Figure 1. Average earned infusions for all groups across nicotine doses tested. Data points represent the 3-day averages of infusions earned across the last three sessions. Error bars represent standard errors. Significant effect of age is represented by *. Adults earned significantly more infusions than adolescents at the 10 µg/kg/infusion nicotine dose (t (21.35) = 4.08, P < .05).There was also a significant main effect of dose (see text). Significant difference from 3 µg/kg/infusion within each age and sex group is represented by †.

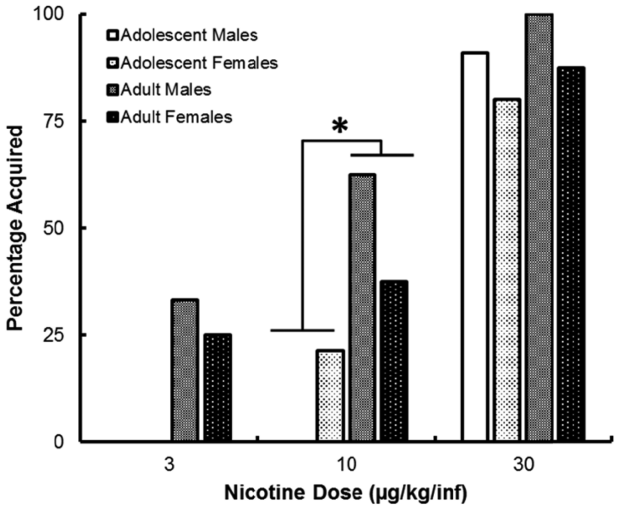

Figure 2. Percentage of rats meeting criterion for self-administration within each group. There was a significant effect of dose on the proportion of rats meeting criterion (G^2(2) = 37.25, P < .05). At the 10 µg/kg/infusion dose, there was a significant effect of age (G^2(1) = 6.4, P < .05 and there was a trend (P < .1) at the 3 µg/kg/infusion dose. Significant effect of age within a dose is represented by *.

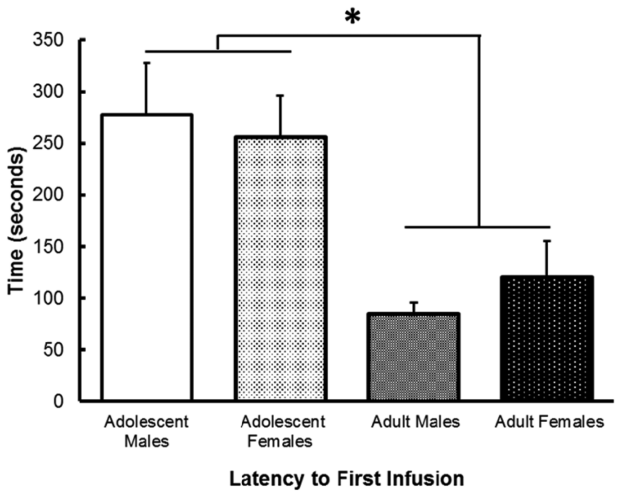

Figure 3. Average latency to the first infusion for the final three sessions. Adolescents took significantly longer than adults to earn the first infusion at the 30 µg/kg/infusion dose (F (1, 33) = 4.50, P < .05; represented by the *).

Impact of Age, Sex, and Dose on the Proportion and Rate to Acquire Self-Administration

A log-linear analysis confirmed that there was a significant effect of dose on the proportion of rats that met criterion for self-administration (G^2(2) = 37.75, P < .05, Figure 2). Follow-up 2 × 2 (age, sex) tests at each dose found no effect of age or sex at 30 µg/kg/infusion, a significant effect of age at 10 µg/kg/infusion (G^2(1) = 6.4, P < .05), and a nonsignificant trend for age at 3 µg/kg/infusion (P < .1).

