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Enhanced nicotine reward in adulthood after exposure to nicotine during early adolescence in mice☆

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ABSTRACT

Approximately one million adolescents begin smoking cigarettes every year. Studies show that adolescents may be particularly vulnerable to various aspects of nicotine dependence. Work on rodents demonstrates parallel findings showing that adolescence is a time of changed sensitivity to both rewarding and aversive effects of nicotine. However, it is unclear if these effects are long-lasting and whether they contribute to a lifetime of nicotine addiction. In this study we have characterized the effects of adolescent nicotine exposure on the rewarding properties of nicotine in adulthood using the CPP model. Specifically, we have addressed whether the phase of adolescence (early, middle, or late adolescence) plays a role in the susceptibility to the enhanced rewarding effects of nicotine. Furthermore, we have investigated the long-term effects of adolescent nicotine exposure on nicotine reward in adulthood and have correlated these behavioral adaptations with possible molecular mechanisms. We observed that early adolescence in the mouse is a unique phase for elevated sensitivity to nicotine reward using a CPP model. In addition, exposure to nicotine during this phase, but not during late adolescence or adulthood, resulted in a lasting enhancement of reward in adulthood. Finally, we have shown that early adolescent nicotine exposure significantly elevates nAChR function in adulthood. Overall, we demonstrate that early adolescence represents a period of development, distinct from middle and late adolescence, during which nicotine exposure can cause persistent changes in behavior and molecular adaptations.

1. Introduction

Adolescence is a period known for high levels of drug experimentation. Specifically, tobacco is one of the most commonly used drugs during this time. For example, Johnston et al. [15] report that the incidence of smoking is high among American adolescents and that the age of initiation has been declining in recent years. Furthermore, clinical studies demonstrate that adolescents show signs of nicotine dependence after minimal tobacco exposure [16]. Epidemiological observations show that those who begin smoking at an early age are less likely to have successful quit attempts than those who begin smoking as adults [9,17]. These results suggest that adolescents are more susceptible to nicotine dependence than adults and that exposure to nicotine may have distinctive effects during adolescence making adolescents more vulnerable than adults to their long-term effects and contributing to increased risk to develop drug dependence in adulthood.

Work on rodents has produced parallel findings and showed that adolescence is a time of changed sensitivity to the effects of nicotine, namely reward and withdrawal. For example, adolescent rodents have demonstrated an enhanced preference for nicotine [6,18,29] and an attenuated intensity of both physical and affective withdrawal signs [18,22,24]. Specifically, in adolescent male rodents, it appears that the rewarding effects of nicotine may play a larger role in the initiation of smoking behavior at an early age [18]. Thus, there is increasing evidence that the short period of early adolescence is a developmental time in which rodents are particularly sensitive to the rewarding effects nicotine and are less sensitive to its aversive properties.

Few reports addressed however the long-term behavioral impact of adolescent exposure later in life. Rats pretreated with nicotine during adolescent or periadolescent period increase self-administration of nicotine as adults, suggesting that they have become more sensitive to the reinforcing effects of the drug [3]. In addition, enhanced vulnerability to the rewarding effects of

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Abbreviations: PND, post-natal day; AUC, area under the curve; nAChR, nicotinic acetylcholine receptor; s.c., subcutaneous injection; CPP, conditioned place preference.

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nicotine as measured by the conditioned place preference test was reported after exposure to the drug during the adolescent period of development in rats [2,28]. However, these studies did not explore the role of adolescent age (early versus late) and the dose or duration of drug exposure (one single protocol of nicotine administration) in this increased sensitivity to nicotine in later age. Furthermore, Adriani et al. [2] reported that chronic nicotine treatment in adolescents, but not adults rats, produces persisting changes in several nicotinic acetylcholine receptor (nAChR) subunit mRNAs and binding in regions associated with reward. In adult rats pretreated with nicotine during periadolescence, an increase in gene expression of the α5, α6, and β2 subunits was found. However, the functional implication of this differential age effect was not reported.

The goal of the present work was to characterize the effects of adolescent nicotine exposure on the rewarding properties of nicotine in adulthood using the CPP model. To date, studies have not examined multiple phases of adolescence to determine the greatest window of vulnerability. Furthermore, studies have not addressed whether factors such as dose and duration of exposure influence nicotine-induced behavior in adulthood. In addition to characterizing the rewarding component of nicotine dependence, we have also addressed a possible molecular mechanism for these differences by examining the functional response of the neuronal nAChR in adulthood following adolescent nicotine exposure.

2. Materials and methods

2.1. Animals

Experimentally naïve male ICR mice were obtained from Harlan Laboratories (Indianapolis, IN). They were housed five per cage and given free access to food and water. Based on previous characterization of rodent adolescence [26], the following ages were used in our studies: early adolescent (PND 28–34), middle adolescent (PND 35–48) late adolescent (PND 49–58) and adult mice (PND 70+). All experiments were performed during the light cycle (between 7:00 A.M. and 7:00 P.M.). Animals were maintained in an American Association for Accreditation of Laboratory Animal Care approved facility and the study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2. Drugs

(−)-Nicotine bitartrate was purchased from Sigma Aldrich (St. Louis, MO). All doses were given s.c. and were expressed as free base.

