Psychopharmacology (2003) 169:141–149
DOI 10.1007/s00213-003-1486-y

ORIGINAL INVESTIGATION

Edward D. Levin · Amir H. Rezvani · Daniel Montoya · Jed E. Rose · H. Scott Swartzwelder

Adolescent-onset nicotine self-administration modeled in female rats

Received: 18 December 2001 / Accepted: 16 March 2003 / Published online: 23 May 2003

Abstract *Rationale:* Although the great majority of tobacco addiction begins during adolescence, little is known about differential nicotine effects in adolescents versus adults. *Objectives:* A rat model was used to determine the impact of the age of onset on nicotine self-administration. *Methods:* In expt 1, nicotine self-administration of female Sprague-Dawley rats over a range of acute doses (0.01–0.08 mg/kg per infusion) was determined in adolescent (beginning at 54–62 days) versus adult (beginning at 84–90 days). In expt 2, chronic nicotine self-administration over 4 weeks from adolescence into adulthood was compared with the chronic self-administration beginning in adulthood. In expt 3, adolescent-adult differences in nicotine effects on body temperature and locomotor responses were determined. *Results:* Adolescent-onset rats showed a significant main effect of increased nicotine intake compared with adult-onset rats in an eight-fold range of acute unit doses/infusion. Significant age differences were also seen in the chronic level of nicotine self-administration. Over 4 weeks, the adolescent-onset group had nearly double the rate of nicotine self-administration of the benchmark nicotine dose (0.03 mg/kg per infusion) compared to the adult-onset group. This increased nicotine intake persisted into adulthood. Adolescent rats had significantly greater response than adults to the hypothermic effects of nicotine, but had significantly less response than adults to the reduction in locomotor activity seen after nicotine. *Conclusions:* Adolescent-onset nicotine self-administration in female rats was associated with significantly higher levels of nicotine self-administration versus rats, which began nicotine self-administration in adulthood. This greater self-administration persists into adulthood and may underlie greater propensity of adolescents to nicotine addiction.

Keywords Nicotine · Addiction · Self-administration · Adolescence

E. D. Levin (✉) · A. H. Rezvani · D. Montoya · J. E. Rose · H. S. Swartzwelder
Neurobehavioral Research Laboratory, Department of Psychiatry, Duke University Medical Center,
341 Bell Building, Box #3412, Durham, NC 27710, USA
e-mail: edlevin@duke.edu
Tel.: +1-919-6816273
Fax: +1-919-6813416

Introduction

Adolescence is the final period of neurodevelopment. It is also quite often the initial period of drug self-administration, often starting with tobacco use. In contrast to the great body of literature concerning persisting effects of prenatal nicotine exposure, there has been little research on the persisting effects of nicotine exposure during adolescence (Eissenberg and Balster 2000). Given the prevalence of teenage smoking and the critical nature of adolescence for the final phases of neurodevelopment, it is important to begin characterizing persisting neurobehavioral effects of adolescent nicotine exposure. The causative relationship of beginning nicotine self-administration during adolescence to the liability to addiction is difficult to determine in humans. The same genetic and environmental factors that promote nicotine addiction may also cause people to start smoking earlier. Randomized experimental studies to determine the effect of starting nicotine use at different ages cannot be ethically conducted in humans.

The great majority of tobacco use begins during adolescence, and smokers who start during adolescence are more likely to be life-long smokers than those who start in adulthood (Rigotti 1990; Centers for Disease Control and Prevention 1991; US Public Health Service 1994). The earlier a person starts, the more likely that person is to become a heavier smoker (Everett et al. 1999). Eighty-eight percent of current smokers in the USA smoked their first cigarette before age 18, 60% before age 14 (Glynn et al. 1993; US Public Health Service 1994), and 11% by age 10 (Everett et al. 1999). Despite the fact that it is illegal to sell tobacco to people under the age of 18 in the USA, the fact remains that

adolescents do obtain it. Also, it is legal to sell tobacco to adolescents of younger ages in other countries. It has been found in a recent study in Spain that early initiation to smoking was positively correlated with higher intake later in adulthood (Fernandez et al. 1999). Adolescent smokers may have a limited history of nicotine use, but they do take in physiologically active doses of nicotine and show defined withdrawal effects upon cessation, demonstrating the importance of nicotine in the maintenance of smoking at an early age even when smoking is intermittent (McNeill et al. 1986; Corrigall et al. 2001; Zack et al. 2001). Clearly, adolescence is the period when most people begin smoking. Nicotine effects during adolescence can be critical in determining the tenacity of addiction through the rest of life (Eissenberg and Balster 2000).

Adolescent nicotine effects may critically differ from those in adulthood because of important late phase neural development during adolescence (Spear 2000b). Adolescence is defined as the period beginning with the onset of sexual development and ending when full adult size is attained (Dorland 1994). In humans, this includes the teenage years, while in the rat this period lasts from approximately 4 weeks of age when signs of sexual development begin. The end point of adolescence in humans and animal models is a matter of controversy and most likely depends on the function under consideration (Spear 2000b). We have used a working definition of adolescence extending from the onset of sexual maturation to the attainment of full adult body size. Adolescent-onset drug self-administration can be easily modeled in the rat as demonstrated by Spear's recent work with ethanol (Spear 2000a).