An omnibus ANOVA did not reveal main effects or interactions of age or sex on number of days to fulfill acquisition criterion (P > .05, data log-transformed for positive skew, analyses only include rats that met criterion for self-administration and only tested in groups where at least a third of the of animals met criterion). A follow-up 2 × 2 ANOVA conducted at 30 µg/kg/infusion did not reveal an effect of age or sex on rate to acquire self-administration (adolescent males: 6.6 ± 0.9 days [n = 10]; adolescent females: 4.5 ± 1.1 days [n = 8]; adult males: 6.6 ± 1.1 days [n = 8]; and adult females: 5.4 ± 1.4 days [n = 7]). In the adult rats that did acquire at the 10 µg/kg/infusion dose, the rate of acquisition was similar to the rate of acquisition at the higher dose (adult males: 5.8 ± 1.8 days [n = 6]; and adult females: 4.3 ± 1.7 days [n = 3]).

Intra-Session Responding

Previous studies have shown that rats regulate their intake of nicotine throughout the period of availability within the self-administration session[17] and that despite there being no apparent differences in overall responding, there may be differences within a session, including the latency to earn the first infusion of the session.[28] Although at 30 µg/kg/infusion nicotine both adolescent and adult rats met the criteria for self-administration and the total number of infusions earned across the 1-hour session was similar in both age groups, there were subtle differences in the intra-session distribution of responses (Supplementary Figure S3). Comparing responding across three 20-minute epochs within a session, a three-factor ANOVA (epoch × age × sex) revealed a significant effect of epoch (F (2,66) = 39.848, P < .05), as well as a significant interaction of epoch × age (F (2,66) = 9.508, P < .05), but there was no three-way interaction, or epoch × sex interaction.

A 2 × 2 ANOVA revealed that the latency to first infusion at 30 µg/kg/infusion was longer in the adolescent rats than adult rats (log transformed for positive skew, F (1,33) = 4.999, P < .05; Figure 3). There was no effect of sex.

Discussion

The goal of this study was to examine adolescent nicotine self-administration in comparison to adults, as well as determine whether sex affected self-administration in either age group. Specifically, this study set out to compare nicotine self-administration between adolescent and adult rats, especially at low doses of nicotine. The main

finding of this study is that at a low dose of nicotine, adult male and female rats earn more infusions than adolescent males and females. Furthermore, the lowest nicotine dose supporting self-administration in the majority of adults was 10 µg/kg/infusion, but it was 30 µg/kg/infusion for the adolescent rats, suggesting that the threshold dose of nicotine supporting self-administration may be higher during adolescence. This difference in the number of nicotine infusions earned between adolescents and adults has important implications for tobacco regulation policy. Human smokers predominantly begin smoking during adolescence; however, most studies utilizing an animal model of nicotine self-administration use adult male animals. As potential nicotine standards for tobacco products are considered under the Family Smoking Prevention and Tobacco Control Act, it will be essential to understand how information generated from adult smokers will apply to adolescents. We previously reported that in adult rats, the threshold dose of nicotine required to maintain self-administration is similar to the threshold dose that supports acquisition of self-administration.[16] Thus, based on the present findings, it would appear that if a nicotine standard was set below the threshold nicotine content established in studies on adult cigarette smokers, this standard would fall below the threshold for adolescents as well. Even at doses that might be around or just above threshold, adolescents will self-administer less than adults.

In addition to the differences between adolescent and adult rats in responding for low doses of nicotine, the present studies also demonstrate that at a suprathreshold dose of nicotine adolescent and adult rats self-administer a similar number of infusions. A dose of 100 µg/kg/infusion nicotine was also tested in adolescent rats to determine whether this dose, which is on the descending portion of the dose-response curve in adults, might still be on an ascending portion of the dose-response curve. Adolescents self-administering at this high dose did not earn more infusions compared to the 30 µg/kg/infusion nicotine dose. However, adult animals were not tested at this dose here, so direct comparisons between developmental stages cannot be made. Future studies will need to examine the differences, if any, between adults and adolescent animals at high doses of nicotine.

