Cholinergic transmission during nicotine withdrawal is influenced by age and pre-exposure to nicotine: Implications for teenage smoking

Laura O'Dell, University of Texas at El Paso

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Teenage Brains: Think Different?

Editors
B. J. Casey, New York, N.Y.
B. E. Kosofsky, New York, N.Y.
P. G. Bhide, Tallahassee, Fla.
Teenage Brains: Think Different?
The present special topic issue focuses on the role of environmental influences encountered during teenage years on the structure, biochemistry and function of the mature brain. It represents a unique compilation of articles by leading experts in the fields of psychology, neurobiology, genetics, neurology and psychiatry. The papers span a wide range from animal models, epidemiology and molecular biology to advanced human functional neuroimaging. Three new studies offer unprecedented insights into brain mechanisms that may predispose teenage boys to risky behavior and an unhealthy disregard for disincentives and punishment. The publication brings together perspectives of experts on the critical importance of the interaction of the teenage brain with the ever-changing and complex physical, social and intellectual environment in shaping the adult brain and mind.
The publication is not only highly recommended to neuroscientists, psychiatrists, pediatricians, psychologists, social workers and health-related professionals, but also to anybody who wishes to gain an insight into the unique potentials and vulnerabilities of the teenage brain and teenage behavior.
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78 figures, 21 in color, and 36 tables, 2014
Contents

Adolescent Brain Development

147 The Developmental Mismatch in Structural Brain Maturation during Adolescence

161 Regional Hippocampal Volumes and Development Predict Learning and Memory

175 Decreases in Energy and Increases in Phase Locking of Event-Related Oscillations to Auditory Stimuli Occur during Adolescence in Human and Rodent Brain
Ehlers, C.L.; Wills, D.N.; Desikan, A.; Phillips, E.; Havstad, J. (La Jolla, Calif.)

196 The Role of the Anterior Insula in Adolescent Decision Making
Smith, A.R.; Steinberg, L.; Chein, J. (Philadelphia, Pa.)

210 Behavior and Neural Correlates of Empathy in Adolescents
Overgaauw, S.; Giroglu, B.; Rieffe, C.; Crone, E.A. (Leiden)

220 Teens Impulsively React rather than Retreat from Threat

228 Feedback Processing in Adolescence: An Event-Related Potential Study of Age and Gender Differences
Grose-Fifer, J.; Migliaccio, R.; Zottoli, T.M. (New York, N.Y.)

239 The Impact of Puberty and Social Anxiety on Amygdala Activation to Faces in Adolescence
Ferri, J.; Bress J.N.; Eaton N.R.; Proudfoot G.H. (Stony Brook, N.Y.)

250 Altered Gene Expression and Spine Density in Nucleus Accumbens of Adolescent and Adult Male Mice Exposed to Emotional and Physical Stress
Warren, B.L.; Sial, O.K.; Alcantara, L.F.; Greenwood, M.A.; Brewer, J.S.; Rozofsky, J.P.; Parise, E.M.; Bolaños-Guzmán, C.A. (Tallahassee, Fla.)
261 Shifts in Hormonal Stress Reactivity during Adolescence Are Not Associated with Changes in Glucocorticoid Receptor Levels in the Brain and Pituitary of Male Rats

269 BDNF Modulates Contextual Fear Learning during Adolescence
Dincheva, I. (New York, N.Y.); Pattwell, S.S. (Seattle, Wash.); Tessarollo, L. (Frederick, Md.); Bath, K.G. (Providence, R.I.); Lee, F.S. (New York, N.Y.)

Drug Abuse in Adolescence

277 The Role of Dopamine D1 and D2 Receptors in Adolescent Methylenidate-Conditioned Place Preference: Sex Differences and Brain-Derived Neurotrophic Factor
Cummins, E.D.; Griffin, S.B.; Duty, C.M.; Peterson, D.J.; Burgess, K.C.; Brown, R.W. (Johnson City, Tenn.)

