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Adolescent vs. adult-onset nicotine self-administration in male rats: Duration of effect and differential nicotinic receptor correlates☆

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Abstract

Adolescence is the life stage when tobacco addiction typically begins. Adolescent neurobehavioral development may be altered by nicotine self-administration in a way that persistently potentiates addiction. Previously, we showed that female adolescent rats self-administer more nicotine than do adults and that the increased nicotine intake then persists through the transition to adulthood [E.D. Levin, A. Rezvani, D. Montoya, J. Rose, H. Swartzwelder, Adolescent-onset nicotine self-administration modeled in female rats, Psychopharmacology 169 (2003) 141–149.]. In the current study, male Sprague–Dawley rats were given access to nicotine via the standard operant IV self-administration procedure (nicotine bitartrate dose of 0.03 mg/kg/infusion). One group of male rats started during adolescence the other group started in young adulthood. After the end of the four-week period of self-administration brain regions of the rats were assessed for $\alpha4\beta2$ nicotinic receptor binding. We found that male rats, like females, show higher nicotine self-administration when starting during adolescence as compared to starting in adulthood ($p<0.001$). Indeed, the effect in adolescent males was even greater than that in females, with more than triple the rate of nicotine self-administration vs. the adult-onset group during the first 2 weeks. The adolescent onset nicotine-self-administering rats also had significantly greater high affinity nicotinic receptor binding in the midbrain and the striatum, whereas hippocampal binding did not differ between the age groups. Striatal values significantly correlated with nicotine self-administration during the first 2 weeks in the adult-onset group but not the adolescent-onset rats, suggesting that the differences in self-administration may depend in part on underlying disparities in synaptic responses to nicotine. After the initial 2 weeks, nicotine self-administration in male rats declined toward adult-like levels, as the adolescent rats approached adulthood. This study showed that adolescent male rats self-administer significantly more nicotine than do male adult rats, but that adolescent-onset nicotine self-administration in male rats declines over weeks of continued use to approach adult-onset levels. In a previous study, we found that female rats also show greater nicotine self-administration with adolescent onset vs. adult onset, but that the females continued higher rates of self-administration into adulthood. Our results thus reinforce the concept that the adolescent brain is unusually receptive to the effects of nicotine in a manner that reinforces the potential for addiction.

Keywords: Adolescent; Nicotine; Male; Nicotinic receptor binding; Striatum

1. Introduction

Extensive research has shown that 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 (for recent reviews see [6,14,26,31]). This observation suggests that smoking is more addictive during adolescence than in adulthood. However, this hypothesis is difficult to assess in humans because of self-selection bias and ethical constraints. Self-selection bias occurs because people, who for genetic or environmental reasons, are more prone to become addicted to nicotine, may for the same reasons start using it earlier. Ethical constraints make it impossible to conduct randomized assignment of human adolescents to nicotine self-administration and control conditions.

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Animal models can obviate these problems with random assignment to adolescent and adult nicotine self-administration. This project used the rat model of IV nicotine self-administration to determine the effect of adolescent-onset nicotine self-administration on the amount of nicotine intake.

Nicotine dependence has been successfully modeled in the rat self-administration procedure since the work of Corrigall and co-workers developed the methods for reproducible rat nicotine self-administration [9–13], using an operant situation in order to obtain nicotine delivery. This work has now been replicated in other laboratories including our own [16,17,23,34]. The Corrigall model of nicotine self-administration takes advantage of the IV route of administration used in many animal models of drug abuse. This differs from the typical inhalation route most often engaged by human tobacco users. Nicotine self-administration by inhalation has not yet successfully been achieved in rats. It may never be since rats are obligate nose breathers. However, the IV route simulates the rapid rise in arterial nicotine that occurs via the typical pulmonary route of exposure in humans [20]. The self-administration model has proven valuable to identify mechanisms of nicotine addiction such as those unmasked by the blocking of specific nicotinic receptors [42] and the role of mesolimbic dopamine pathways [12].