There have been recent studies that associate an increased likelihood of sustained smoking with adolescent-onset nicotine use (Eissenberg and Balster 2000; Pomerleau and Pomerleau 2000), suggesting that if smoking begins during adolescence, it may be more addictive than if it begins in adulthood. However, this hypothesis cannot be assessed in humans because of self-selection bias and ethical constraints. Self-selection bias occurs because the factors that make some people more prone to nicotine addiction may also promote its initial use during childhood or adolescence. Ethical constraints make it impossible to conduct randomized assignment of human adolescents to nicotine self-administration and control conditions. Rat models can obviate these problems. The reinforcing effects of nicotine have been successfully modeled in the rat self-administration procedure. Nicotine self-administration research in rats has become quite active in recent years, since the work of Corrigall and co-workers demonstrating nicotine self-administration in rats (Corrigall and Coen 1989; Corrigall 1992; Corrigall et al. 2000). Rats will work to obtain nicotine delivery. This finding has now been replicated in other laboratories as well (Donny et al. 1995; Shoaib et al. 1997). Adriani et al. (2002) found that during early adolescence (post-natal days 24–35) mice showed a marked preference for drinking a nicotine-laced solution and showed nicotine-induced hyperactivity compared to older mice. However, a role for taste factors cannot be ruled out. Most of the earlier work has examined nicotine self-administration in male rodents. However, there are considerable problems with smoking addiction in females. Thus, the current study focused on the understudied aspects of nicotine self-administration in female rats. The rat model can be used to determine the causative action of starting nicotine self-administration during adolescence on control over nicotine intake with random assignment of adolescent and adult subjects to nicotine self-administration and control procedures. The current studies used the rat model of nicotine self-administration to compare in females the effect of adolescent-onset versus adult-onset nicotine self-administration on the maintenance of nicotine intake.

Materials and methods

Subjects

Female Sprague-Dawley strain albino rats (Zivic-Miller, Allison Park, Pa., USA) were used in these studies. They were singly housed in approved standard laboratory conditions in a Duke University Vivarium facility near the testing room to minimize any stress induced by transporting the rats. They were kept on a 12:12 reverse day:night cycle so that they were in their active phase during behavioral testing. The procedures used in this study were approved by the Duke University Animal Care and Use Committee and conform to the 1996 edition of the Animal Care Guide.

Drug preparation and administration

Solutions of nicotine ditartrate were prepared weekly in pyrogen-free glassware in sterilized isotonic saline. The doses used were calculated as a function of the nicotine base weight. The pH of the solutions was adjusted to 7.0 using NaOH and then the solutions were passed through a 0.22 μm filter (Millipore Crop). All solutions were kept refrigerated in the dark between experiments.

Drug self-administration

Adolescent and adult female Sprague-Dawley rats were implanted with chronically indwelling intravenous jugular catheters at approximately 30 or 60 days of age. Rats were individually housed in a colony maintained at 22°C with a reverse 12-h light-dark cycle (lights on from 1800 to 0600 hours). Adolescent and adult rats were received from the supplier at the same time so that the period of time in our colony was equal. The rats arrived fitted with jugular catheters by the supplier. The rats were trained in IV self-administration method we have found to be effective in other sets of rats for cocaine self-administration (Levin et al. 2000). Catheters were flushed daily with a 0.3 ml solution containing 25 IU/ml heparinized saline and 0.4 mg Gentamicin as an antibiotic. Twenty-four hours after arrival, rats were handled for 8 min several times per day for 3 consecutive days. During this time, the rats had unrestricted access to water, and were fed approximately 20 g of chow daily. Following this 3-day acclimation period, behavioral training began and food consumption was restricted to approximately 16 g of chow per day to maintain the rats at approximately 85% of their free-feeding weights adjusted for growth. The rats were fed fixed amounts of food each day to control their weight. The quick onset of testing was necessary to fit onset of nicotine self-administration within the adolescent period.

Table 1 Nicotine doses for self-administration in expt 2

Weeks	1					2					3					4				
Days	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg per infusion)	0.03		0.01 or 0.09			0.03		0.01 or 0.09			0.03		0.01 or 0.09			0.03		0.01 or 0.09		

For behavioral training, rats were placed in dual lever test chambers (Med Associates, Vt., USA). Each chamber was equipped with a tone generator, house light, cue light above each lever, and a metal tether to cover the drug delivery line. A Pentium computer programmed with MED-PC software controlled experimental events and data collection. Each catheter was connected to a High Speed Micro-Liter Syringe Pump (Med Associates) with polyethylene tubing and was fitted with a blunt edged 23-gauge needle. During each session, the rats wore jackets (Lomir Biomedical Inc., Quebec, Canada) to connect them to the tethers and to prevent chewing of the drug delivery lines.

Initially, the rats were trained daily to press the levers on an FR1 schedule for food pellet reinforcers. Either the right or left lever was designated active for each rat such that half the animals were reinforced for responding on the right lever and half for responding on the left. The cue light over the active lever was illuminated to indicate which side was correct. Responses on the active lever resulted in the immediate delivery of one 45-mg food pellet and activation of the feedback tone for 0.5 s. Each session lasted for one hour. Cessation of lever press training occurred when the rat has pressed the active lever a minimum of 80 times for food pellet reinforcers during three consecutive sessions.

Following the lever press training for food reinforcers (three to four sessions), rats began nicotine self-administration. No nicotine priming injections were given. For three to four sessions nicotine (0.03 mg/kg per infusion) was paired with delivery of food reinforcers. Then only nicotine was given as a reinforcer. A lever press on the active side resulted in the activation of the feedback tone for 0.5 s, the immediate delivery of one 50-µl infusion of nicotine in less than 1 s. Each infusion was immediately followed by a one-minute timeout in which the house and cue lights went out and responses were recorded but not reinforced. The adolescent rats started nicotine self-administration at 40–46 days of age and the adults at 70–76 days of age. Two levers were available to be pressed in every session of nicotine self-administration. Only one caused the delivery of nicotine the other did not and served as a control.

Statistical analysis

The self-administration data were assessed by a mixed between and within subjects design analysis of variance. The between subjects factor was age of onset of nicotine self-administration (adolescent and adult) and the within subjects factors were repeated sessions of testing or nicotine infusion dose. Testing cohort was used as a control factor for different batches of rats tested at different times. Significant interactions were followed-up by tests of the simple main effects. An alpha level of $P<0.05$ was used as a cutoff for statistical significance.