287 Dopamine D1-D2 Receptor Heteromer Regulates Signaling Cascades Involved in Addiction: Potential Relevance to Adolescent Drug Susceptibility
Perreault, M.L.; O'Dowd, B.F.; George, S.R. (Toronto, Ont.)

297 Persistent Loss of Hippocampal Neurogenesis and Increased Cell Death following Adolescent, but Not Adult, Chronic Ethanol Exposure
Broadwater, M.A.; Liu, W.; Crews, F.T. (Chapel Hill, N.C.); Spear, L.P. (Binghamton, N.Y.)

306 Physiological Correlates of Neurobehavioral Disinhibition that Relate to Drug Use and Risky Sexual Behavior in Adolescents with Prenatal Substance Exposure

Prenatal Drug Exposure

316 Structural Brain Imaging in Children and Adolescents following Prenatal Cocaine Exposure: Preliminary Longitudinal Findings
Akyuz, N.; Kekapure, M.V. (New York, N.Y.); Liu, J.; Sheinkopf, S.J. (Providence, R.I.); Quinn, B.T.; Lala, M.D. (New York, N.Y.); Kennedy, D.; Makris, N. (Charlestown, Mass.); Lester, B.M. (Providence, R.I.); Kosofsky, B.E. (New York, N.Y.)

329 Prenatal Drug Exposure Moderates the Association between Stress Reactivity and Cognitive Function in Adolescence

338 Effects of Prenatal Cocaine Exposure on Social Development in Mice
Kabir, Z.D. (New York, N.Y.); Kennedy, B. (Minneapolis, Minn.); Katzman, A. (Iowa City, Iowa); Lahvis, G.P. (Portland, Oreg.); Kosofsky, B.E. (New York, N.Y.)

347 Cholinergic Transmission during Nicotine Withdrawal Is Influenced by Age and Pre-Exposure to Nicotine: Implications for Teenage Smoking

356 Author Index
357 Subject Index
Cholinergic Transmission during Nicotine Withdrawal Is Influenced by Age and Pre-Exposure to Nicotine: Implications for Teenage Smoking

Luis M. Carcoba  James E. Orfila  Luis A. Natividad  Oscar V. Torres  Joseph A. Pipkin  Patrick L. Ferree  Eddie Castañeda  Donald E. Moss  Laura E. O’Dell

Department of Psychology, University of Texas at El Paso, El Paso, Tex., USA

Key Words
Acetylcholine · Nicotine · Withdrawal · Adolescent · Nucleus accumbens

Abstract
Adolescence is a unique period of development characterized by enhanced tobacco use and long-term vulnerability to neurochemical changes produced by adolescent nicotine exposure. In order to understand the underlying mechanisms that contribute to developmental differences in tobacco use, this study compared changes in cholinergic transmission during nicotine exposure and withdrawal in naïve adult rats compared to (1) adolescent rats and (2) adult rats that were pre-exposed to nicotine during adolescence. The first study compared extracellular levels of acetylcholine (ACh) in the nucleus accumbens (NAC) during nicotine exposure and precipitated withdrawal using microdialysis procedures. Adolescent (postnatal day, PND, 28–42) and adult rats (PND60–74) were prepared with osmotic pumps that delivered nicotine for 14 days (adolescents 4.7 mg/kg/day; adults 3.2 mg/kg/day; expressed as base). Another group of adults was exposed to nicotine during adolescence and then again in adulthood (pre-exposed adults) using similar methods. Control rats received a sham surgery. Following 13 days of nicotine exposure, the rats were implanted with microdialysis probes in the NAC. The following day, dialysis samples were collected during baseline and following systemic administration of the nicotinic receptor antagonist mecamylamine (1.5 and 3.0 mg/kg, i.p.) to precipitate withdrawal. A second study compared various metabolic differences in cholinergic transmission using the same treatment procedures as the first study. Following 14 days of nicotine exposure, the NAC was dissected and acetylcholinesterase (AChE) activity was compared across groups. In order to examine potential group differences in nicotine metabolism, blood plasma levels of cotinine (a nicotine metabolite) were also compared following 14 days of nicotine exposure. The results from the first study revealed that nicotine exposure increased baseline ACh levels to a greater extent in adolescent versus adult rats. During nicotine withdrawal, ACh levels in the NAC were increased in a similar manner in adolescent versus adult rats. However, the increase in ACh that was observed in adult rats experiencing nicotine withdrawal was blunted in pre-exposed adults. These neurochemical effects do not appear to be related to nicotine metabolism, as plasma cotinine levels were similar across all groups. The second study revealed that nicotine exposure increased AChE activity in the NAC to a greater extent in adolescent versus adult rats. There was no difference in AChE activity in pre-exposed versus naïve adult rats. In conclusion, our results suggest that nicotine exposure during adolescence enhances baseline ACh in the NAC. However, the finding that ACh levels
were similar during withdrawal in adolescent and adult rats suggests that the enhanced vulnerability to tobacco use during adolescence is not related to age differences in withdrawal-induced increases in cholinergic transmission. Our results also suggest that exposure to nicotine during adolescence suppresses withdrawal-induced increases in cholinergic responses during withdrawal. Taken together, this report illustrates important short- and long-term changes within cholinergic systems that may contribute to the enhanced susceptibility to tobacco use during adolescence.