Because the great majority of smokers begin using tobacco during adolescence [6] it is essential to determine the cause-and-effect relationships between the age of first nicotine use and the tenacity of later addiction. It is clear that people who begin smoking earlier in life are likely to be more dependent on nicotine [30], and it may be that people who are inherently prone to nicotine addiction are more likely to begin smoking earlier. Alternatively, early exposure to nicotine during adolescence may itself have persistent effects leading to more tenacious nicotine addiction. Recent evidence has shown that adolescents progress into nicotine dependence much more quickly than previously thought [24] and indeed, many individuals may show signs of dependence and withdrawal after only a few cigarettes [15].

Only in an animal model, where randomized assignment of treatment is made, can the role of age and nicotine be adequately determined. The core age span of adolescence in a rat has been variously described as being 28–42 days of age, but signs can range over a more extended age range from 23–55 days after birth [36]. However, the precise description of adolescence depends on the function under study. The definition of adolescence with regard to nicotine effects will emerge with the literature characterizing the age-effect functions of nicotine response. Recently, a variety of investigators have studied the differential responses of adolescent rodents to nicotine. The current results are consistent with previous work from our lab and others in the field. There is an emerging literature published over the past several years showing the differential responsivity of adolescents to nicotine including enhanced conditioned place preference [4,40] and high rates of nicotine self-administration [2,5,23]. This may be related to greater and more long-lasting nicotine effects on nicotinic receptor up-regulation [1,3].

The current study continues our development of a rodent model of nicotine psychopharmacology during adolescence [23] and was designed to evaluate the specific vulnerability of adolescents to acute and persisting effects of nicotine, as well as nicotinic receptor mechanisms that may dictate age-dependent differences in nicotine responsiveness. The nicotinic alpha4 beta2 was chosen for analysis because of the literature showing the involvement of this receptor subtype in the basis of nicotine reinforcement and self-administration [12,29].

2. Methods

2.1. Subjects

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. Male Sprague-Dawley albino rats (Taconic Farms, Gemantown, NY, USA) 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. The day–night cycle was reversed cycle (lights on 18:00–6:00) so that they were in their active phase during behavioral testing. All rats (N=13/age group) had *ad lib* access to water and were fed the same type of rat chow once daily throughout the study to keep them at approximately a lean healthy weight with food amounts adjusted from 8 for the young adolescents increasing to 16 g per day as they became adults. The rats were fed daily after the operant session. The adolescent rats grew from an average of 109 g to 141 g, while the adults maintained their weight on this diet averaging 233 g at the start of the study and 229 g at the end of the study.

2.2. Drug preparation and administration

Solutions of nicotine bitartrate 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 Corp). All solutions were kept refrigerated in the dark between experiments.

2.3. Drug self-administration

Adolescent and adult rats were received from the supplier at the same time so that the period of time in our colony was equal. Chronically indwelling intravenous jugular catheters were implanted at approximately 32 (adolescent) or 64 (adult) days of age. They were flushed twice daily: before nicotine sessions, with 100 units/ml heparinized sterile saline; then, after sessions, with 100 units/ml heparinized sterile saline containing 20 mg/ml Gentamicin as an antibiotic. (Each catheter flush had a volume between 0.2 and 0.3 ml; post-session infusions included between 4 and 6 mg Gentamicin). The adolescent rats weighed an average of 109±4 g and the adults weighed an average of 233±5 g at the point of catheter implant surgery. The anesthesia was a ketamine/domitor combination. The catheters had sufficient length inside the vein and between the vein and the external port

to allow for growth of the animals and still maintain their integrity. The extension of the catheter inside the veins of adolescents was approximately 0.7 cm and 1.2 cm in the veins of the adults. The catheters were made in our laboratory from Micropenthane tubing (size MRE-040) from Braintree Associates (Braintree, MA, USA). Phenobarbitol injection tests through the catheter were used to verify patency. Twenty-four hours after arrival, rats were handled for 8 min several times per day for 3 consecutive days before implantation of the catheter. Following this 3-day acclimation period, behavioral training began.

For behavioral training, rats were placed in dual lever test chambers (Med Associates, Vermont, 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, VT, USA) 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 a FR-1 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 1 hour.