2.3. Nicotine-induced conditioned place preference (CPP)

An unbiased CPP paradigm was utilized in all studies. Mice were assigned randomly in the different treatment groups. Place conditioning boxes consisted of two distinct sides (20 cm × 20 cm × 20 cm); one black and one white each with unique flooring. A partition separated the two sides with an opening that allowed access to either side of the chamber, and this partition could be closed for pairing days.

Handling habituation: On Wednesday–Friday of the week prior to the start of the place conditioning procedure, each mouse in the CPP studies was handled once per day for approximately two min. Handling experience plays an important role in the ability of nicotine to produce a CPP [14].

Preconditioning Phase: On day 1, animals were placed in the boxes and allowed to move freely from side to side for 15 min, and time spent in each side was recorded. Mice were then randomly assigned into treatment groups in an unbiased design.

Conditioning phase: Animals were placed in the chambers for 20 min, with the saline group receiving saline in both sides of the boxes and drug groups receiving nicotine (0.05, 0.1, 0.5, 0.7 or 1 mg/kg) on one side and saline on the opposite side. Drug-paired sides were randomized among all groups. Conditioning lasted for 3 days, with animals in the drug group receiving drug each day [either in the morning (8:00 A.M.) or the afternoon (1:00 P.M.)].

Test phase: On the test day, no injections were given. Time spent on each side was recorded, and data were expressed as time spent on drug-paired side minus time spent on saline-paired side. A positive number indicated a preference for the drug-paired side, while a negative number indicated an aversion to the drug-paired side. A number at or near zero indicated no preference for either side.

2.4. Adolescent nicotine exposure studies

Mice were exposed to nicotine according to the protocol described in Table 1. Briefly, three patterns of exposure were tested in early adolescent mice: acute, intermittent, and frequent. The frequent exposure paradigm was also tested in late adolescent mice and adult mice. In addition to various exposure patterns, early adolescent mice were also tested using two doses of nicotine exposure (0.1 or 0.5 mg/kg s.c.). Only the 0.5 mg/kg s.c. dose was used in testing the late adolescent and adult mice due to data gathered with early adolescent studies. Following nicotine exposure, mice were returned to their home cages and allowed to mature for 7 weeks so that early adolescents were now fully developed adults (PND 70). After this same maturation period, adults had now reached the age of PND 120. Late adolescent mice matured for 3 weeks so that they were also PND 70 at the time of CPP testing. In these experiments, a dose of nicotine of 0.5 mg/kg s.c. was used as the conditioning dose in our CPP model.

2.5. Rubidium efflux studies

A separate group of early adolescent and adult mice were injected with nicotine 0.5 mg/kg s.c. or saline under the frequent administration protocol (Table 1) for evaluation of nAChR function. After 7 weeks of maturation, mice were rapidly decapitated and four brain regions were dissected for use in the assay. The effects on nAChR function were investigated in the ventral striatum and prefrontal cortex, regions involved in the mesolimbic reward

Table 1
Protocol for nicotine or saline pretreatment. Three injection patterns were used: acute, intermittent, and frequent. All s.c. injections were based on weight of the mouse and given twice a day.

Age and duration of exposure	Frequency of injections	Dose (mg/kg)	Age of CPP testing
Early adolescence – acute	One day; PND 28; 2 total injections	0.1 and 0.5	PND 70–74
Early adolescence – intermittent	Every 3 days; PND 27, 30, 33, 36; 8 total injections	0.1 and 0.5	PND 70–74
Early adolescence – frequent	Every day; PND 27–33; 14 total injections	0.1 and 0.5	PND 70–74
Late adolescence – frequent	Every day; PND 50–56; 14 total injections	0.5	PND 70–74
Adulthood – frequent	Every day; PND 74–80; 14 total injections	0.5	PND 120–124

