



## Research report

# Adolescents and adults differ in the immediate and long-term impact of nicotine administration and withdrawal on cardiac norepinephrine



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## ABSTRACT

Cardiovascular responses to smoking cessation may differ in adolescents compared to adults. We administered nicotine by osmotic minipump infusion for 17 days to adolescent and adult rats (30 and 90 days of age, respectively) and examined cardiac norepinephrine levels during treatment, after withdrawal, and for months after cessation. In adults, nicotine evoked a significant elevation of cardiac norepinephrine and a distinct spike upon withdrawal, after which the levels returned to normal; the effect was specific to males. In contrast, adolescents did not show significant changes during nicotine treatment or in the immediate post-withdrawal period. However, beginning in young adulthood, males exposed to adolescent nicotine showed sustained elevations of cardiac norepinephrine, followed by later-emerging deficits that persisted through six months of age. We then conducted adolescent exposure using twice-daily injections, a regimen that augments stress associated with inter-dose withdrawal episodes. With the injection route, adolescents showed an enhanced cardiac norepinephrine response, reinforcing the relationship between withdrawal stress and a surge in cardiac norepinephrine levels. The relative resistance of adolescents to the acute nicotine withdrawal response is likely to make episodic nicotine exposure less stressful or aversive than in adults. Equally important, the long-term changes after adolescent nicotine exposure resemble those known to be associated with risk of hypertension in young adulthood (elevated norepinephrine) or subsequent congestive heart disease (norepinephrine deficits). Our findings reinforce the unique responses and consequences of nicotine exposure in adolescence, the period in which most smokers commence tobacco use.

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## 1. Introduction

It is increasingly clear that the heightened vulnerability of the adolescent to nicotine addiction entails underlying biological differences in nicotine responsiveness, with the adolescent brain showing exquisite sensitivity to nicotine (Dwyer et al., 2009; Lydon et al., 2014; Slotkin, 2008). Much less attention has been paid to the potential role of differential responses to nicotine withdrawal between adolescents and adults. Adolescents are resistant to activation or inhibition of reward pathways during withdrawal (Natividad et al., 2012) and tend to show lesser withdrawal signs, affective problems or anxiety compared to adults (O'Dell et al., 2006, 2007; Wilmouth and Spear, 2006). Although the mechanisms underlying these differences remain to be elucidated, there is

increasing evidence that differences in neurotransmitter responses to withdrawal underlie the behavioral disparities (Carcoba et al., 2014; Slotkin, 2002).

The current study focuses on peripheral autonomic components that may distinguish nicotine administration and withdrawal in the adolescent vs. the adult. Withdrawal from smoking in adults triggers autonomic stress responses, notably sympathetic activation, that are thought to contribute to difficulties in smoking cessation (Kotlyar et al., 2006; Sofuoglu et al., 2003); these include exacerbation of cardiac and vascular responses to acute stress (al'Absi et al., 2002; Vanderkaay and Patterson, 2006). In contrast, smoking cessation in adolescents does not trigger heart rate increases (Rubinstein et al., 2009) and may actually reduce heart rate (Killen et al., 2001). There is little known about potential central or peripheral neuronal mechanisms that may underlie these differences, nor is there any information as to whether nicotine exposure and/or withdrawal in adolescence might evoke persistent changes in autonomic function. Adolescent nicotine uniquely elicits long-term alterations in the function of central monoamine systems (Slotkin et al., 2007a; Slotkin and Seidler, 2009; Trauth et al., 2001), raising the

Abbreviations: ANOVA, analysis of variance.

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possibility that lasting effects might also occur in peripheral sympathetic activity. Here, we compared the effects of nicotine administration and withdrawal in adolescent and adult rats, assessing cardiac norepinephrine levels during treatment, during withdrawal, and for months after the termination of exposure. We also evaluated the specific role of withdrawal stress in the effects on cardiac norepinephrine by comparing continuously-administered nicotine (osmotic minipump infusions) to repeated nicotine injections, which elicit inter-dose withdrawal episodes (Abreu-Villaça et al., 2003; Slotkin, 1998, 2002). Our studies provide some of the first evidence for immediate and persistent biological differences in the autonomic effects of nicotine between adolescents and adults.