The sequence of testing was as follows: 3 sessions of lever pressing for food reinforcement, 3 sessions of nicotine paired with food reinforcement and then 20 sessions of nicotine reinforcement alone over a period of 4 weeks. Following the lever press training for food reinforcers (3 sessions), rats began nicotine self-administration. No nicotine priming injections were given. For 3 sessions, nicotine (0.03 mg/kg/infusion) was paired with delivery of food reinforcers. Only then was nicotine 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. Just following the three sessions of pellet only training and three sessions of pellet plus nicotine training the nicotine only response was tested. The adolescent rats ($N=13$) started nicotine only self-administration at 40–46 days of age and the adults ($N=13$) 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. The benchmark infusion dose of nicotine was (0.03 mg/kg/infusion). This was used for the first 2 days of each week. On the following 3 days of each week, doses of either one-third (0.01 mg/kg/infusion) or three times (0.09 mg/kg/infusion) the original dose were administered. The dose given on days 3–5 of each week was counterbalanced over a 4-week period with both ABBA and BAAB orders being used. Half of the animals were given access to 0.01 mg/kg/infusion during days 3–5 of weeks 1 and 4 and the other half were given access to this dose during weeks 2 and 3. The other dose of 0.09 mg/kg/infusion was given during the other weeks in this ABBA and BAAB design. Finally, on the first 2 days after week 4 of the study all the rats had daily sessions using the benchmark 0.03 mg/kg/infusion dose. One day after the final session of nicotine self-administration while they were still under the restricted feeding procedure used through the whole study the rats were sacrificed and their brains analyzed for nicotinic receptor binding. The adolescent-onset rats weighed 140±4 g and the adult-onset rats weighed 229±8 g at the end of the study.

2.4. Receptor binding

After the completion of the nicotine self-administration measures, animals were decapitated and the striatum, midbrain and hippocampus were flash-frozen in liquid nitrogen and stored at −45 °C until assayed. Tissues were thawed and homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in 20–30 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) and were sedimented at 40,000 ×g for 10 min. The membrane pellet was re-suspended (Polytron) and washed using a smooth glass homogenizer fitted with a Teflon pestle and aliquots of the resuspension were used for measurements of ligand binding and membrane protein.

We utilized [^{3}H]cytisine, a ligand that binds selectively to the α4β2 nicotinic receptor, the predominant subtype in mammalian brain [19] and which shows differential sensitivity to nicotine in the adolescent vs. the adult [37]. Each assay contained a final concentration of 1 nM [^{3}H]cytisine (specific activity, 35 Ci/mmol; PerkinElmer Life Sciences, Boston, MA) in a total volume of 250 μl of a buffer consisting of 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 50 mM Tris (pH 7.4). Incubations lasted 75 min at 4 °C, with or without 10 μM nicotine to displace specific binding. Labeled membranes trapped by filtration on glass fiber filters that were presoaked with 0.15% polyethyleneimine, washed twice and then counted for radioactivity.

2.5. Statistical analysis

The self-administration data were assessed by analysis of variance. An alpha level of $p<0.05$ was used as a cutoff for statistical significance. The between subjects factor was age (adolescent ($N=13$) vs. adult onset ($N=13$)) and the repeated measure was week of testing. The dependent measure was infusions per session. For assessment of the relationship of nicotinic receptor binding to nicotine self-administration analysis of covariance was used with a between subjects factor and a covariant of nicotine self-administration during the first 2 weeks of the trial ($N=12$ Adolescent-onset and $N=11$ Adult-onset). For the co-variance analysis the log of nicotine self-administration infusions was used to reduce the heterogeneity of variance.

