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A unique role for striatal serotonergic systems in the withdrawal from adolescent nicotine administration

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Abstract

Adolescent smokers experience more severe withdrawal symptoms upon smoking cessation than do adults, even when daily smoking has occurred for only a short period or with low levels of consumption. Animal models of nicotine withdrawal indicate involvement of striatal serotonin (5-HT) systems in nicotine reward, withdrawal and craving. We evaluated indices of striatal 5-HT and dopamine (DA) synaptic activity, neurotransmitter levels and turnover (metabolite/transmitter ratio), after continuous nicotine infusions to adolescent rats from postnatal days 30 to 47, using a dose rate (6 mg/kg/day) that produces plasma levels typical of smokers. Withdrawal was accompanied by a significant and persistent loss of striatal 5-HT synaptic activity, evidenced by an initial decline in turnover followed by a reduction in 5-HT content without a compensatory increase in turnover. Similar effects were seen for striatal DA activity. These effects were superimposed on a loss of stimulatory 5-HT cellular responses and promotion of inhibitory responses as identified in an earlier work with this model. None of these alterations was seen during withdrawal in adult rats given the same regimen. The unique adolescent withdrawal effects were not seen when the adolescent nicotine treatment period was shortened to early adolescence (days 30–37), even if the administration paradigm was changed to twice-daily injections to maximize withdrawal stress. Our results are consistent with unique effects of adolescent nicotine withdrawal on striatal 5-HT and DA systems, and point to a potential for serotonin-specific reuptake inhibitors as alternatives to nicotine replacement therapy for smoking cessation in adolescents.

Keywords: Adolescence; Dopamine; Nicotine withdrawal; Serotonin; Striatum

1. Introduction

Adolescence is associated with neurochemical and behavioral traits that contribute to increased susceptibility to substance abuse, dependence and addiction, including tobacco [6,13–15,31,35]. Most cigarette smokers begin as adolescents and early onset of smoking is highly correlated with subsequent heavy consumption and inability to quit [11]. The psychoactive ingredient of tobacco, nicotine, has unique effects on the adolescent brain that are distinguishable from those in the adult, comprising prolonged upregulation of nicotinic cholinergic receptors, persistent alterations in synaptic responses to a wide variety of neurotransmitters, and even outright neurotoxicity, and many of the effects are seen even with short-term exposures to low doses [1,4,31,38–44].

Recent work points to the importance of withdrawal in adolescent responses to nicotine. Adolescents show a rapid onset of nicotine dependence, with loss of autonomy over tobacco use and pronounced withdrawal symptoms upon abstinence [13–15,27,28], sometimes after consumption of as little as two cigarettes a day [13]. Withdrawal symptoms provide a major driving force sustaining adolescent tobacco use, including the emergence of craving, cognitive dysfunction and dysphoria, even to the extent of outright depression [8,10,13–16,18–20,27,28]. The affective changes point to the potential targeting of serotonin (5-HT) pathways, the projections most highly associated with the symptomatology and therapy of depressive disorders [23,26]. In animal models, adolescent nicotine administration, at exposures that reproduce the plasma levels of nicotine found in smokers, was found to evoke damage to 5-HT

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine, serotonin; ANOVA, analysis of variance; DA, dopamine; PN, postnatal; SSRIs, serotonin-specific reuptake inhibitors.

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terminals in the hippocampus and striatum [41,42]. However, the most substantial effects were seen during withdrawal, which was associated with a profound defect in 5-HT-mediated synaptic signaling, with stimulatory responses suppressed in favor of inhibitory output [34,43], an effect that was unique to 5-HT and was not shared by dopamine (DA) or norepinephrine [3].

The current study focuses on the striatal effects of withdrawal from adolescent nicotine administration. Along with DA, striatal 5-HT pathways are thought to play important roles in addiction and reward [9,12,17,21] and are an important site for nicotine-elicited reward function [29]. Nicotine effects on striatal 5-HT contribute to nicotine withdrawal symptoms [30], specifically those involving craving [45], one of the major factors in nicotine withdrawal in adolescents [13–16,27,28]. 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 requirement to sustain reward function [24]. We assessed 5-HT levels and fractional turnover, measures of presynaptic activity, in striatal projections during withdrawal from adolescent nicotine administration, with the results contrasted to those in the brainstem, a region containing 5-HT cell bodies. Turnover was calculated from the ratio of 5-hydroxyindole acetic acid (5-HIAA), the major 5-HT metabolite, to 5-HT [33,42]. Effects on 5-HT systems were also contrasted to those on DA levels and turnover, again using the metabolite ratio method [33,42], assessing dihydroxyphenylacetic acid and homovanillic acid, and calculating the ratio as [total metabolites]/DA. For the primary experiments, we utilized a standard adolescent nicotine administration paradigm, exposure to 6 mg/kg/day from postnatal days (PN) 30–47, which produces nicotine plasma levels of approximately 25 ng/ml [41]. The effects were compared to those elicited by the same dose given for the same duration to adult rats (PN90–107). Then, in additional experiments, we examined the dose and duration thresholds for withdrawal effects by shortening the adolescent treatment to PN30–37 and reducing the dose to 2 or 0.6 mg/kg/day. Finally, we pursued a different pattern of exposure, administering nicotine by twice daily subcutaneous injections instead of by continuous infusions.