pathway. The hippocampus was studied due to this region's involvement in learning and memory, often associated with CPP models. Finally, nAChR function was evaluated in the thalamus, a region rich in nAChRs, especially the β2 containing subunits, which are known to be involved in nicotinic reward. In our dissection method, the thalamus contains the ventral tegmental area, also important in reward. Unless otherwise noted, all reagents were purchased from Sigma Chemical Co., St. Louis, MO. Synaptosomes were prepared according to Marks et al. [20,21]. Briefly, synaptosomes were prepared by hand homogenizing tissue in cold 0.32 M sucrose (1 ml/g tissue). After centrifugation, pellets were resuspended in cold load buffer (140 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM HEPES hemisodium salt, 20 mM glucose, pH 7.4). A 25-μl aliquot of the synaptosome suspension was incubated for 40 min with 10 μl load buffer containing approximately 4 μCi $^{86}RbCl$ (Perkin Elmer Life Sciences, Boston, MA). After the synaptosomes were filtered onto glass fiber filters under gentle vacuum, the filters were rinsed with 0.5 ml of load buffer and placed on the perfusion apparatus for washing with perfusion buffer (135 mM NaCl, 5 mM CsCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 1.5 mM KCl, 1 g/l bovine serum albumin, 50 nM tetrodotoxin, 25 mM HEPES hemisodium salt, pH 7.4) for six min. The filter containing synaptosomes was subsequently perfused continuously. Filters were stimulated for 1 min with various concentrations of nicotine (1, 5, 10, 20, 30, 50, 100, 200, or 300 μM) prepared in perfusion buffer followed by a 3-min wash with perfusion buffer alone. Twelve-second fractions were collected in 12 mm × 75-mm test tubes beginning 6 min into the perfusion. Samples were counted for 1 min each in a Wallac Wizard 3 in. 1480 Automatic Gamma Counter (PerkinElmer, Shelton, CT). The magnitude of $^{86}Rb^+$ efflux response was calculated based on the increase in counts above baseline after stimulation of the tissue with nicotine. Data were calculated as fractional release (cpm/total cpm loaded on filter) for each fraction collected. The baseline was calculated for each mouse by fitting to an exponential equation the fractional release in fractions immediately preceding and following the peak. The area under the curve was calculated for each mouse using this mathematically derived baseline and the fractional release values in the peak.

2.6. Statistical analysis

Statistical analysis of CPP studies was performed with mixed-factor analysis of variance (ANOVA) with post hoc Tukey's test when appropriate. Nicotine stimulated $^{86}Rb^+$ efflux was analyzed with a two-way ANOVA followed by Tukey post hoc tests. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Nicotine-induced conditioned place preference in various age groups

Previous data from this laboratory have shown that early adolescent male mice display enhanced rewarding effects in the CPP model as compared to adult male mice [18]. However, other phases of adolescence have not yet been examined. We chose to examine three stages of adolescence (early, middle, and late) using developmental periods, which have been established in the literature [26]. These divisions have been thoroughly assessed in rodents and are thought to correlate well with aspects of human adolescence [26]. In Fig. 1, we show the results from a nicotine-induced CPP study which assessed reward in four age brackets. As expected from previous results, early adolescent mice exhibited significant preference for nicotine at low doses of 0.05 and 0.1 mg/kg. However, middle and late adolescents lacked a significant effect

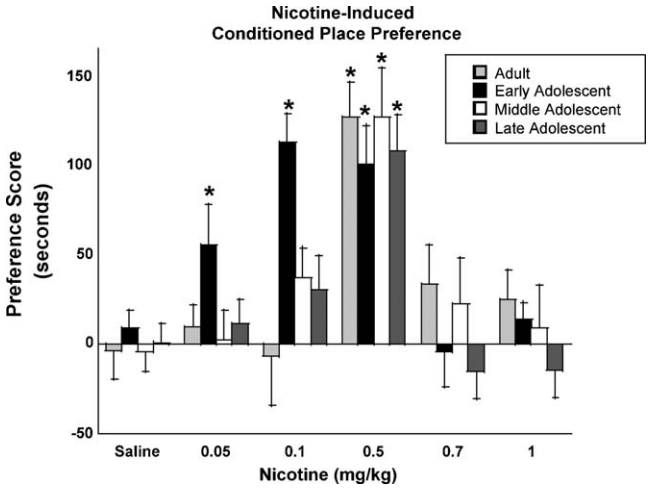

Fig. 1. Dose–response relationship for nicotine-induced CPP in mice of different ages. Male mice from four age groups were injected s.c. with various doses of either saline or nicotine. Ages were as follows: adult (PND 70), late adolescent (PND 49), middle adolescent (PND 35) and early adolescent (PND 28). Each point represents the mean ± SEM of eight mice. $^*p < 0.05$ from saline group.

at these doses in the CPP test. These age groups, as well as adult mice, only exhibited significant preference at a dose of 0.5 mg/kg nicotine. These results suggest that early adolescence is a unique period for experiencing heightened rewarding effects of nicotine as measured in the CPP test. Since data in our first experiment suggested early adolescence was a unique period for nicotine reward, we decided to focus on this phase of development for subsequent studies.

3.2. Effect of early adolescent nicotine exposure on nicotine reward in adulthood

To assess the effects of adolescent nicotine exposure on nicotine's rewarding effects in adulthood; we pretreated mice with s.c. injections of nicotine during early adolescence and then allowed them to mature to adulthood (PND 70) before CPP testing. Three different exposure protocols and two doses of nicotine (0.1 and 0.5 mg/kg) were tested (Table 1). These protocols were