3. Results

Initial responding for food pellets was not found to differ ($F(1,24)=0.076$, $p=0.78$) between adolescents (52±7, mean± sem) and adult rats (48±14). Both adolescents and adults clearly demonstrated preference for the lever, which delivered the pellet with the adolescents distributing 78% of their responses on the correct lever and the adults 75% to the correct lever. When nicotine delivery (0.03 mg/kg/infusion) was combined with pellet delivery, an age-related effect began to emerge. The adolescents self-administered 13.3±3.0 nicotine infusions/session while the adults self-administered 6.5±1.8 infusions/session. This difference was not quite significantly different ($F(1,24)=3.71$, $p<0.07$). As with the pellet-only stage of training, both age groups showed clear preferences for the active lever during the pellet+nicotine delivery, with the adolescents rising to 90% responding on the active lever and the adults staying at about the same level as previously with 78% active lever responding. Chronic nicotine self-administration, studied over 4 weeks from adolescence into adulthood, was compared with self-administration beginning in adulthood in male Sprague-Dawley rats at the benchmark nicotine dose of 0.03 mg/kg/infusion for the first 2 days during each of the 4 weeks. There continued to be a highly significant preference of the rats for the active (nicotine delivering) vs. inactive lever ($F(1,24)=32.50$, $p<0.0001$). A significant ($F(1,24)=23.97$, $p<0.001$) difference between the two age groups was seen, with the adolescent-onset group displaying more than triple the rate of nicotine self-administration as compared to the adult-onset group during the initial 2 weeks. Subsequently, the adolescent self-administration reduced toward adult-like levels during the second 2 weeks of the study as the adolescent rats themselves approached adulthood (Fig. 1). The age differences were restricted to pressing on the active lever.

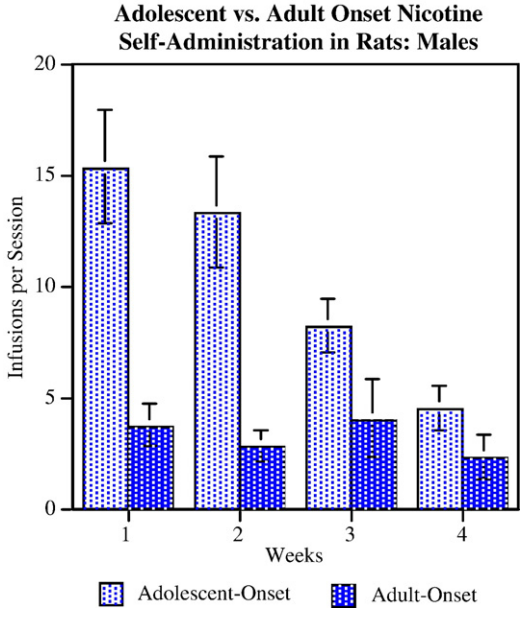

Fig. 1. Nicotine IV self-administration (0.03 mg/kg/infusion) in adolescent-onset and adult-onset ($N=13$/age group) male rats during each week, infusions per session (mean±sem).

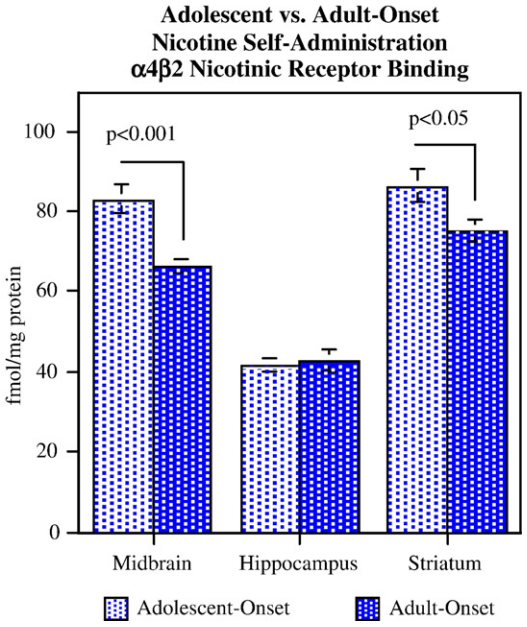

Fig. 2. Regionally selective α4β2 nicotinic receptor binding in adolescent-onset ($N=12$) and adult-onset ($N=11$) nicotine self-administering male rats (mean± sem).

Adolescents pressed the active lever (24.9±3.9) significantly more than adults (4.8±1.4), whereas response on the inactive lever did not significantly differ between the two groups with the adolescents having 8.4±2.3 and the adults 3.8±1.3.