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Introduction

Adolescents display high rates of tobacco use initiation and are more likely to continue smoking into adulthood. Surprisingly little is known about the underlying biological mechanisms that promote tobacco use during adolescence and the long-term consequences of adolescent nicotine exposure. During tobacco cessation, withdrawal from nicotine produces a series of biochemical changes characterized by the emergence of negative affective states that promote compulsive tobacco use and relapse [1]. Given the importance of withdrawal in tobacco use, a better understanding of the mechanisms that mediate withdrawal will yield important information that may guide the development of better cessation treatments for smokers of different ages.

Preclinical studies have demonstrated that the negative affective states and behavioral signs of nicotine withdrawal are lower during adolescence. For example, nicotine-treated adolescent rats [2, 3] and mice [4] display fewer physical symptoms of withdrawal compared to adults. Moreover, withdrawal-associated deficits are less pronounced in adolescent versus adult rats in procedures assessing changes in brain reward function and place aversion [2, 5]. These studies suggest that adolescents suffer relatively less severe consequences of nicotine withdrawal than adults.

The mechanisms that mediate nicotine withdrawal appear to involve dopaminergic deficits in the mesolimbic pathway. This pathway originates in the ventral tegmental area and terminates in several forebrain structures, including the nucleus accumbens (NAC). Previous research has shown that nicotine-dependent adult rats display a decrease in extracellular levels of dopamine during withdrawal [6–8]. Recent work in our laboratory demonstrated that adolescent rats display reduced withdrawal-associated deficits in NAC dopamine versus nicotine-dependent adults [9]. Similarly, nicotine-treated adolescent rats display a reduced decrease in NAC dopamine following administration of a kappa-agonist compared to adults [10]. Subsequent studies revealed that adolescents are resistant to the decreases in NAC dopamine produced by withdrawal because of enhanced excitation via glutamate and reduced inhibition via GABA on dopamine cell bodies in the ventral tegmental area [11]. These studies suggest that age differences produced by nicotine withdrawal are mediated, in part, by dopamine transmission in the mesolimbic pathway.

There is also evidence suggesting that cholinergic transmission in the NAC is altered during nicotine withdrawal. Specifically, adult rats experiencing nicotine withdrawal display an increase in extracellular levels of acetylcholine (ACh) in the NAC [8]. This effect is also observed during withdrawal from amphetamine, cocaine, ethanol and morphine [12, 13]. Conditioned taste aversion and mild stress also increase NAC ACh, while lowering dopamine in this region [14]. These findings suggest that increases in ACh and decreases in dopamine in the NAC serve as biomarkers of negative affective states produced by withdrawal from drugs of abuse.