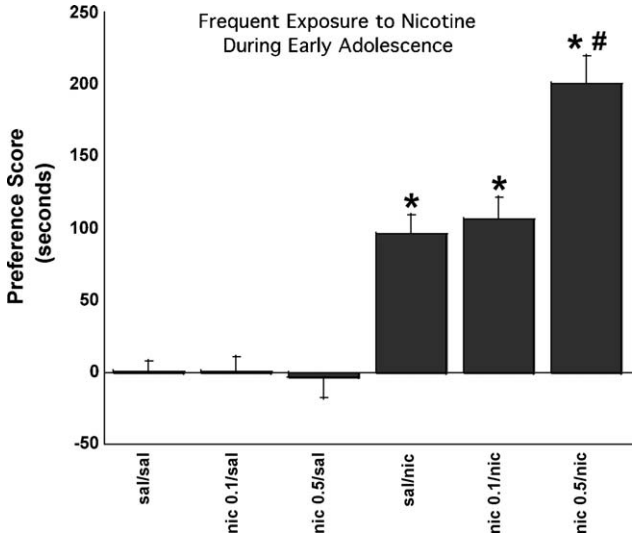

Fig. 2. Effect of frequent (14 injections) early adolescent nicotine exposure on nicotine-induced reward in adulthood. The y-axis represents preference score and the x-axis expresses adolescent treatment followed by treatment in the CPP paradigm. The conditioning dose used for CPP was 0.5 mg/kg s.c. Each bar represents the mean ± SEM of seven to eight mice. $^*p < 0.05$ from respective saline control; $^\#p < 0.05$ from sal/nic group.

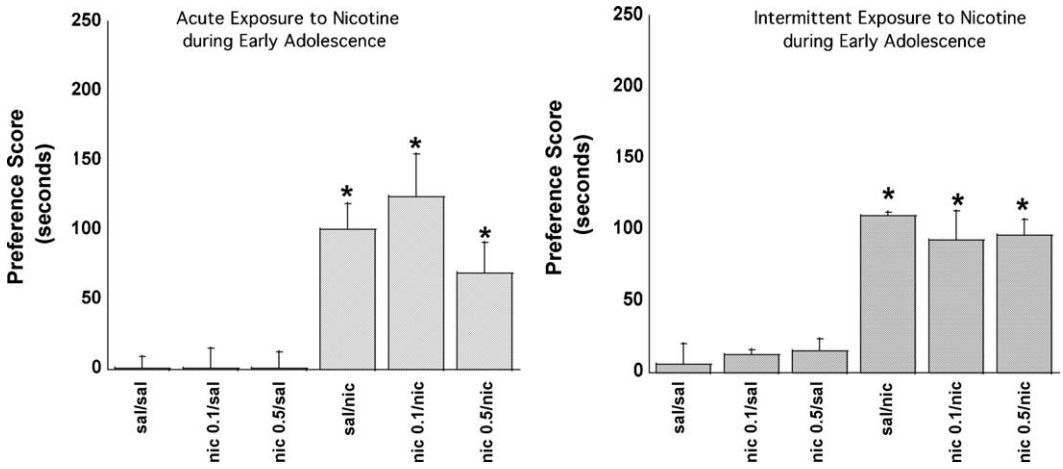

Fig. 3. Effect of acute (two injections) (a) and intermittent (eight injections) (b) early adolescent nicotine exposure on nicotine-induced reward in adulthood. The *y*-axis represents preference score and the *x*-axis expresses adolescent treatment followed by treatment in the CPP paradigm. Each bar represents the mean ± SEM of seven to eight mice. $^{*}p < 0.05$ from respective saline control; $^{\#}p < 0.05$ from sal/nic group.

selected in order to mimic patterns of adolescent experimentation with cigarette smoking: short/acute; intermittent, and frequent/chronic.

3.3. Frequent exposure to nicotine during early adolescence

Fig. 2 shows the results from the CPP study after a 7-day exposure period to nicotine. All mice conditioned with nicotine in the CPP paradigm during adulthood developed a significant preference to nicotine at a dose of 0.5 mg/kg. However, pretreatment with the higher dose of nicotine (0.5 mg/kg) in adolescence evoked a significant increase in the rewarding effects of nicotine in adulthood as compared to saline pretreated controls.

3.4. Acute and intermittent exposure to nicotine during early adolescence

Once again, all mice conditioned with nicotine in the CPP model displayed a significant preference for nicotine. However, neither acute nor intermittent (Fig. 3a and b) nicotine pretreatment resulted in a significant difference in the reward response in adulthood. This suggests that a more chronic pattern of adolescent nicotine exposure is required to induce lasting changes in subsequent behavioral responses.

3.5. Enhanced rewarding effects in adulthood are unique to early adolescent nicotine exposure

One question that arises is whether or not this enhanced effect is due to nicotine exposure in early adolescence specifically or if this is effect occurs after nicotine exposure at any age. To address this question, we exposed adult and late adolescent mice to the higher dose of nicotine (0.5 mg/kg) and tested them in our CPP model after a period of maturation as shown in Fig. 4. Once again, all mice conditioned with nicotine in the CPP model elicited significant preference for the drug, but there were no differences based on pretreatment. These data support our previous findings in which early adolescence is a unique period for heightened vulnerability to nicotine's rewarding effects.