Experiment 1: acute dose-response

First, the rats (adolescent onset $n=8$, adult onset $n=7$) underwent two weeks of training for nicotine self-administration at the benchmark dose 0.03 mg/kg per infusion. Then, the rats underwent a randomized infusion dose-response study with the following doses of nicotine: of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 and 0.08 mg/kg per infusion. Each dose was tested on a single day in expt 1 without return to baseline. Chronic changes in dose were assessed in expt 2 (see below). The rats were given each dose in one-hour sessions similar to baseline training, whereby pressing the active lever resulted in the activation of the feedback tone for 0.5 s, and the infusion of nicotine (in <1 s), without any food pellet reinforcers. Each infusion (FR1) was followed by a one-minute timeout. The dose-response assessment began when the adolescent-onset rats were 54–62 days of age and the adult-onset rats were 84–90 days of age. These ages were chosen because during ages 54–62 days female rats typically begin sexual maturity but have not yet attained full adult body size similar to human adolescents and by days 84–90 they have nearly attained their full adult body size in young adulthood.

Experiment 2: chronic nicotine self-administration

As in the previous experiment other sets of rats of the same age ranges were trained to self-administer nicotine beginning in adolescence ($n=13$) or adulthood ($n=7$). After initial acquisition, they were tested for chronic nicotine self-administration over a period of four weeks at a rate of five sessions per week. At the beginning of the chronic nicotine self-administration phase of the study the adolescent-onset rats were 50–62 days old and the adult-onset rats were 80–85 days old. During the first 2 days of each week the rats self-administered nicotine at the standard dose of 0.03 mg/kg per infusion. On days 3–5 of each week, the rats were switched to nicotine doses of either 0.01 or 0.09 mg/kg per infusion (Table 1).

Half of the rats in each age group were switched to 0.01 mg/kg per infusion first and the other half to 0.09 mg/kg per infusion first. On days 3–5 of the second week, the doses were reversed. On days 3–5 of the third and fourth weeks, the order of infusion of 0.01 and 0.09 mg/kg per infusion were reversed, such that each rat self-administered the higher and lower nicotine doses in an ABBA or BAAB order. The chronic self-administration of the benchmark 0.03 mg/kg per infusion was analyzed with 2-week block as a repeated measure. Because of the different design of the presentation of the 0.01 and 0.09 mg/kg per infusion switch doses in the later part of each week, they were assessed in a separate repeated measures analysis with both 2-week block and dose as repeated measures.

Experiment 3: adolescent-adult differences in non-conditioned response to nicotine: body temperature and locomotor activity

In the body temperature study, we examined the effects of acute nicotine ditartrate on body temperature in adolescent (36 day old, $n=6$) and adult female rats (91 day old, $n=10$). Nicotine ditartrate (0, 0.05 and 0.10 mg/kg) was injected SC in a repeated measures counterbalanced design with at least 2 days between doses. Colonic temperature was measured by a rectal thermoprobe (Physitemp Instruments Inc. Clifton, N.J., USA) lubricated with dibucaine (Parke Davis) connected to a digital thermometer. The sensor is at the end of a flexible plastic cord (RET-1) that is coated with lubricant and inserted approximately 2 cm into rectum of the rat. The temperature was taken 30 s after insertion, 10 min after nicotine or vehicle (saline) administration.

In the locomotor activity study, we gave drug naive adolescent female Sprague-Dawley rats 0 or 0.2 mg/kg nicotine ditartrate salt SC (20 min before testing) and assessed for locomotor activity in the figure-8 maze over the period of 1 h. There were nine rats in each of four groups (2 ages×2 nicotine doses). The maze consisted of a continuous enclosed alley 10 cm×10 cm in the shape of an 8, which was 70 cm long and 42 cm wide. There was a central arena

21 cm×16 cm with a ceiling 20 cm high with two blind alleys extending 20 cm from either side. Eight photobeams crossed the maze alleys to index locomotor activity. One was located on each of the two blind alleys and three on each of the two loops of the figure-8. There was no prehabituation to the environment before injections, so that nicotine effects on the exploration of the environment could be determined. The number of photobeam breaks in each 5-min block in a 1-h session were tallied by a microcomputer. The mean, linear and quadratic trends across twelve 5-min time periods in the 1-h test session were analyzed.

Results

Experiment 1: acute dose-response

During the training sessions with 0.03 mg/kg per infusion in which nicotine was given along with food reward there was no significant difference (P=0.42) in the number of reinforcers earned by the adolescent and adult rats. The adolescents self-administered 19.2±4.4 (mean±SEM) reinforcers while the adults self-administered 16.7±4.5. After removing delivery of food reinforcers, the adolescents self-administered nicotine significantly [$F(1,7)$=11.76, P<0.025] more than the adults. The adolescents self-administered 13.3±3.4 nicotine infusions/session, while the adults self-administered only 8.2±1.7 nicotine infusions/session (Fig. 1). It is possible that the adolescent rats showed a slower extinction to the withdrawal of food reinforcement than adults and this accounted for the greater self-administration of nicotine alone. However, in this experiment and in expt 2 (see below) the significant increase in nicotine self-administration persisted in the adolescent-onset rats relative to adult-onset rats far beyond the withdrawal of food reinforcement.