2. Methods

2.1. Animals and treatments

All experiments were carried out in accordance with the declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. For minipump infusions in adolescent Sprague-Dawley rats (Zivic Laboratories, Pittsburgh, PA), on PN30, each animal was anesthetized lightly with ether, a 3 × 4 cm area on the back was shaved and an

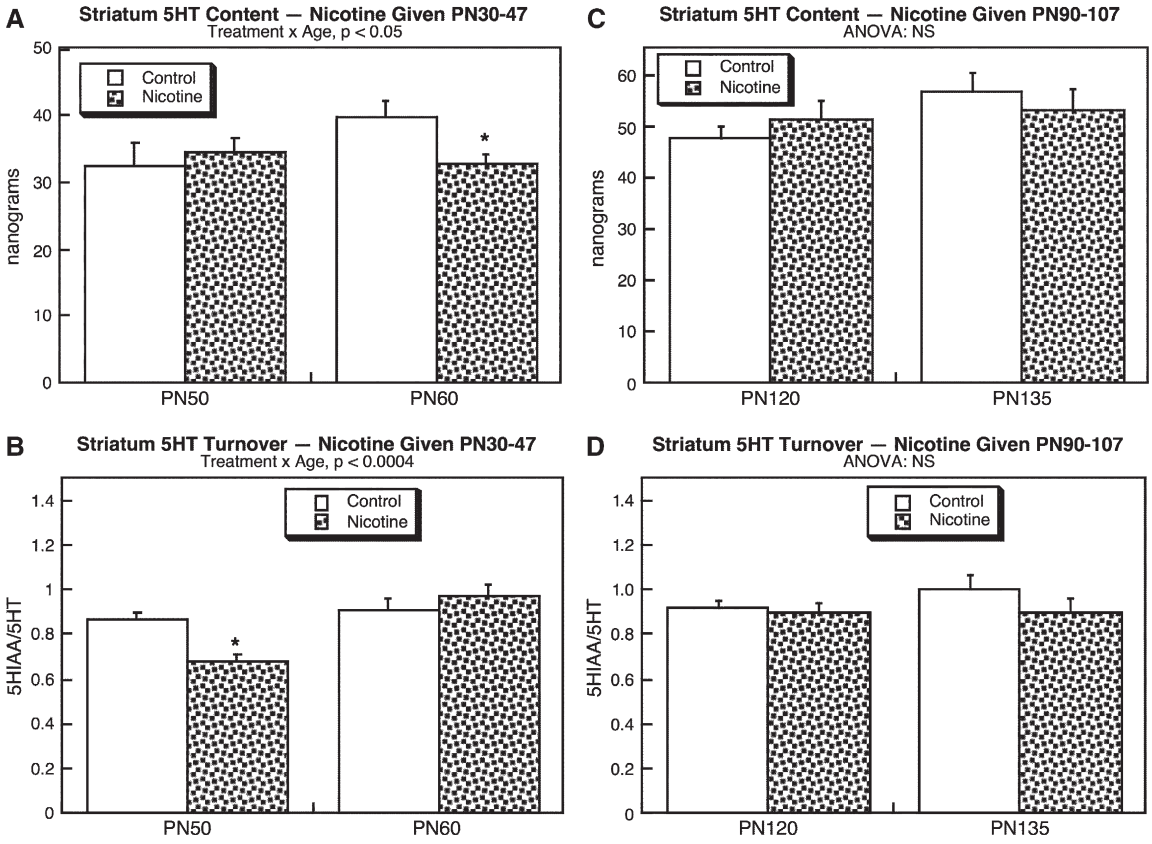

Fig. 1. Striatum 5-HT content and turnover in adolescent (A, B) and adult (C, D) rats during withdrawal after 17 days of continuous nicotine exposure (6 mg/kg/day). Results of multivariate ANOVA are shown at the top of each panel and asterisks (*) denote individual ages at which the nicotine group differs from the corresponding control.