During days 3–5 of each week's sessions either one-third (0.01 mg/kg/infusion) or three times (0.09 mg/kg/infusion) of the benchmark dose was given. The age-related effects were also seen with the infusion doses one-third and three times the benchmark 0.03 mg/kg/infusion dose. With the low 0.01 mg/kg/infusion dose the adolescent-onset rats had significantly ($F(1,24)=7.19$, $p<0.025$) more self-administered infusions per session (7.3± 1.0) averaged over the 4 weeks than the adult-onset rats (3.4±1.1). Significantly ($F(1,24)=21.53$, $p<0.0001$) greater responding in the adolescent-onset group (8.6±1.1) was also seen with the higher 0.09 mg/kg/infusion dose compared to the adult-onset group (2.6±0.7). As with the benchmark 0.03 mg/kg/infusion dose responding for nicotine in the adolescent-onset group decreased from the first 2 weeks to weeks 3–4. On the first 2 days after week 4 of the study all the rats had daily sessions using the benchmark 0.03 mg/kg/infusion dose. No significant age of onset related differences were seen in these sessions.

One day after the self-administration period, the rats were sacrificed and their brains assessed for nicotinic receptor binding. Nicotinic receptor binding showed significant differences between the two age groups (Fig. 2), seen most clearly in the midbrain, where the adolescent-onset group showed values nearly 30% higher than those in the adult self-administration group. A smaller, but significant difference was also seen in the striatum but there were no differences in receptor binding in the hippocampus.

There was also a differential effect for the relationship between nicotinic receptor binding and nicotine self-administration in adolescent vs. adult rats. In the striatum, there was a significant

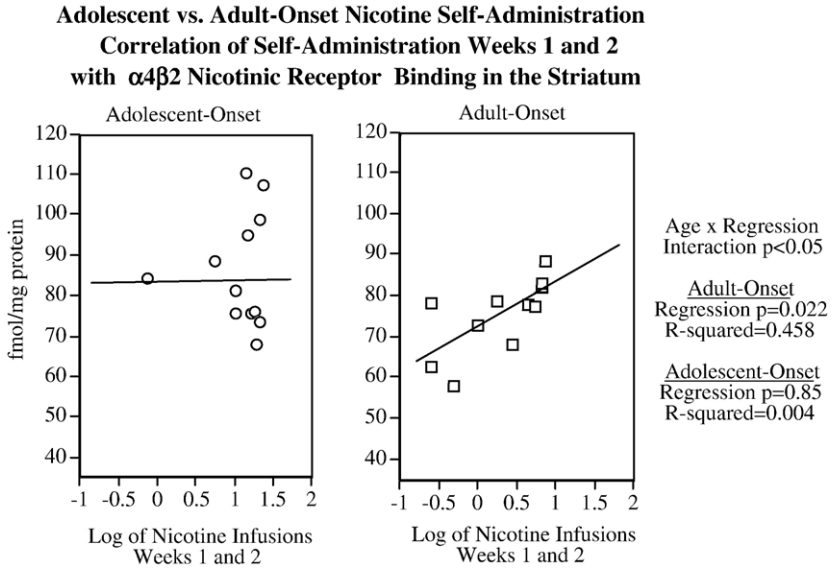

Fig. 3. Relationship between nicotine self-administration and α4β2 nicotinic receptor binding in the striatum of adolescent-onset (N=12) and adult-onset (N=11) nicotine self-administration during weeks 1 and 2 of nicotine access.

($F(1,19)=5.18$, $p<0.05$) interaction between the amount of nicotine self-administered at each age during the first 2 weeks of nicotine self-administration and the extent of receptor binding at the end of the experiment (Fig. 3). This reflected the fact that, although there was a significant ($F(1,9)=7.59$, $p<0.025$) correlation between nicotine self-administration and nicotinic receptor binding in the adult group ($R^2=0.458$), there was no such relationship in the adolescent ($F(1,10)=0.04$, $p=0.85$, $R^2=0.004$). This differential relationship between nicotinic receptor binding and nicotine self-administration did not seem to be due to nicotine effects on receptor number immediately prior to the time of assay inasmuch as the relationship between nicotine self-administration and receptor number was not apparent in the adults after additional experience with nicotine during weeks 3–4 of self-administration (adult-onset: $p=0.45$, $R^2=0.072$; adolescent onset: $p=0.76$, $R^2=0.01$).