The increase of nicotine self-administration in the adolescent-onset group continued during the subsequent dose-response test (0.01–0.08 mg/kg per infusion). Overall, as a main effect of age, the adolescent-onset group self-administered (10.4±2.6 infusions/session) significantly [$F(1,7)$=7.78, P<0.05] more nicotine infusions per session than the adult-onset group (7.5±1.3 infusions/session). The total dose of nicotine self-administered per session was also significantly [$F(1,7)$=9.27, P<0.025] higher in the adolescent-onset rats (0.467±0.106 mg/kg per session) than in the adult-onset nicotine self-administering rats (0.339±0.061 mg/kg per session). The main effect of infusion dose was highly significant [$F(7,49)$=14.82, P<0.0001] with increasing total amount of nicotine self-administered per session with increasing nicotine doses per infusion. There was not a significant age×nicotine dose interaction. Further investigation will be necessary to determine possible age-related differences in the reaction to changes in unit dose of nicotine infusion.

Experiment 2: chronic nicotine self-administration

The adolescent-onset nicotine self-administration paradigm was then used to determine the persistent effect on chronic nicotine self-administration as the animals grew into adulthood. As shown in Fig. 2, starting nicotine self-administration in adolescence versus starting in adulthood caused significantly greater amount of chronic four-week nicotine administration [$F(1,18)$=5.17, P<0.05]. The increased nicotine self-administration in adolescent-onset versus adult-onset rats persisted throughout the four weeks of the test (Fig. 3). By the end of this period the adolescent-onset rats were themselves adults. At the end of the chronic exposure the adolescent-onset group was 82 days old and the adult-onset group was 112 days old. Yet the significantly increased nicotine self-administration persisted into adulthood.

Each week of the chronic 4-week study began with 2 days of testing at the benchmark 0.03 mg/kg per infusion

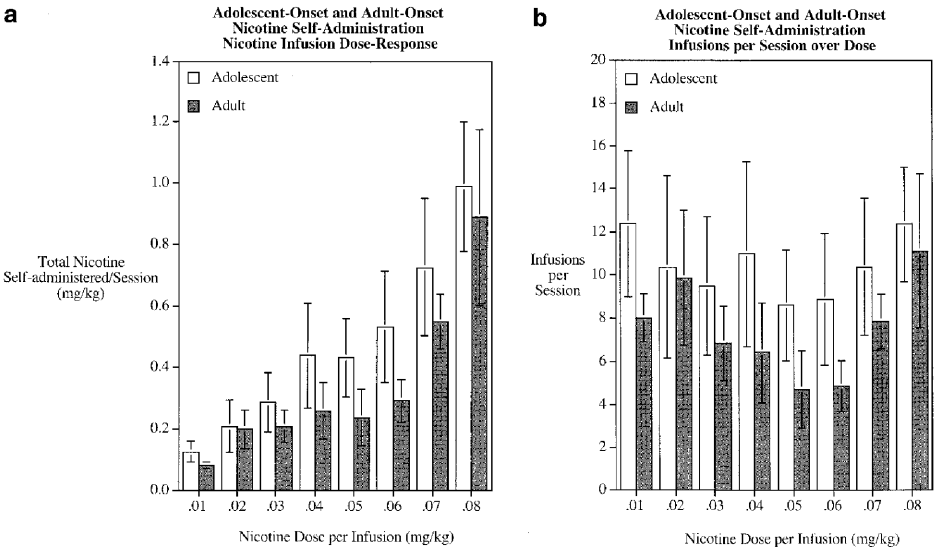

Fig. 1a, b Titration of total nicotine dose with acutely increasing unit dose with adolescent-onset and adult-onset nicotine self-administration (mean±SEM). **a** Amount of nicotine infused per session (n=7–8 per age group, main effect of age P<0.025). **b** Number of infusions per session. The dose-response assessment began when the adolescent-onset rats were 54–62 days of age and the adult-onset rats were 84–90 days of age (n=7–8 per age group, main effect of age P<0.05)

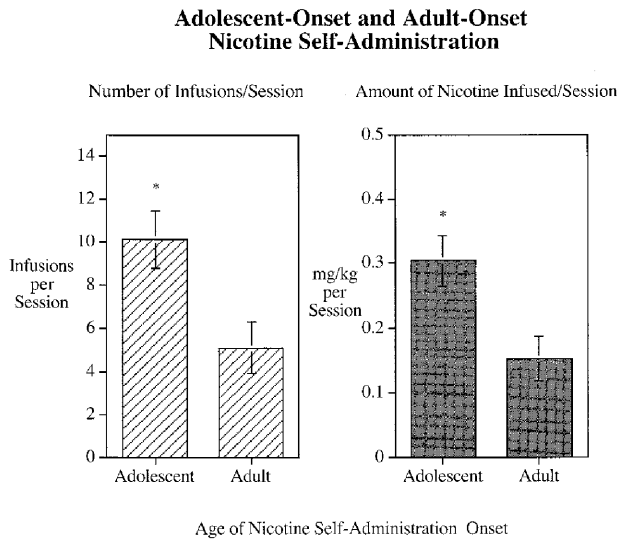

Fig. 2 Infusion rate and total amount of nicotine infused per session with chronic 4-week adolescent-onset and adult-onset nicotine self-administration (mean±SEM). Adolescent onset: days 54–82; adult onset: days 84–112 (*P<0.05 adolescent onset versus adult onset)

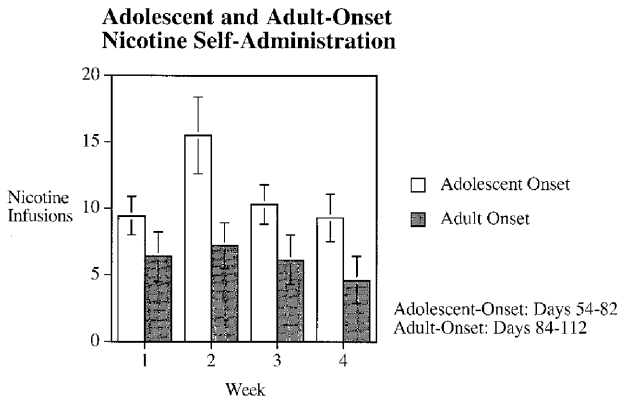

Fig. 3 Infusion rate per session of 0.03 mg/kg per infusion of nicotine for each week over the 4-week chronic adolescent-onset and adult-onset nicotine self-administration (mean±SEM). Adolescent onset: days 54–82; adult onset: days 84–112

dose. The switch to either low (0.01 mg/kg per infusion) or high (0.09 mg/kg per infusion) doses during the later part of each of the four weeks of testing slightly increased the response rate in the adult-onset rats such that a significant age-related difference was not seen. The mean number of nicotine infusions per session in these latter sessions each week for the adolescent-onset rats was 10.2±0.8 (mean±SEM) and for the adult-onset rats was 8.6±1.6.