To our knowledge, no study has compared cholinergic transmission in the NAC during nicotine withdrawal in adolescent and adult rats. Moreover, the long-term effects of adolescent nicotine exposure on cholinergic transmission in the NAC have not been studied during nicotine exposure or withdrawal from this drug. To address these issues, the present study conducted a series of two-group comparisons between adult rats and either (1) adolescents or (2) adults that were exposed to nicotine during adolescence (pre-exposed adults). Within each experimental group, the rats were either nicotine treated or received a sham pump surgery. The first study compared extracellular levels of ACh in the NAC during nicotine exposure and withdrawal. In order to determine whether group differences in nicotine metabolism may have influenced our results, a second study compared blood plasma levels of cotinine (a nicotine metabolite) following 14 days of nicotine exposure. This study also compared acetylcholinesterase (AChE) activity levels as a metabolic marker of cholinergic transmission across our experimental groups.

Experimental Procedures

Animals

Male Wistar rats (n = 4–12 per group) were used. The adolescents were approximately postnatal day (PND)28 and the adults were PND60 at the time of the pump im-
plantation surgery. All rats were given free access to food and water throughout the study. Rats were pair-housed in a humidity- and temperature-controlled (20–22°C) vivarium using a 12-hour light/dark cycle with lights on at 8:00 p.m. The home cage consisted of a rectangular Plexiglas hanging cage (41.5 cm long x 31.7 cm wide x 32.1 cm high) with pine bedding. The food and water were located above the animals' living space on a wire platform enclosed within a filtered cover. The animals were bred in the animal vivarium of the Psychology Department from a stock of outbred rats from Harlan Inc. (Indianapolis, Ind., USA). The rats were bred onsite in order to minimize stress that might have occurred in the adolescents if they had been shipped and tested too close to their weaning period. All procedures were approved by the University of Texas at El Paso Animal Care and Use Committee and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Drugs**

(−)-Nicotine hydrogen tartrate and mecamylamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Mecamylamine was dissolved in 0.9% sterile saline and injected intraperitoneally in a volume of 1 ml/kg. All drugs were administered at physiological pH of 7.2–7.4.

**Surgical Procedures**

The rats were first anesthetized with an isoflurane/oxygen mixture (1–3% isoflurane). They were then prepared with 14-day osmotic pumps that were implanted subcutaneously parallel to the spine (Alzet model 2ML2, 1.0 μl/h; Durect Corporation, Cupertino, Calif., USA). The pumps were filled with nicotine (4.7 mg/kg/day for adolescents or 3.2 mg/kg/day for adults, expressed as base). The adolescents were exposed to nicotine between PND28–42 and the adults were exposed between PND60–74. A separate group of rats received 14 days of nicotine exposure during adolescence (4.7 mg/kg/day) and then received another 14 days of exposure later during adulthood (3.2 mg/kg/day). The nicotine pump was surgically removed after 14 days of exposure during adolescence to ensure the same level of nicotine exposure across experimental conditions. The naive (not pre-exposed) adults received a sham surgery during adolescence to control for the influence of the surgical procedures that may have influenced our comparisons to the pre-exposed adults. The concentration of nicotine in the pump was adjusted according to the weight of the rat at the time of the surgery. The nicotine concentrations were based on previous studies showing that adolescent rats require approximately 1.5 times more nicotine to produce similar plasma nicotine levels to adults [2, 15]. Following implantation, the incision was closed with 9-mm stainless steel wound clips and treated with a topical antibiotic ointment.

**Experimental Groups**

The controls for each group received a sham surgery and were not exposed to nicotine. Our experimental approach essentially involved a two-group comparison between adult rats and either (1) adolescents or (2) adults that were exposed to nicotine during adolescence (pre-exposed adults). The experimental conditions were as follows:

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**Study 1: Cholinergic Transmission in the NAc during Nicotine Withdrawal**