We did not observe correlations between the two parameters in the hippocampus or midbrain, nor were there differential relationships related to the age of self-administration for these regions.

4. Discussion

Our findings are consistent with the view that the adolescent is especially susceptible to nicotine reinforcement. During adolescence the male rats in the current study self-administered approximately three times the amount of nicotine per kilogram of body weight than adults. The nicotine self-administration in the adolescent-onset rats declined to near adult-onset levels as the adolescents grew into adulthood.

The lack of age-related differences in responding for food supports the specificity of the age differences in nicotine motivated responding. This lack of age-related difference in food-motivated responding also argues against differential impact of food restriction in the different age groups. The food restriction fully allowed for growth of healthy lean animals that did not proceed to obesity as is usually the case with *ad lib* feeding in male rats.

Greater nicotine self-administration was seen in adolescent male rats not only relative to adults that began nicotine self-administration at the same time but also compared to their own levels of self-administration as they aged into adulthood. This was not due to simple catheter failure as the rats grew. Tests of catheter patency demonstrated their continuing integrity. Rather, pharmacokinetic and/or pharmacodynamic effects of nicotine, which differ between adolescents and adults, may have driven this effect. Pharmacokinetic factors include more rapid catabolism of nicotine during adolescence [35]. Pharmacodynamic factors include altered sensitivities of dopamine, norepinepherine, sertotonin and acetylcholine system responses during adolescence [35,38,39,37]. Either or both pharmacokinetic and pharmacodynamic factors may contribute to the more avid nicotine self-administration during adolescence. Further pharmacological studies will be key in determining the relative involvement of these non-neural vs. neural factors. Determining the mechanisms will be important in developing better therapeutic treatments to aid smoking cessation in adolescents.

The current results are consistent with previous work from our lab and others in the field. There is an emerging literature published over the past several years showing the differential responsivity of adolescents to nicotine. Adolescent rats showed enhanced conditioned place preference for nicotine compared with adults [4,40]. High rates of nicotine self-administration in adolescent rodents has been seen in other research [2]. Adolescent nicotine self-administration is particularly enhanced by co-administration of acetaldehyde [5]. In adolescence, nicotine effects on nicotinic receptor regulation seem to be especially long-lasting even after relatively brief exposure to nicotine [1]. Adriani et al. found that daily nicotine injections for 10 days starting on day 34 after birth caused an increase in

nicotinic receptor gene expression, which was evident even after the adolescents had grown into adulthood 5 weeks later [3].

The current study showed that as in the previous study with female rats [23] male rats show higher levels of nicotine self-administration when drug access is begun in adolescence as compared with adult-onset. However, there are important sex differences that emerge as self-administration continues from the adolescent stage into young adulthood. Initially, it appears that adolescent males are more susceptible than adolescent females, with more than three times the adult-onset levels of nicotine self-administration as compared to only a doubling in females [23]. However, only the females show a persisting effect. In the current study, in adolescent-onset males the very high levels of nicotine self-administration decreased as the rats aged into adulthood, so that by the fourth week of exposure the adolescent-onset males self-administered only slightly more nicotine more than the adult-onset males. In contrast, our previous study showed in females that adolescent-onset nicotine self-administration maintained the higher levels of self-dosing even when the adolescents became adults. However, it is important to remember that the male and female rats were run in two separate studies. The design of the studies was generally quite similar with the same doses used and training procedures used. There were some differences. In the prior study the female rats had their catheters implanted by the vendor, whereas in the current study with male rats the catheters were implanted in our own laboratory. Also, in the current study with males the adolescent-onset of nicotine self-administration was 40–46 days whereas in the previous study with females it averaged somewhat later at 54 days for the four-week time-effect function.

The observed sex differences in adolescent onset nicotine self-administration may reflect sex-selectivity of conditioned reinforcement of the sensorimotor aspects of nicotine self-administration that persist into adulthood. It is possible role that sex differences in conditioned reinforcement for nicotine may underlie the greater persistence of adolescent-onset nicotine self-administration into adulthood. There are sex-differences in conditioned reinforcement in human cigarette smokers, where women show a greater effect of nonpharmacological cues for smoking than men [28]. A similar effect is seen in female vs. male rats self-administering nicotine [7]. If this contributes to the differences seen here, the persistence of conditioned reinforcement to nicotine self-administration that develops during adolescence would then be more persistent in females than in males, a difference that can be tested using differential conditioned cue structure in the rat self-administration model [18,27].