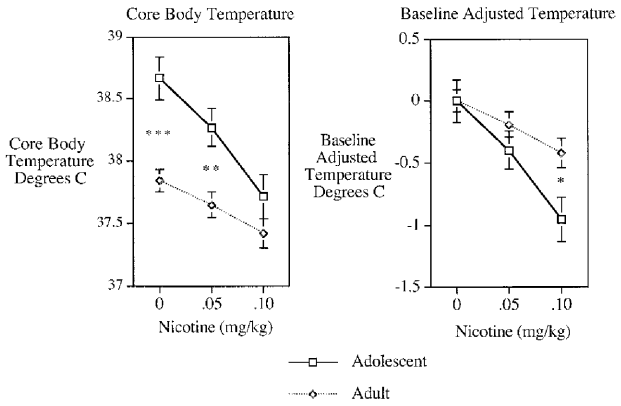

Fig. 4 Hypothermic effects of acute 0.05 and 0.1 mg/kg nicotine administration (mean±SEM) 10 min after SC injection in adolescent and adult rats (*P<0.025, **P<0.005, ***P<0.0005, adolescent versus adult)

Experiment 3: adolescent-adult differences in non-conditioned response to nicotine: body temperature and locomotor activity

Body temperature

In an acute nicotine study with adolescent and adult female Sprague-Dawley rats we found that SC injections of nicotine ditartrate caused a significant dose-related decrease in body temperature. There was a significant overall nicotine-induced reduction in body temperature (P<0.001), but the adolescents (n=6) showed a greater decline than the adults (n=10). With injection of saline (P<0.0005) or the low 0.05 mg/kg dose of nicotine (P<0.005) the adolescents had a significantly higher temperature than the adults. With the 0.1 mg/kg dose they had body temperature reduced to adult levels (Fig. 4). Because there was a significant baseline difference in temperature between adolescents and adults without nicotine treatment, an additional analysis was conducted on the nicotine-induced temperature differences from the mean temperature for each age group after vehicle injections. As shown in Fig. 4, even when the temperature scores were adjusted for differences in baseline temperature, there was a significantly (P<0.025) greater hypothermic effect of nicotine in the adolescent compared with the adult rats.

Locomotor activity

Drug naive adolescent (36 day old) and adult (91 day old) female Sprague-Dawley rats were administered 0 or 0.2 mg/kg nicotine ditartrate SC (20 min before testing) and assessed for locomotor activity in the figure-8 maze over the period of 1 h. There were nine rats in each group

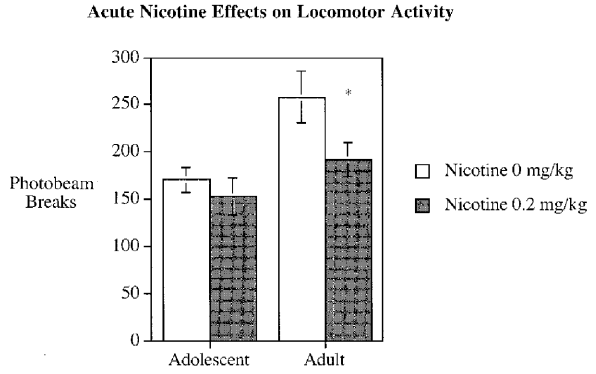

Fig. 5 Locomotor activity after an initial acute dose of 0.2 mg/kg nicotine versus vehicle saline control adolescent and adult rats (mean±SEM). *Open bars* are for control vehicle saline injection data and the *shaded bars* are for the nicotine injection data (*P<0.05 nicotine 0 mg/kg versus 0.2 mg/kg)

of four (2 ages×2 nicotine doses). As can be seen in Fig. 5, nicotine (0.2 mg/kg) caused a significant decrease in locomotor activity in the adults compared to vehicle treatment. In contrast, there was no apparent effect of nicotine on locomotor activity in the adolescent rats administered with the same dose of nicotine. There was a significant age effect (P<0.005) with the vehicle-treated adolescents having lower activity counts than the adults. However, despite the lower baseline levels of activity there was still considerable room for nicotine-induced reduction in activity in the adolescent group. Only the mean activity showed this differential effect. No differences were seen as a function of the habituation over the twelve 5-min blocks of the hour-long session.

Discussion

The principal findings of this study are that when rats begin nicotine self-administration during adolescence they self-administer much more nicotine than rats that begin during adulthood. This pattern of self-administration caused substantially higher total nicotine self-administration even when the adolescent-onset rats reached adulthood. These data suggest that this greater nicotine self-administration may put those who begin nicotine use during adolescence at greater risk for long-lasting nicotine addiction.