3.6. Neuronal nicotinic receptor function following early adolescent nicotine exposure

We have previously reported that central nAChRs from naïve adolescent rodents displayed increased functional response to nicotine as compared to adults [8,18]. Therefore, we wanted to examine whether the increased behavioral response in adulthood reflected an increase in nAChR function during this time frame as well. For this experiment, early adolescent and adult mice were

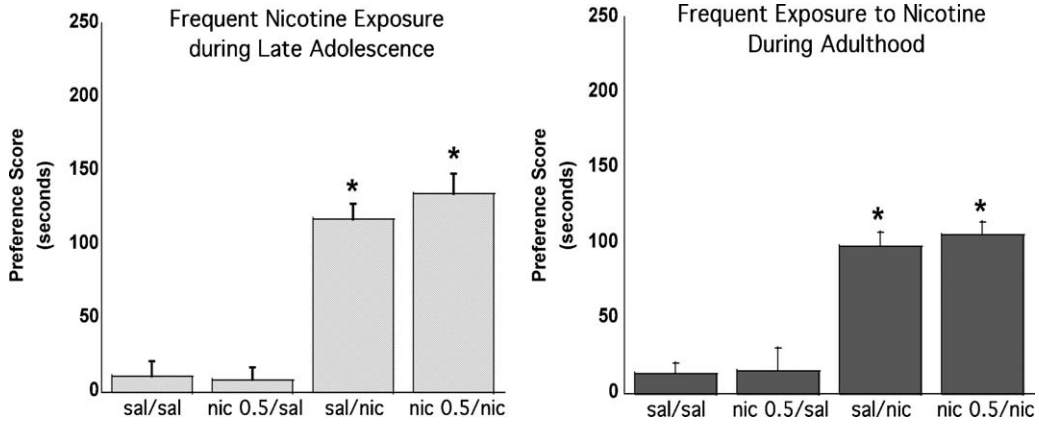

Fig. 4. Effect of late adolescent and adulthood nicotine exposure on nicotine-induced reward. The *y*-axis represents preference score and the *x*-axis expresses adolescent treatment followed by treatment in the CPP paradigm. A frequent pattern of nicotine exposure in late adolescence (a) and adulthood (b) was tested. Each bar represents the mean ± SEM of eight mice. $^{*}p < 0.05$ from respective saline control.

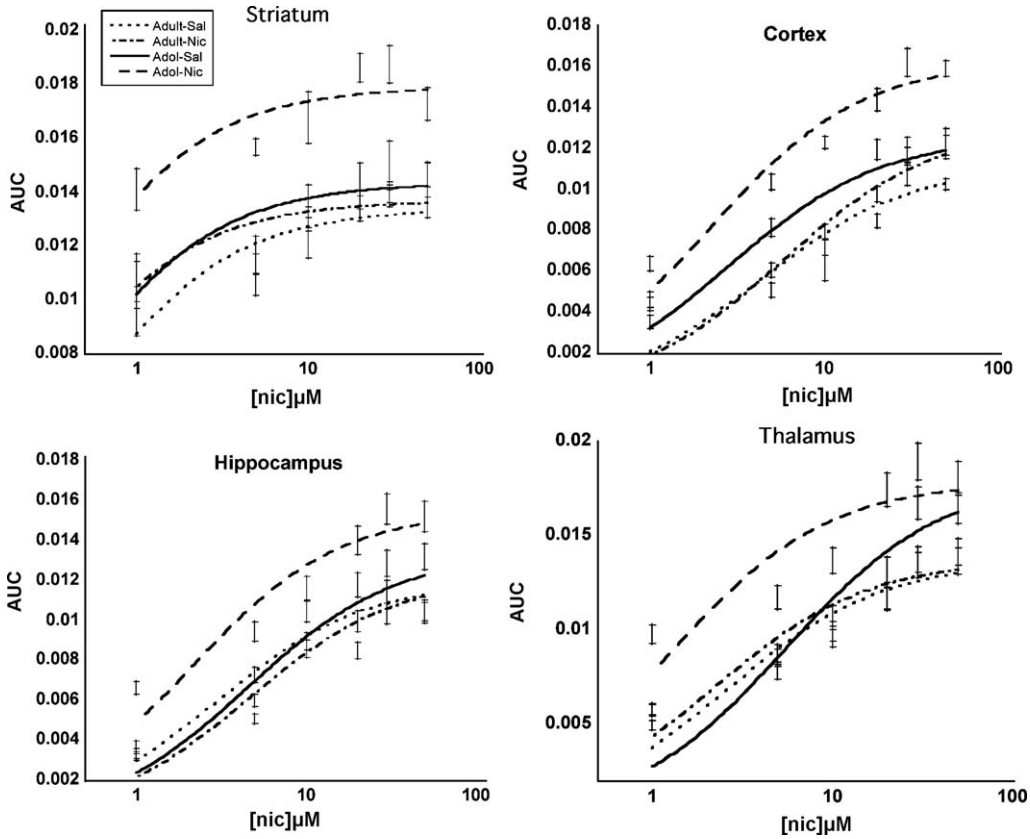

Fig. 5. Effect of adolescent and adulthood nicotine pretreatment on nicotinic receptor function. Mice were pretreated with either saline or 0.5 mg/kg nicotine s.c. for 7 days during adulthood or adolescence. Dose response curves were generated 7 weeks following pretreatment. Synaptosomes were stimulated with various doses of nicotine for 1 min to generate dose–response curves. Area under the curve is shown on the *y*-axis and nicotine dose is shown on the *x*-axis. Data are represented by a Michaelis–Menten curve fit where $y = m1x/(m2 + x)$. Results are expressed as mean AUC ± SE of 10 mice.

pretreated with nicotine (0.5 mg/kg s.c.) under the frequent exposure pattern (7 days). After a 7-week maturation period, rubidium efflux assays were used to assess nAChR function in four brain regions.