Alternatively, this sex difference may be due to underlying effects of nicotine on late-stage neurobehavioral development during adolescence. Sex differences in up-regulation of nicotinic receptors have already been shown for experimenter-imposed adolescent nicotine administration [39]. However, other research has found that nicotine administration during adolescence does not cause the increase in nicotinic receptors as much it does in adults [8]. One critical difference in this study from the earlier one [39] is that 1 week of daily nicotine injections was given in the Collins study while 4 weeks of chronic osmotic minipump infusions was given in the Trauth study. Another important difference is that the Trauth study examined the effects of adolescent nicotine administration on nicotinic receptor binding when the animals became adults, while the Collins study examined adolescent nicotine effects on nicotinic receptor binding within the adolescent period.

We also found underlying differences in $\alpha4\beta2$ nicotinic receptor binding after self-administration with adolescent onset vs. adult onset. The adolescent-onset rats showed greater receptor binding in the midbrain and striatum than adult-onset rats. Interestingly, the adolescent-onset rats showed these regionally selective receptor binding differences even though during the 2 weeks prior to sacrifice no difference in nicotine self-administration was seen between the two age groups. Significant age-related differences in nicotine administration were present only during the first 2 weeks. The *history* of nicotine self-exposure may be relevant to the effect. Previously, we have shown that fixed doses of nicotine in adolescent male rats produce more persistent nicotinic receptor upregulation relative to vehicle treated controls compared to adult male rats given the same dose [39]. This effect was particularly notable in the midbrain, the region found in the current study to have the greatest differences between the two age groups. Indeed, the prominent difference in nicotinic binding in the midbrain may be relevant to the nuclei which give rise to the ascending dopamine projections involved in motivational function, cognition, mood and drug abuse liability. However, our results also indicated a second type of receptor involvement in age-related differences in the response to nicotine: a correlation between self-administration and $\alpha4\beta2$ receptors in the striatum of the adult, but not the adolescent. In this case, the receptor binding at the end of the study was predictive of the initial rate of self-administration in the first 2 weeks: adult rats with higher receptor binding in the striatum were the ones with higher initial nicotine self-administration in the first 2 weeks. Because the effects of nicotine on receptor binding decay more rapidly in the adult [39], this relationship cannot be the direct result of the amount of nicotine self-administered, and indeed, there was no significant ($p=0.45$) relationship detected between striatal receptor binding and nicotine self-administration during weeks three and four, the period immediately preceding the binding determination. The striatum is an important site for nicotine-elicited reward function [32] and nicotine effects on striatal monoamine systems contribute to nicotine withdrawal symptoms [33], especially the craving that tends to subvert attempts at quitting smoking [43]. In keeping with this interpretation, striatal reward-associated pathways are desensitized to non-smoking related inputs in smokers, so that further nicotine intake becomes a required to sustain reward function [25]. Our results suggest that these components may be less predictive of nicotine self-administration in the adolescent than in the adult, or alternatively that the already-noted major differences augmenting the responses to nicotine in the adolescent overshadow the underlying contribution of striatal pathways. Future work is necessary to determine whether this

relationship in adults is more related to the ventral striatum including the nucleus accumbens, which is important for the rewarding effects of drugs of abuse [22] or the dorsal striatum, which is important for the programming of stereotyped and compulsive behavior and craving [21,41].

In conclusion, our results indicate that adolescent males, like females, are prone to self-administer substantially more nicotine than in adulthood. Although the adolescent effect is initially more prominent in males than females, the effect in females appears to be more persistent. These findings bolster the fact that basic biologic differences underlie the greater susceptibility of adolescents to nicotine addiction, over and above any societal or socioeconomic factors. The fact that the pattern of self-administration differs substantially between males and females mirrors findings that are emerging for the development of nicotine dependence in adolescent smokers [15] and may play a role in the success or failure of smoking cessation strategies that utilize nicotine replacement therapy.

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