**Microdialysis Procedures**

Thirteen days after pump implantation, the rats were reanesthetized and stereotaxically implanted with microdialysis probes aimed at the NAc. The probes were purchased from CMA Microdialysis (model CMA 12, 20 kDa; Holliston, Mass., USA) with an active membrane length of 2 mm. The probes were perfused for at least 1 h prior to implantation at a rate of 0.5 μl/min with artificial cerebrospinal fluid composed of 145 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 5.4 mM D-glucose and 0.25 mM ascorbic acid (adjusted to a pH of approx. 7.2). The probes were implanted into the NAc using the following coordinates from bregma (adolescent = AP +2.2, ML ±0.8, DV –7.1; adult = AP +1.7, ML ±1.4, DV –8.1). The placements were derived from our previous studies using these age ranges [9]. The hemisphere that was implanted with the probe was counterbalanced to control for possible hemispheric differences across experimental conditions. Following surgery, the rats were individually housed in a similar-sized test cage as their home cage with food and water available throughout the following test day.

After surgery, the perfusate flow rate was maintained at 0.2 μl/min overnight. The next morning the flow rate was increased to 1.5 μl/min for 1 h to allow for equilibration of the probes. Dialysate samples were then collected at 10-min intervals for 1-h sampling periods during baseline and then following systemic administration of saline...
followed by 2 doses of mecamylamine (1.5 and 3.0 mg/kg, i.p., expressed as salt). The doses of mecamylamine were chosen based on previous studies demonstrating age differences in dopamine transmission produced by nicotine withdrawal [9, 11]. After collection, each sample was immediately frozen on dry ice and then stored at −80°C until they were analyzed 1–2 weeks later.

ACh Analysis

ACh was separated by a 12-cm reverse-phase ESA ACh-3 analytical column at 38°C. The ACh was converted to hydrogen peroxide and betaine by an ESA ACh solid-phase reactor containing AChE and choline oxidase. The estimation of ACh levels was assessed by electrochemical detection, as described previously [16]. Mobile phase was pumped through the system at a pump speed of 0.35 ml/min. The mobile phase contained 100 mM Na3HPO4, 0.5 mM trimethylammonium chloride, 0.005% Reagent MB (ESA) and 20 mM 1-octanesulfonic acid (Sigma-Aldrich), which was adjusted to a pH of 8.0 with 85% H3PO4 and degassed through a 0.2-μm filter. ACh levels were quantified with a model 5040 ESA analytical cell with a platinum target electrode set at +300 mV. The EC detector range (ESA Coulochem II) was set to 2 nA for ACh detection with a 10-second filter. Peaks were analyzed and recorded on a computerized data capture system. The detection limit for ACh was 7 fmol/min/10 μl. Peak identities were verified by their absence following removal of the solid-phase reactor column.

Histological Verification of Probe Placements

At the end of the experiment, the rats were sacrificed and the brains were frozen on dry ice. The probe placements were verified from coronal sections of the NAc. In order to be included in the neurochemical analysis, the probe placements had to fall within the NAc and the animals' baseline values had to fall within 2 standard deviations from the group mean.

Study 2: Metabolic Markers of Cholinergic Transmission in the NAc during Withdrawal

AChE Activity Assay

A separate group of rats received the same nicotine pump exposure as in study 1. Fourteen days after pump implantation, the rats were sacrificed and the brains were placed in ice-cold phosphate (Na) buffer at a pH of 8.0 containing 0.02 M MgCl2. Once each brain was cooled, the NAc was quickly dissected on a petri dish that was kept on ice. The NAc tissue was then weighed and frozen at −80°C until the samples were analyzed within the next couple of days. The AChE assays were conducted according to the spectrophotometric method of Ellman et al. [17] except at pH 7.4. The NAc was homogenized as a 16.7% w/v (1:6 w/v) in 0.1 M (Na)PO4 in a ground glass tissue homogenizer. The homogenate was then diluted 1:10 (1:60 total dilution) in PBS and that suspension was used as the source of AChE activity. A 150-μl aliquot of the 1:60 dilution of brain homogenate was added to 150 μl of Ellman's reagent and 4.0 ml of additional PBS. Finally, 290 μl of the AChE/Ellman's mixture was added into each well of a 96-well plate. The AChE reaction was initiated by the addition of 10 μl of acetylthiocholine Br solution (30, 6 or 3 mM) to make a final volume of 300 μl in each well, which made a 1-cm light path. The final concentrations of the acetylthiocholine substrates in the wells were 3.3, 6.67 or 33.3 μM and the change in absorbance at 412 nm was recorded for 4 min. In this assay, the final enzyme dilution was 1:1,781 w/v from wet weight tissue. Blanks were run using HPLC water instead of substrate. Km and Vmax values were calculated by least squares linear regression of 1/V versus 1/[S]. Finally, enzyme activity was expressed in moles of substrate converted min−1 gram−1 of wet weight tissue using Ellman's extinction coefficient.