Fig. 5 shows the dose–response curves from the receptor function study. In all four brain regions tested, mice that were treated with nicotine in early adolescence displayed a significant leftward shift in the curve as compared to saline pretreated controls indicating an increased functional response at the receptor level. In contrast, mice which were pretreated with nicotine in adulthood showed the same level of response as their respective saline controls. When we combine the data into total area under the curve as shown in Fig. 6, we see that the functional response of the receptor is significantly enhanced in all four brain regions after early adolescent, but not adult, nicotine exposure. Specifically, both the striatum and thalamus regions showed a 22% increase in nAChR function. The cortex and hippocampus displayed slightly higher increases at 26% and 25% respectively. These data suggest that early adolescent nicotine exposure also has persistent effects on nicotinic receptor function. Since the nAChR is the initial target of nicotine, this is likely to be involved in the mechanism explaining an enhanced susceptibility to nicotine dependence in adolescence.

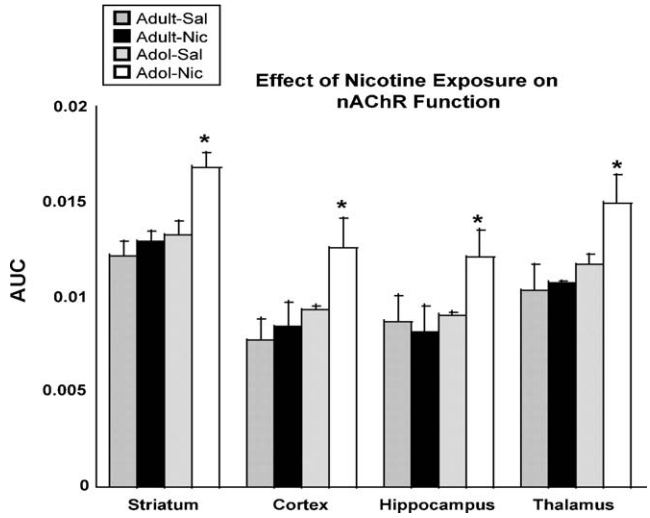

Fig. 6. Total area under the curve for all doses of nicotine in the rubidium efflux assay in four brain regions. Results are expressed as mean AUC ± SE of 10 mice/dose. $^{*}p < 0.05$ from adult mice.

4. Discussion

The aim of the present study was to examine how adolescent nicotine exposure alters the subsequent response to nicotine dependence in adulthood. More specifically, we investigated how various factors such as the timing, dose, and duration of exposure influences the response to nicotine-induced reward using a CPP model. Finally, we investigated whether these behavioral alterations correlate with changes in nAChR function. Our results indicate that early adolescence (PND 28–34) is indeed a unique period for experiencing an enhanced sensitivity to nicotine reward as compared to middle and late adolescence. In addition, we found that adolescent nicotine exposure resulted in elevated nicotine reward in adulthood in a dose- and duration-dependent manner. Finally, using a rubidium efflux assay, we demonstrated that

nicotine exposure in early adolescence, but not in adulthood, caused a long-lasting increase in neuronal nAChR function, which may underlie an augmented sensitivity to the rewarding properties of nicotine.

4.1. Early adolescence is a unique period for enhanced sensitivity to nicotine reward

These results confirm previous findings in our lab [18], indicating that early adolescent mice are more sensitive to nicotine reward as compared to adults in a CPP model. In addition, our study is the first to demonstrate that this enhanced sensitivity is lost in middle and late adolescent mice as well as adult mice. Other studies have also demonstrated this increased sensitivity to nicotine reward in early adolescence in a rat model [3,6,30]. Several factors could be involved in the age-dependent differences we have observed. It is possible that early adolescent mice display an enhanced ability for learning as compared to later adolescent stages and adults. Another possibility that would explain differences between the age groups is the role of anxiety and response to novelty. Adolescents are thought to be less anxious than adults, which may play a role in this paradigm since it involves a novel environmental context. However, adult and adolescent mice in our experimental conditions do not differ in their basal anxiety levels when tested in the elevated plus maze paradigm (data not shown). It would be of interest to test younger mice as well. It is possible that with the full onset of puberty and thus hormones, the long-term effects of nicotine change and that nicotine could elicit similar long-term effects in younger pre-adolescent animals.