Although adolescents and adults earned a similar number of infusions across the hour-long self-administration session at the above-threshold dose of 30 µg/kg/infusion, there were subtle, but potentially important, differences in the pattern of when the infusions were earned (Figure 3 and Supplementary Figure S3). In particular, the adolescent rats, independent of sex, took more than twice as long to earn the first infusion of the session. In human smokers, the time to first cigarette in the morning is often taken as the best measure of nicotine dependence.[29] Interestingly, as we have observed with latency in the present experiment, time to first cigarette is longer in adolescent smokers than adult smokers.[30,31] Furthermore, previous studies have shown that in adult rats, shorter latency to first nicotine infusion is correlated with higher breakpoint on a progressive ratio schedule,[28] and thus, like time to first cigarette in human smokers, latency to first infusion might be a good measure of motivation to obtain nicotine.

Developmental differences in nicotine metabolism could be a source of difference in the threshold dose of nicotine that supports initiation of self-administration behavior seen here. Vieira-Brock et al.[32] and Craig et al.[33] found that following a subcutaneous injection of nicotine, blood and brain nicotine levels were lower in adolescent male rats compared to adult male rats. Similarly, following an intravenous injection Craig et al.[33] observed that although the initial peak in blood nicotine levels did not differ between adolescents and adults, the levels declined more rapidly in adolescents. These authors interpreted their data as reflecting both a larger volume of distribution and a higher plasma clearance in adolescents. Therefore, significant differences in nicotine pharmacokinetics across development may influence the dose at which nicotine is able to exert primary reinforcing effects, as well as impact the amount of nicotine self-administered. In this context, it is worth noting that humans or animals with higher rates of nicotine clearance tend to have higher rates of nicotine consumption,[34,35] and therefore at doses of nicotine above the threshold for self-administration it might be expected that adolescent rats would self-administer more nicotine if clearance rate was the principal difference between age groups. However, this was not observed in the present study.

Several important technical differences from previously published studies must be considered when evaluating these data and placing them in the context of the existing literature. One important methodological issue is prior operant training. In order to study acquisition of nicotine self-administration, it is important to avoid pre-training with sucrose or food, common in other adolescent studies of nicotine self-administration,[4,5,9–11] as well as priming injections of nicotine at the start of self-administration sessions.[6] Conditioning the operant as a reinforcer will likely alter behavior when nicotine is subsequently paired with that operant.[19,36] To complicate interpretations further, nicotine has previously been shown to enhance motivation to obtain food[37] and sucrose.[38] Thus, although some prior studies have observed nicotine self-administration in adolescent rats at lower doses of nicotine than observed in the present study (eg, Lynch[6] and Li et al.[5]), these studies all have used prior operant training with other reinforcers. Shram et al.[12] did not use prior training and their findings are similar to those reported here.

Another technical issue important for these studies comparing adolescent and adult rats is the feeding status of the rats. Most self-administration studies in adult rats use rats that are mildly food-restricted, as this seems to increase motivated behavior in operant procedures.[39] However, it is problematic to equate food restriction between adult rats, which have a stable daily food intake, and adolescent rats, whose food intake is increasing along with their rapid growth. Furthermore, restricting food availability in adolescent rats may impact growth and developmental changes.[24] Therefore, in the present studies, we avoided food restriction and rats had *ad libitum* access to food except during the daily 1-hour self-administration sessions, as was the case in some other studies in adolescent rats.[3,5,6,12]

The adolescent period in rats is brief, and we chose to begin our studies in early adolescence to provide for 16 days of self-administration that are unambiguously within the adolescent period. It is conceivable that results might have been different if self-administration was initiated later in the adolescent period. Levin et al.[4,10] tested adolescent rats starting at different ages, but the procedure in those studies was complicated by prior food training and the failure of self-administration to be maintained, making it difficult to draw conclusions from those studies. Nevertheless, Levin et al.[4,10] found that self-administration tended to be greater at the earlier adolescent ages.