Cotinine Assay

Trunk blood was collected at the time the rats were sacrificed. The blood was collected into test tubes and the plasma was separated from the whole blood via centrifugation at 2,000 rpm. The plasma was then separated and cotinine levels were analyzed using a commercially available 96-well plate ELISA kit from OraSure Technologies Inc. (Bethlehem, Pa., USA). The cotinine levels were estimated from internal standards using a SpectraMax Plus spectrophotometer from Molecular Devices Inc. (Sunnyvale, Calif., USA).

Statistical Analysis

The data were analyzed using SPSS Statistics for Windows, version 19.0 (Armonk, N.Y., USA). Our statistical approach involved overall ANOVAs followed by post hoc testing where appropriate using Fisher's LSD tests $(p < 0.05)$. The data in figures 1 and 3 (basal ACh and AChE activity) were analyzed using ANOVA with treatment (control vs. nicotine) and experimental group (adult, adolescent and pre-exposed adult) as between-subject factors. The data in figure 2 (ACh levels) were analyzed using repeated measures ANOVA with time as a within-subject factor and treatment and age group as between-subject factors. The experimental approach of this study is essen-
Fig. 1. The data reflect baseline levels of ACh (±SEM) in the NAc of adolescent (control n = 6; nicotine treated n = 7), adult (control n = 6; nicotine treated n = 6) and pre-exposed adult rats (control n = 4; nicotine treated n = 8). Asterisks denote a main effect of nicotine treatment relative to controls and the dagger denotes a significant difference from adult rats (p < 0.05).

Fig. 2. The data reflect extracellular levels of ACh (±SEM) in the NAc of adolescent (control n = 6; nicotine treated n = 7), adult (control n = 6; nicotine treated n = 6) and pre-exposed adult rats (control n = 4; nicotine treated n = 8). Mec. = Mecamylamine. Each point reflects a 10-min sampling period following saline administration and then administration of 2 doses of mecamylamine (1.5 and 3.0 mg/kg) to precipitate withdrawal (shaded areas). Asterisks denote a significant difference from respective controls (p < 0.05).

Results

Study 1
Baseline ACh

Figure 1 denotes baseline levels of ACh in the NAc of control and nicotine-treated adolescent, adult and pre-exposed adult rats. Overall, the results revealed that nicotine treatment increased baseline ACh compared to controls, an effect that was highest in adolescent rats. There was no interaction between treatment and experimental conditions (F2, 31 = 2.6; p = n.s.). However, there was a main effect of treatment (F1, 31 = 18.2; p < 0.001), illustrating that nicotine exposure produced an increase in baseline ACh regardless of age group (p < 0.05). There was also a main effect of experimental group (F1, 31 = 9.0; p < 0.001), with nicotine-treated adolescents displaying higher basal ACh compared to both adult groups, regardless of nicotine pre-exposure (p < 0.05). There were no differences in control rats across experimental groups.
adult rats. The overall analysis revealed a significant interaction between treatment and experimental group \((F_{2,31} = 30.15; p < 0.05)\). The post hoc analyses revealed that nicotine-treated adolescents and adults displayed an increase in ACh compared to control rats during each sampling period \((p < 0.05)\). However, this effect was not observed in pre-exposed adults. There were no differences in control rats across experimental groups.