4.2. Early adolescent nicotine exposure causes increased rewarding effects in adulthood

These data show that mice exposed to nicotine in early adolescence display heightened sensitivity to nicotine's rewarding effects in adulthood in a dose- and duration-dependent manner. Specifically, a daily administration of nicotine during early adolescence causes long-lasting behavioral changes, while acute and intermittent exposure does not yield this result. In addition, the higher dose of nicotine tested (0.5 mg/kg s.c.) was required to yield this effect while the lower dose (0.1 mg/kg s.c.) was not sufficient for long-term alterations in nicotine reward. Furthermore, these persistent behavioral changes are specific to early adolescent nicotine exposure. The observed alterations are not due to prior nicotine exposure in general as indicated by the study testing adulthood nicotine exposure (Fig. 4). Taken together, these data suggest the long-term effects of nicotine in rodents depend on factors such age of adolescence, duration and dose of the drug. Our results are in parallel with those of i.v. self-administration study of Adriani et al. [3] in rats. Although the two paradigms measure different aspects of reward, our data with the conditioned place preference extend the finding to a paradigm evaluating reward and contextual learning.

4.3. Long-term increases in nAChR function result from early adolescent nicotine exposure

Finally, we have sought to investigate possible molecular mechanisms underlying this behavioral plasticity. In a previous report, we found that naïve adolescent mice displayed increased nAChR function in four prominent brain regions [18]. We chose to extend this finding by exposing early adolescent mice to nicotine and testing them for nAChR function in adulthood after a period of drug abstinence. Indeed, these data indicate that early adolescent nicotine exposure significantly elevates nAChR function in adulthood in all these four brain regions. In contrast, nicotine exposure in the adult does not have this long-lasting effect. Although the precise reasons for this functional upregulation are yet to be determined, several factors such as functional properties, distribution, and number of nAChRs may be contributing. Indeed, recent studies [1,3,4,25] showed that chronic nicotine treatment in adolescents cause differential upregulation of nAChRs binding and mRNA levels in brain regions associated with reward when animals were tested later in adulthood. Alternatively, it is possible that an age-dependent gain in receptor function (increased ion flux through nAChRs) occurs in response to chronic nicotine exposure in young animals. Indeed, the data presented here would support an altered receptor physiology and may play a role in our observations.

5. Conclusion

Human adolescent reports suggest that certain aspects of nicotine dependence can be observed after short exposure periods to low doses of nicotine. Indeed, studies indicate that dependence can be seen after smoking only a few cigarettes [12,13]. Furthermore, the progression from drug use to abuse is more likely when initiation begins at an early age [5,27]. Moreover, a late onset of drug use was found to serve as a protective factor for adulthood drug addiction problems [7,27]. These reports clearly reveal that exposure to nicotine during this time frame may have long-lasting behavioral consequences. Although results from animal models need to be interpreted with caution, our data seem to have produced parallel findings and exposed underlying neurobehavioral mechanisms focusing on neuronal nAChRs, the primary target of nicotine.

In our studies, relatively low levels of nicotine and short patterns of exposure of the drug were utilized demonstrating that even experimentation with the drug could have significant consequences on brain nicotinic mechanisms. Using the same strain of mice, we have recently shown that a 2.5 mg/kg administration (s.c.) of nicotine results in a C_{max} of 314 ± 170 ng/ml [11]. Our study used a dose of nicotine five times lower than that in the previous study (0.5 mg/kg). Since the dose and plasma nicotine levels are linearly correlated [19], we would expect an estimated plasma nicotine level in our study to be approximately 63 ng/ml, which is more likely to represent adolescent experimentation with cigarette smoking. Taken together, these findings demonstrate that short exposure to relatively low levels of nicotine is likely to have persistent and detrimental effects on subsequent effects of nicotine.

Importantly, these studies have shown that adolescent nAChRs exhibit increased functional response to nicotine. Moreover, even short-term adolescent nicotine exposure causes a persistent increase in receptor function which lasts well into adulthood. Indeed, these data correlate well with an enhanced responsiveness in our CPP model. Another factor to consider is that increased receptor function following adolescent nicotine exposure may also translate into downstream consequences more directly involved in plasticity effects. Changes in dopaminergic and serotonergic transporters in the striatum have been reported after exposure to nicotine in adolescent rats [10]. Long-term effects may be mediated by nicotine-induced changes in gene expression. Two likely candidates that should be further investigated in this regard are the transcription factors CREB (cAMP response element binding protein) and ΔFosB, both of which are known to be induced by nicotine [23,31]. It is not clear however that adolescent nicotine exposure will differentially affect these various neurochemical pathways important to nicotine dependence.

In conclusion, early adolescence is distinct from middle and late adolescent development in that it represents a period during which nicotine exposure can cause persistent changes in both

behavior and molecular adaptations in subsequent stages of life. Our work confirms that delaying the onset of cigarette smoking is protective against developing lifetime nicotine addiction and dependence. More effective prevention strategies and messages need to be implemented so that adolescents are aware of the harmful consequences of smoking at an early age.