The greater self-administration of nicotine in the adolescent-onset group may reflect a lessened perception of the aversive effects of nicotine or different hedonic set-point in rats that started self-administration during adolescence. Alternatively, adolescent rats may have pharmacokinetic differences in nicotine distribution and metabolism. Recently, Trauth et al. (1999, 2000, 2001) demonstrated lasting neurochemical effects in rats after adolescent nicotine exposure including desensitization of nicotinic-induced catecholamine release. They also found that adolescent rats (45 days old) had only about 25% the blood level of nicotine after similar chronic nicotine as adults (105 days old), possibly due to faster metabolism. However, not all responses of adolescents to nicotine are diminished. In expt 3, the adolescent rats showed an exaggerated hypothermic response to nicotine compared with adults. If diminished nicotine blood levels were the only mechanism for higher rates of nicotine self-administration, one would expect all effects to be diminished in adolescents. It is important to note that nicotine was administered in expt 3 by a different route than expts 1 and 2 (SC rather than IV) and was given passively rather than actively self-administered. However, age-related differences in the rate of nicotine metabolism is unlikely to be unique to either SC or IV exposure or active or passive administration. Another possibility is that greater nicotine-induced suppression of activity in adults than adolescents as was seen in expt 3 (Fig. 5), may have been responsible for less lever pressing in the adult-onset rats. However, this does not seem to be the case, as nicotine-induced response suppression would also be predicted to have had greater effects with the higher doses, which it did not. Finally, the greater nicotine self-administration of the adolescent-onset rats may have resulted from a more general ability of adolescent rats to learn more quickly. For example, we have shown that during adolescence rats learn the radial-arm maze for food reinforcement more quickly than either younger or older rats (Chambers et al. 1996). With greater learning abilities adolescent rats may learn addiction more avidly.

Interestingly, the effect of starting nicotine self-administration during adolescence continued into adulthood. In expt 1, the acute nicotine dose-response assessment began when the adolescent-onset rats were 54–62 days of age and the adult-onset rats were 84–90 days of age. In expt 2, at the end of the chronic exposure the adolescent-onset group was 82 days old and the adult-onset group was 112 days old. There was still a significant increase in nicotine self-administration in the adolescent onset group. Thus, the age of onset of nicotine self-administration can have long-term effects on the amount of nicotine self-dosing. Adolescent rats may self-administer more nicotine than adults at first to overcome their faster catabolism of nicotine. However, in the current study, we found that adolescent-onset rats continue to self-administer more nicotine even when they become adults. The higher response rate established during adolescence continues into adulthood even though their nicotine pharmacokinetics slows to the adult level.

Experiment 2 was conducted over a period of 4 weeks, which is more chronic than the daily dose changes in expt 1. It is true that 4 weeks is a short time compared to the decades of smoking seen in humans, but it was long enough to document the persistence of the increased nicotine self-administration in the adolescent-onset rats into adulthood.

Interestingly, an inverted U-shaped dose-effect function of diminished lever pressing for nicotine was not seen in the current study (Fig. 1b). This may have been due to the transient nature of the dose switching in which

different doses were only given for one session. Also, a visual conditioned cue (light stimulus) was given with each infusion to facilitate self-administration. With this paradigm a broader dose range may be necessary for determining an inverted U-shaped function.

There was some evidence for adjustment of responding with changes in the middle dose range (0.02–0.06 mg/kg per infusion) in the adult-onset rats. As shown in Fig. 1a, b the adult-onset rats showed only a 48% increase in total amount of nicotine self-administered in the face of a 300% increase in nicotine dose per infusion. This degree of control in the adult-onset rats is impressive especially given the low FR (FR1) used in the current study. In contrast, over the same dose range the adolescent-onset rats showed a 389% increase in total amount of nicotine self-administered. The adolescent-onset rats self-administered considerably more nicotine overall. The main effect of age across dose was clearly significant, with the adolescent-onset rats self-administering substantially greater amounts of nicotine. However, in expt 1, the age×dose interaction was not significant. Additional research will be necessary to determine whether there are reliable age-related differences in the reaction to changes in unit dose of nicotine infusion.

Diminished control over nicotine intake in adolescents would be consistent with the reported ineffectiveness of nicotine replacement therapy in adolescents seeking replacement treatment. Hurt and colleagues (Hurt et al. 2000) found that transdermal nicotine replacement therapy which is effective in promoting smoking cessation in adults was not effective in adolescent smokers. Other types of cessation therapy are needed in conjunction with pharmacotherapy for adolescent smokers.

Age-related differences in learning may be important in the observed age-related differences in nicotine self-administration seen in the current study. Adolescent rats have been shown to learn faster than younger animals or adults (Chambers et al. 1996). This effect documented in the radial-arm maze may carry over to other sorts of conditioning such as drug self-administration. Acquisition of drug self-administration is a form of learning. The effect of nicotine-induced improvement of cognitive function may further facilitate conditioning to self-administration in adolescents. Recently, it has been shown that in human adolescents (Zack et al. 2001), as in human adults (Warburton et al. 1992), nicotine improves cognitive performance. Nicotine-induced cognitive improvement has also been documented in rat models (Levin and Simon 1998; Rezvani and Levin 2001). Future studies will characterize this effect in adolescent versus adult rats.

Important for considering facilitated onset of nicotine self-administration in adolescents is the finding that adolescents have a greater perservation after reinforcers are withdrawn in tasks requiring active responding (Spear and Brake 1983). It may have been the case that adolescents had greater responding to the lever formerly delivering food when it was changed to deliver nicotine only, even though there was no age difference in food-motivated responding in the current study. This would influence the mechanistic interpretation of the effect seen, but the fact remains that for whatever reason, adolescents might first engage in nicotine intake, for example food motivation in rats or peer pressure in human adolescents, they continue to take nicotine in a higher rate than adults who begin taking nicotine. If attenuated extinction in adolescents were entirely responsible for the doubling of the nicotine self-administration, which extends into adulthood, this must be a very important phenomenon for potentiating nicotine use in adolescents. The influence of concurrent reinforcers will be the focus of future studies in this line of research.

Not only the use of food reinforcement at the initial phase of training, but also the use of food restriction throughout testing may be important for explaining the age differences seen in nicotine self-administration. Carroll and Lac (1993) have shown that rats will self-administer greater amounts of drugs of abuse if they are trained under a food restricted state. This motivational enhancement may be more pronounced in adolescent rats than in adults. The rats in the current study were fed sufficiently to gain weight in a normal fashion; so increased deprivation in the adolescent rats was probably not in itself the cause of the increased nicotine self-administration.