**Study 2**

**AChE Activity**

Figure 3 depicts AChE activity in the NAc of adolescent, adult and pre-exposed adult rats. The results revealed that nicotine-treated adolescents displayed an increase in AChE activity that was higher than both groups of adult rats, regardless of nicotine pre-exposure. The analysis revealed a significant interaction between treatment and experimental condition \((F_{2,33} = 4.19; p < 0.05)\), with nicotine-treated adolescent rats displaying higher AChE activity levels compared to control rats \((p < 0.05)\) and both adult groups \((p < 0.05)\). There were no differences in control rats across experimental conditions.

**Cotinine Levels**

Figure 4 depicts cotinine levels \((\text{ng/ml} \pm \text{SEM})\) in blood plasma collected from nicotine-treated adolescent, adult and pre-exposed adult rats. The results revealed that there were no significant differences in cotinine levels across experimental groups after 14 days of nicotine exposure \((F_{2,27} = 0.89; p = \text{n.s.})\).

**Discussion**

**Summary**

A major finding of this report is that nicotine exposure produced an increase in basal levels of ACh in the NAc that was largest in adolescent rats compared to adults. Another important finding is that under withdrawal conditions, an increase in ACh was observed in the NAc that was similar in adolescent and naïve adult rats. Interestingly, the latter effect was absent in adult rats that were exposed to nicotine during adolescence. These findings suggest that adolescence is a unique period of development characterized by the following: (1) a short-term increase in cholinergic transmission produced by nicotine exposure during adolescence and (2) a long-term suppression of cholinergic responses during withdrawal in adult rats that were exposed to nicotine during adolescence.
The Role of ACh in the Process of Nicotine Addiction

The process of tobacco addiction involves activation of nicotinic ACh receptors (nAChRs) that consist of specific subunit configurations such as a4β2 and a7 [18]. Regarding the initial acquisition of nicotine use, a preclinical report demonstrated that blockade of nAChRs in the NAc disrupts the development of drug-seeking behavior in rats [19]. Thus, the initial phases of acquisition of tobacco use are likely to involve nAChRs in the NAc. Following chronic drug use, preclinical studies have demonstrated a dysregulation in brain reward mechanisms, including (but not limited to) changes in nAChRs and ACh release in the NAc. For example, a neurochemical 'marker' of nicotine withdrawal is increased ACh levels in the NAc [8]. Increased ACh levels are also observed during withdrawal from other drugs such as amphetamine, cocaine, ethanol and morphine [20, 21]. Further, conditioned taste aversion and mild stress also increase NAc ACh levels while lowering dopamine levels in this region [22]. These findings suggest that increases in ACh combined with a decrease in dopamine levels in the NAc serve as biomarkers of withdrawal from chronic nicotine exposure. The present study contributes to this literature by examining changes in cholinergic systems in the NAc following nicotine exposure and withdrawal from this drug during the adolescent period (short-term effects) and later in adulthood following exposure to nicotine during adolescence (long-term changes).

Short-Term Effects of Nicotine Exposure and Withdrawal during Adolescence

Preclinical studies have revealed that there are fundamental differences in the mechanisms that drive nicotine use among adolescents and adults [23–25]. The present study extends previous work by demonstrating that adolescent nicotine exposure produces an increase in basal cholinergic transmission, and that the increases in ACh produced by nicotine withdrawal are similar across age groups. Studies in other laboratories have compared changes in nAChR following nicotine exposure in adolescent and adult rats. This work has revealed that the changes in nAChRs are age, sex, receptor subtype and region dependent [26–29]. As an example, one report demonstrated that across several brain regions a4β2 and a7 nAChRs were more prominently increased following nicotine exposure in adult versus adolescent rats [28]. However, another report demonstrated a more prominent increase across several brain regions in nAChRs following nicotine exposure in adolescent versus adult rats [29]. Our results suggest that one potential mechanism for the changes in nAChRs may involve age-dependent changes in cholinergic tone following nicotine exposure. This is based on our finding that nicotine exposure produced a larger basal increase in ACh levels and AChE activity in adolescent versus adult rats. The implications of these effects with regard to enhanced vulnerability to tobacco use during adolescence warrants future investigation using animal models of nicotine dependence in rodents of different ages.