References

[1] Abreu-Villaca YA, Seidler FJ, Qiao D. Short-term exposure adolescent nicotine exposure has immediate and persistent effects on cholinergic systems: critical periods, patterns of exposure, dose thresholds. Neuropsychopharmacology 2003;28:1935–49.

[2] Adriani W, Deroche-Gamonet V, Le Moal M, Laviola G, Piazza PV. Preexposure during or following adolescence differently affects nicotine-rewarding properties in adult rats. Psychopharmacology (Berl) 2006;184:382–90.

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

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

[5] Anthony JC, Petronis KR. Early-onset drug use and risk of later drug problems. Drug Alcohol Depend 1995;40:9–15.

[6] Belluzzi JD, Lee AG, Oliff HS, Leslie FM. Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. Psychopharmacology 2004;174:389–95.

[7] Breslau N, Peterson EL. Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences. Am J Public Health 1996;86:214–20.

[8] Britton AF, Vann RE, Robinson SE. Perinatal nicotine exposure eliminates the peak in nicotinic acetylcholine receptor response in adolescent rats. J Pharmacol Exp Ther 2007;320:871–6.

[9] Chen J, Millar WJ. Age of smoking initiation: implications for quitting. Health Rep 1998;9:39–46.

[10] Collins SL, Wade D, Ledon J, Izenwasser S. Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats. Eur J Pharmacol 2004;502:75–85.

[11] Damaj MI, Siu EC, Sellers EM, Tyndale RF, Martin BR. Inhibition of nicotine metabolism by methoxysalen: pharmacokinetic and pharmacological studies in mice. J Pharmacol Exp Ther 2007;320:250–7.

[12] DiFranza JR. Hooked from the first cigarette. J Fam Practice 2007;56:1017–22.

[13] DiFranza JR, Savageau JA, Fletcher K, O'Loughlin J, Ockene JK, Rigotti NA, et al. Measuring the loss of autonomy over nicotine use in adolescents: the DANDY study. Arch Pedriat Adolesc Med 2002;156:397–403.

[14] Grabus SD, Martin BR, Brown SE, Damaj MI. Nicotine place preference in the mouse: influences of prior handling, dose and strain and attenuation by nicotinic receptor antagonists. Psychopharmacology 2006;184:456–63.

[15] Johnston LD, O'Malley OM, Bachman JG. Secondary school students. National Survey Results on Drug Use from the Monitoring the Future Study 1975–1997, vol. 1. Washington, DC: National Institute on Drug Abuse; 1998.

[16] Kandel DB, Chen K. Extent of smoking and nicotine dependence in the United States: 1991–1993. Nicotine Tob Res 2000;2:263–74.

[17] Khuder SA, Dayal HH, Mutgi AB. Age at smoking onset and its effect on smoking cessation. Addict Behav 1999;24:673–7.

[18] Kota D, Martin BR, Robinson SE, Damaj MI. Nicotine dependence and reward differ between adolescent and adult male mice. J Pharmacol Exp Ther 2007;322:399–407.

[19] Lichtensteiger W, Ribary U, Schlumpf M, Odermatt B, Widmer HR. Prenatal adverse effects of nicotine on the developing brain. Prog Brain Res 1988;73:137–57.

[20] Marks MJ, Grady SR, Collins AC. Downregulation of nicotinic receptor function after chronic nicotine infusion. J Pharmacol Exp Ther 1993;266:1268–76.

[21] Marks MJ, Farnham DA, Grady SR, Collins AC. Nicotinic receptor function determined by stimulation of rubidium efflux from mouse brain synaptosomes. J Pharmacol Exp Ther 1993;264:542–52.

[22] O'Dell LE, Bruijnzeel AW, Smith RT, Parsons LH, Merves ML, Goldberger BA, et al. Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. Psychopharmacology 2006;186:612–9.

[23] Pich EM, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R, Chiamulera C. Common neural substrates for the addictive properties of nicotine and cocaine. Science 1997;275:83–6.

[24] Shram MJ, Funk D, Li Z, Le AD. Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine. Psychopharmacology 2006;186:201–8.

[25] Slotkin TA, Bodwell BE, Ryde IT, Seidler FJ. Adolescent nicotine treatment changes the response of acetylcholine systems to subsequent nicotine administration in adulthood. Brain Res Bull 2008;76:152–65.

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

[27] Taoli E, Wydner EL. Effect of the age at which smoking begins on frequency in adulthood. N Engl J Med 1991;325:968–9.

[28] Torres OV, Tejeda HA, Natividad LA, O'Dell LE. Enhanced vulnerability to the rewarding effects of nicotine during the adolescent period of development. Pharmacol Biochem Behav 2008;90:658–63.

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

[30] Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP. Nicotine-induced conditioned place preference in adolescent and adult rats. Physiol Behav 2002;77:107–14.

[31] Walters CL, Cleck JN, Kuo Y, Blendy JA. μ-Opioid receptor and CREB activation are required for nicotine reward. Neuron 2005;46:933–43.