There are many aspects of the self-administration procedure itself that vary between studies. Daily 1-hour sessions were used in the present studies, whereas some prior studies used longer sessions, including 23-hour access.[3,6,7] Sessions of 1 hour are clearly sufficient to demonstrate the primary reinforcing properties of nicotine,[19,36] though they fail to model the effects of more continuous exposure to nicotine.[40] Studies examining acquisition of self-administration have typically utilized low fixed ratio schedules (FR1–FR3), but some have tested progressive ratio (PR) schedules as well.[5,6] The strain of

rat (and even supplier) may also be important; Sprague-Dawley rats were used in the present studies, whereas previous studies have used other strains, such as Long-Evans rats or Lewis rats. Shram et al.[12] used both Wistar and Long-Evans rats and reached conclusions similar to those presented here.

The present study employed the use of a cue light in the self-administration procedure that is, by itself, not reinforcing, at least initially, as we have shown previously[19] and confirmed in the present study by the lack of discriminative responding at the lowest dose of nicotine in both adolescents and adults. Previous studies have documented that when nicotine is delivered along with other mildly reinforcing cues, nicotine enhances responding for those cues.[19,22,36] This reinforcement-enhancing action of nicotine has been shown in adolescent rats.[41] Thus, using cues that might be mildly reinforcing, such as the cues used in many of the prior nicotine self-administration studies in adolescent rats (especially if the cues were paired with other reinforcers during training),[4,9–11] will not provide clear information on the primary reinforcing actions of nicotine, but rather have this action confounded with the reinforcement-enhancing action of nicotine. Although the reinforcement-enhancing actions of nicotine may contribute to nicotine use in adolescents, it is important to understand the roles of the different actions of nicotine in driving behavior and the present studies indicate that adolescent rats are less sensitive to the primary reinforcing actions of nicotine than are adults.

Another variable is how the adolescent rats are obtained.[42] Most studies, including the current one, used rats that were shipped from suppliers in early adolescence, typically right after weaning. This might be stressful and possibly contribute to the results. Other studies have used pregnant rats shipped from suppliers, with the rats then being born "in house"; shipping of the pregnant rats may provide intrauterine stress and also contribute to the results obtained. This was the approach taken by Shram et al.,[12] and their results were comparable to the present results. Although the ideal procedure might be an in-house breeding program, we are not aware of any comparable nicotine self-administration studies that have used adolescent rats that did not involve shipping the animals (Natividad et al.[7] did use rats bred in house, but the design and goals of that study were quite different.). Regarding shipping, a recent study,[43] found that rats shipped from a supplier on P21 and rats bred in house showed a similar profile of activity in a novel environment when tested on P23/24. Therefore at present, there is no reason to expect that the manner in which the adolescent rats are acquired (eg, shipping or bred in house) would alter the conclusions of the present studies.

We did not observe any differences between males and females either in adolescence or adulthood. Several previous studies have compared nicotine self-administration between adolescent male and female rats, with mixed results. Using a 23-hour access procedure in Lewis rats starting self-administration on P43-45, Chen et al.[3] noted that 7.5 µg/kg/infusion was below threshold for groups of males and females, but higher doses (15 and 30 µg/kg/infusion) produced a similar extent of self-administration behavior in both sexes, similar to what was observed in the present study. However, in contrast to the present study, Chen et al.[3] found that adolescent females responded more than adult females at 30 µg/kg/infusion. Lynch[6] found that females more readily acquired self-administration than males at 5 µg/kg/infusion whereas both sexes showed similar self-administration behavior at 10 µg/kg/infusion. Levin et al.[10] compared adolescent male and female rats that began daily (5 days/week) 45-minute self-administration sessions for 30 µg/kg/infusion beginning at 4, 5, 6, 7, or 8 weeks of age. The complicated dataset showed that significant self-administration occurred during only the first week of sessions at each age, and during that week responding was higher in males compared to females. It is also noteworthy that in the study by Levin et al.,[10] during lever training for food rewards, males earned ~40% more food pellets than females. Li et al.[5] studied adolescent Long-Evans rats of both sexes self-administering 7.5, 15, or 30 µg/kg/infusion on an FR1 followed by FR2 schedule. There were no differences in infusions earned between males and females at any of the doses tested. However, on a subsequent progressive ratio schedule, females tended to earn more infusions than males. Thus, the lack of a difference between adolescent male and female rats in the present study, and a threshold greater than 10 µg/kg/infusion, is in line with the existing literature given the methodological differences across studies. Similarly, our lack of a male-female difference in adult rats is consistent with prior nicotine self-administration studies using low fixed ratio schedules and a neutral stimulus.[44]