Much work has shown that the behavioral effects of nicotine withdrawal are lower in adolescent versus adult rodents [25]. Previous work in our laboratory suggests that dopamine systems modulate age differences produced by nicotine withdrawal. This is based on our finding that nicotine-treated adult rats displayed a robust decrease in NAc dopamine levels during withdrawal that was absent in adolescents [9]. Nicotine-treated adult rats also displayed a smaller decrease in NAc dopamine in adolescents versus adults following administration of a kappa-opiate receptor agonist [10]. Subsequent studies revealed that adolescents showed smaller decreases in NAc dopamine during withdrawal because of enhanced excitation via glutamate and reduced inhibition via GABA on dopamine cell bodies in the ventral tegmental area [11]. The present findings suggest that age differences produced by nicotine withdrawal are not likely to be modulated via cholinergic transmission in the NAc. Collectively, our research suggests that age differences produced by nicotine withdrawal are modulated, in large part, via dopaminergic transmission in the mesolimbic pathway.

Long-Term Effects of Adolescent Nicotine Exposure and Withdrawal Later in Adulthood

There is a large body of literature showing that developmental exposure to nicotine produces an array of behavioral and neurological consequences, especially during the neonatal period [30]. Within this literature, the long-term effects of adolescent nicotine exposure are complex and vary depending on what is measured. With regard to behavioral changes, rodent studies have demonstrated that adolescent nicotine exposure increases the rewarding effects of nicotine later in adulthood [31–33]. However, recent work in our laboratory demonstrated that adolescent nicotine exposure ameliorated the food suppressant effects of nicotine later in adulthood [33]. Also, exposure to nicotine during adolescence enhances anxiety-like behavior [34] but abolishes the corticosterone-activating effect of nicotine later in adulthood [35]. The present study revealed that withdrawal-induced increases in ACh were blunted following exposure to nico-
tine during adolescence. Consistent with this, gestational nicotine exposure produced a long-term suppression of nicotine-induced increases in NAc dopamine levels [36]. Adolescent nicotine exposure also produced a long-term suppression of nicotine-induced increases in striatal dopamine and norepinephrine [37]. Our plasma cotinine data rule out the possibility that the blunted neurochemical effects observed in pre-exposed adults are related to group differences in nicotine metabolism. Another possibility is that adolescent nicotine exposure may have produced neurotoxicity that leads to a blunted neurochemical response during nicotine withdrawal. This idea is based on the finding that adolescent nicotine exposure produces greater neuroteratogenicity compared to exposure to this drug during adulthood [38]. These findings have led researchers to question the use of nicotine replacement therapy in adolescent smokers [39]. Future studies are needed to examine the mechanisms that modulate our neurochemical effects.

Clinical Implications

Our findings have important implications toward developing more effective cessation strategies for smokers of different ages and previous exposure to nicotine. Our data suggest that adolescent nicotine exposure produces long-term changes in cholinergic systems that may reduce the efficacy of smoking cessation medications later in adulthood, such as nicotine replacement therapy. Consistent with this suggestion, clinical studies in adolescents have found that long-term abstinence rates are not closely associated with nicotine replacement therapies [40–42]. With regard to the long-term consequences of nicotine, our data suggest that adolescent nicotine exposure promotes the dysregulation of cholinergic systems that confer drug dependence. This includes enhancing the rewarding effects of nicotine and possibly altering the aversive effects of nicotine withdrawal. Thus, putting a nicotine patch on an adolescent may be harmful because it might facilitate the development of dependence in young tobacco users who do not normally experience nicotine withdrawal. Future studies are needed to better understand the underlying mechanisms that promote enhanced vulnerability to nicotine in an adult that was exposed to nicotine during adolescence. Future studies might also consider the role of sex differences, environmental conditions and prior drug history in developmental differences to nicotine use.

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