In expt 3, it is important to consider that there were baseline age differences in body temperature and locomotor activity with control saline injections. The differences in response to the nicotine test doses may have been due to either age or baseline differences. The higher baseline may have been more likely to decrease with nicotine independent of age.

The current studies were conducted in female rats. Obviously, this more closely models adolescent-onset smoking in teenage girls, a group, which is showing a dramatic rise in smoking rates (US Public Health Service 1994). There are important sex differences in the dynamics of smoking and smoking cessation in adult humans (Perkins 1999; Wetter et al. 1999). Sex differences during the early phases of smoking addiction can be well studied experimentally in the rat model in which the influence of hormonal milieu can be readily analyzed. Donny et al. (2000) tested nicotine self-administration at doses from 0.02 to 0.09 mg/kg per infusion in adult male and female rats and found that they acquired self-administration in a similar fashion. As in the current study, they found that adult male and female rats seem to titrate their intake based on dose/infusion. In contrast to the current study, in the Donny et al. (2000) experiment, the numbers of infusions in 1-h sessions was greater. The number of infusions was approximately the same for 0.01, 0.02 and 0.03 mg/kg per infusion and the number was higher than the number of infusions of 0.06 and 0.09 mg/kg per infusion. They found few sex differences with the number of active response or the amount of nicotine self-administered under a stable fixed ratio schedule and no effect of estrous cycle on nicotine-self-administration in females (Donny et al. 2000). However, females did have a

higher breakpoint on a progressive ratio schedule and acquired self-administration faster at the lowest (0.02 mg/kg) infusion dose. Other data also support the consistency of nicotine effects across the estrous cycle. Kuo et al. found similar effects of nicotine on locomotor activity at the different phases of the estrous cycle in rats (Kuo et al. 1999). Booze et al. did not find repeated nicotine administration to significantly affect the estrous cycle, though they did find that females did show more rapid nicotine-induced motor stereotypies in females compared with males (Booze et al. 1999).

The adolescent period constitutes an active period for the final phase of neurodevelopment (Bayer et al. 1982; Goldman-Rakic 1987; Huttenlocher 1990; Cameron and Gould 1996). Drug exposure during this period may have different effects than adult exposure and the drugs may have persisting effects from disruption of late neural development. Unfortunately, there is sparse and conflicting information concerning drug effects during adolescence in animal models. Adolescents show hyposensitivity to dopamine agonists in terms of locomotor activity, but this might not extend to reinforcing effects (Spear and Brake 1983; Spear 2000b). There is initial information that drugs of abuse have differential effects in adolescents. Ethanol administration during adolescence has much more pronounced amnestic effects compared with adults. This is also seen in terms of enhanced effects on long-term potentiation (Little et al. 1996; Markweise-Foerch et al. 1998; Swartzwelder et al. 1995a, 1995b, 1998). In the current study, we found that adolescents have a more pronounced hypothermic response to nicotine but show diminished response to nicotine effects on locomotor activity.

Age-effect functions have important consequences for both regulation of tobacco availability to minors and for therapeutic treatment for smoking cessation (Eissenberg and Balster 2000). Adolescent-onset nicotine self-administration may be a gateway to abuse of other drugs. The association between adolescent smoking and later use of other drugs has been seen in humans (Hofler et al. 1999), but the order of this association may be due to factors other than causation such as ready availability of tobacco. The causative relationship cannot be determined in clinical studies. Recent research has shown that adolescent nicotine administration in a rodent model significantly alters response to cocaine later in life (Kelley and Middaugh 1999). With the rat model of adolescent-onset nicotine self-administration, we will be able to better understand the causative relationship of adolescent nicotine self-administration and persisting addiction to nicotine and other drugs of abuse.

Acknowledgements This research was supported by the National Institute on Drug Abuse grant DA 11943 and the National Institute of Mental Health grant MH64494.

References

Adriani W, Macri S, Pacifici R, Laviola G (2002) Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology 27:212–224

Bayer SA, Yackel JW, Puri PS (1982) Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science 216:890–892

Booze RM, Welch MA, Wood ML, Billings KA, Apple SR, Mactutus CF (1999) Behavioral sensitization following repeated intravenous nicotine administration: gender differences and gonadal hormones. Pharmacol Biochem Behav 64:827–839

Cameron HA, Gould E (1996) Distinct populations of cells in the adult dentate gyrus undergo mitosis or apoptosis in response to adrenalectomy. J Comp Neurol 369:56–63

Carroll ME, Lac ST (1993) Autoshaping IV cocaine self-administration in rats—effects of nondrug alternative reinforcers on acquisition. Psychopharmacology 110:5–12

Centers for Disease Control and Prevention (1991) Differences in the age of smoking initiation between blacks and whites, United States. Mortal Morbid Weekly Rep 40:754–757

Chambers RA, Moore J, McEvoy JP, Levin ED (1996) Cognitive effects of neonatal hippocampal lesions in a rat model of schizophrenia. Neuropsychopharmacology 15:587–594

Corrigall WA (1992) A rodent model for nicotine self-administration. In: Boulton AA, Baker GB, Wu PH (eds) Animal models of drug addiction (neuromethods). Humana Press, Totowa, N.J., pp 315–344

Corrigall WA, Coen KM (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 99:473–478

Corrigall WA, Coen KM (1994) Nicotine self-administration and locomotor activity are not modified by the 5-HT_3 antagonists ICS 205-930 and MDL 72222. Pharmacol Biochem Behav 49:67–71

Corrigall WA, Coen KM, Adamson KL, Chow BLC (2000) Response of nicotine self-administration to manipulations of mu-opioid and GABA receptors in the ventral tegmental area. Psychopharmacology 149:107–114