The observations in the present study that adolescent rats respond less for a low dose of nicotine than do adult rats, but that the maximal level of responding is similar in adolescent and adult rats at higher doses, appear to be at odds with several previous studies examining nicotine conditioned place preference (CPP).[45–55] These studies generally found that nicotine CPP is observed with lower doses of nicotine in adolescent rats and mice compared to adults. Furthermore, the magnitude of CPP elicited by nicotine is either larger in adolescents compared to adults or similar across ages. Several differences between self-administration and CPP in testing the reinforcing properties of drugs may help explain these discordant data. Among important differences in the two approaches are that self-administration assesses responding for a drug, whereas CPP assesses a preference to be at a site of prior drug injection not linked to an animal's behavior and tested when the animal is drug-free. When tested in a biased design (where drug is paired with the previously nonpreferred chamber), which is the case in most studies examining nicotine CPP, an apparent CPP might reflect an attenuated aversion of the nonpreferred chamber rather than an increased preference of the unconditionally preferred chamber. An attenuation of aversion might occur with an anxiolytic agent, and some studies have suggested that such attenuation may be the explanation of enhanced nicotine CPP in adolescents.[53,55] Even when the design is unbiased, an observed nicotine CPP may be a function of the subset of animals for which nicotine is paired with the nonpreferred chamber.[56] It is important to note that, generally, self-administration is considered to have greater validity as a measure of drug use[40] and CPP is not a measure of drug reinforcement. Ideally, self-administration and CPP experiments would provide converging evidence, but in instances of discordant results, self-administration should be viewed as a better measure of drug reinforcement.

The FDA has authority to establish tobacco product standards to improve public health by reducing the use of tobacco products and one potential strategy is to establish a maximal allowable nicotine level for cigarettes below the threshold required to maintain smoking. Thus, understanding what this threshold may be and the factors that can influence it is critically important.[26,57] A threshold for nicotine reinforcement in humans can only be determined in studies reducing nicotine levels in current cigarette smokers[58]; acquisition studies such as the present one cannot be done in humans, and

therefore it is essential that studies in experimental animals fill this gap. The current study provides important new information regarding how threshold doses supporting nicotine self-administration compare between adolescent and adult rats. The results provide a valuable confirmation of the results of Shram et al.,[11] and extend the previous work to include both male and female rats and use a more naturalistic operant behavior for rats.

Conclusions

The present study demonstrates that the dose-response curve for the acquisition of nicotine self-administration in adolescent male and female Sprague-Dawley rats is shifted to the right compared to adults. The higher threshold dose of nicotine needed to support the acquisition of self-administration in adolescent rats provides evidence that the primary reinforcing properties of nicotine self-administration are reduced compared to adults. These results are important in the context of potential nicotine regulatory policy, as they support the suggestion that setting nicotine regulatory standards based on doses of nicotine that reduce self-administration in adults may also reduce use in adolescents. However, the role of cues and other rewards in sustaining nicotine use in adolescents will be important to investigate in the future, as they may support behavior at low doses of nicotine.

Supplementary Material

Supplementary Figures S1–S3 can be found online at http://www.ntr.oxfordjournals.org

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Declaration of Interests

Portions of these data were presented at the annual meeting of the Society for Nicotine and Tobacco Research, Philadelphia, PA, February 25–28, 2015.

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