Corrigall WA, Zack M, Eissenberg T, Belsito L, Scher R (2001) Acute subjective and physiological responses to smoking in adolescents. Addiction 96:1409–1417

Donny EC, Caggiula AR, Knopf S, Brown C (1995) Nicotine self-administration in rats. Psychopharmacology 122:390–394

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

Dorland WA (1994) Dorland's illustrated medical dictionary, 28th edn. Saunders, Philadelphia

Eissenberg T, Balster RL (2000) Initial tobacco use episodes in children and adolescents: current knowledge, future directions. Drug Alcohol Depend 59:S41–60

Everett SA, Warren CW, Sharp D, Kann L, Husten CG, Crossett LS (1999) Initiation of cigarette smoking and subsequent smoking behavior among US high school students. Prev Med 29:327–333

Fernandez E, Schiaffino A, La Vecchia C, Borras JM, Nebot M, Salto E, Tresserras R, Rajmil L, Villalbi JR, Segura A (1999) Age at starting smoking and number of cigarettes smoked in Catalonia, Spain. Prev Med 28:361–366

Glynn T, Greenwald P, Mills S, Manley M (1993) Youth tobacco use in the United States: problems, progress, goals, and potential solutions. Prev Med 22:568–575

Goldman-Rakic PS (1987) Development of cortical circuitry and cognitive function. Child Dev 58:601–622

Hofler M, Lieb R, Perkonigg A, Schuster P, Sonntag H, Wittchen HU (1999) Covariates of cannabis use progression in a representative population sample of adolescents: a prospective examination of vulnerabilitly and risk factors. Addiction 94:1679–1694

Hurt RD, Croghan GA, Beede SD, Wolter TD, Croghan IT, Patten CA (2000) Nicotine patch therapy in 101 adolescent smokers: efficacy, withdrawal symptom relief, and carbon monoxide and plasma cotinine levels. Arch Pediatr Adolesc Med 154:31–37

Huttenlocher PR (1990) Morphometric study of human cerebral cortex development. Neuropsychologia 28:517–527

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

Kuo DY, Lin TB, Huang CC, Duh SL, Liao JM, Cheng JT (1999) Nicotine-induced hyperlocomotion is not modified by the estrous cycle, ovariectomy and estradiol replacement at physiological level. Chin J Physiol 42:83–88

Levin ED, Simon BB (1998) Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology 138:217–230

Levin E, Mead T, Rezvani A, Rose J, Gallivan C, Gross R (2000) The nicotinic antagonist mecamylamine preferentially inhibits cocaine versus food self-administration in rats. Physiol Behav 71:565–570

Little PJ, Kuhn CM, Wilson WA, Swartzwelder HS (1996) Differential effects of ethanol in adolescent and adult rats. Alcohol Clin Exp Res 20:1346–1351

Markweise-Foerch BJ, Acheson S, Levin ED, Wilson WA, Swartzwelder HS (1998) Differential effects of ethanol on memory in adolescent and adult rats. Alcohol Clin Exp Res 22:416–421

McNeill AD, West RJ, Jarvis M, Jackson P, Bryant A (1986) Cigarette withdrawal symptoms in adolescent smokers. Psychopharmacology 90:533–536

Perkins KA (1999) Nicotine discrimination in men and women. Pharmacol Biochem Behav 64:295–299

Pomerleau C, Pomerleau O (2000) Characteristics of early- and late-onset smokers. Society for Research on Nicotine and Tobacco, Arlington, Va., pp 67

Rezvani AH, Levin ED (2001) Cognitive effects of nicotine. Biol Psychiatry 49:258–267

Rigotti N (1990) How can we help the remaining smokers to quit? Am J Prev Med 6:249–250

Shoaib M, Schindler CW, Goldberg SR (1997) Nicotine self-administration in rats: strain and nicotine pre-exposure effects on acquisition. Psychopharmacology 129:35–43

Spear L (2000a) Modeling adolescent development and alcohol use in animals. Alcohol Res Health 24:115–123

Spear LP (2000b) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463

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

Swartzwelder HS, Wilson WA, Tayyeb MI (1995a) Age-dependent inhibition of long-term potentiation by ethanol in immature versus mature hippocampus. Alcohol Clin Exp Res 19:1480–1485

Swartzwelder HS, Wilson WA, Tayyeb MI (1995b) Differential sensitivity of NMDA receptor-mediated synaptic potentials to ethanol in immature versus mature hippocampus. Alcohol Clin Exp Res 19:320–323

Swartzwelder HS, Richardson R, Markwiese B, Wilson W, Little P (1998) Developmental differences in the acquisition of tolerance to ethanol. Alcohol 15:311–314

Trauth JA, Seidler FJ, McCook EC, Slotkin TA (1999) Adolescent nicotine exposure causes persistent upregulation of nicotinic cholinergic receptors in rat brain regions. Brain Res 851:9–19

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

Trauth J, Seidler F, Ali S, Slotkin T (2001) Adolescent nicotine exposure produces immediate and long-term changes in CNS noradrenergic and dopaminergic function. Brain Res 892:269–280

US Public Health Service (1994) Preventing tobacco use among young people: a report of the Surgeon General. US Government Printing Office, Washington D.C.

Warburton DM, Rusted JM, Muller C (1992) Patterns of facilitation of memory by nicotine. Behav Pharmacol 3:375–378

Wetter DW, Kenford SL, Smith SS, Fiore MC, Jorenby DE, Baker TB (1999) Gender differences in smoking cessation. J Consult Clin Psychol 67:555–562

Zack M, Belsito L, Scher R, Eissenberg T, Corrigall WA (2001) Effects of abstinence and smoking on information processing in adolescent smokers. Psychopharmacology 153:249–257