## 2. Materials and methods

### 2.1. Animal treatments

Although the determinations presented here are entirely new, the tissue samples were archived from earlier studies (Abreu-Villaça et al., 2003, 2004; Slotkin et al., 2008; Slotkin and Seidler, 2009), so that no additional animals were actually used. Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were shipped by climate-controlled truck (transit time <1 h) at least one week prior to commencing drug treatments. Animals were allowed free access to food and water and were housed individually to avoid potential problems with the surgical site that could occur with multiple animals per cage. Adolescent nicotine treatment commenced at 30 days of age. For treatments by minipump infusion, each rat was quickly anesthetized with ether, a  $2 \times 2$  cm area on the back was shaved, and an incision was made to permit subcutaneous insertion of an Alzet osmotic minipump (Durect Corp., Cupertino, CA), either type 1002 (two week infusion) or 1007D (one week infusion). Pumps were prepared with nicotine bitartrate (Sigma Chemical Co., St. Louis, MO) dissolved in bacteriostatic water (Abbott Laboratories, N. Chicago, IL), to deliver an initial dose rate of 6 mg/kg of nicotine (calculated as free base) per day. The incision was closed with wound clips and the animals were permitted to recover in their home cages. Control animals were implanted with minipumps containing only the water and an equivalent concentration of sodium bitartrate. It should be noted that the pumps actually take 17.5 (type 1002 pump) or 8 days (type 1007D) to be exhausted completely (information supplied by the manufacturer) and thus the nicotine infusions terminated at 47 days of age and 38 days of age, respectively; we previously showed that exhaustion of the pumps occurs at the predicted time by direct measurement of plasma nicotine and cotinine levels (Trauth et al., 2000). Plasma nicotine levels achieved with this administration model resemble those seen in typical smokers (25 ng/ml) as characterized previously (Trauth et al., 2000). For nicotine infusions in adults, type 2ML2 pumps were implanted at 90 days of age as already described, again set to deliver either vehicle or nicotine at an initial dose rate of 6 mg/kg per day, with the infusion terminating at 107 days of age, thus producing an exposure parallel to the longer infusion period in adolescents.

Experiments in adolescents utilizing subcutaneous nicotine injections were designed to deliver the same daily dose of nicotine in a bolus, thus providing a maximum pharmacokinetic contrast to the continuous infusion model. We administered nicotine for 7 days beginning at 30 days of age, delivered as two injections of 3 mg/kg 12 h apart, the maximum tolerated dose (Abreu-Villaça et al., 2003). Control animals received injections of bacteriostatic water containing an equivalent concentration of sodium bitartrate.

Studies were conducted at time points encompassing the period of nicotine administration, the posttreatment withdrawal period and long-term changes out to 6 months of age. Animals were

decapitated and tissues were dissected, frozen in liquid nitrogen, and stored at  $-45^{\circ}\text{C}$  until assayed.

### 2.2. Assays

The samples were thawed on ice and deproteinized by homogenization in 0.1N perchloric acid containing 3,4-dihydroxybenzylamine (Sigma) as an internal standard. Homogenates were sedimented at 26,000g for 20 min, the supernatant solutions were decanted, and norepinephrine was then trace-enriched by alumina adsorption, separated by reverse-phase high performance liquid chromatography and quantitated by electrochemical detection (Seidler and Slotkin, 1981); values were corrected for recovery of the internal standard.

### 2.3. Data analysis

To avoid the increase in type 1 errors that could occur from multiple statistical tests on the same data, each set of determinations was first evaluated using multivariate ANOVA considering all relevant variables (treatment, sex, age) in a single test; data were log-transformed because of heterogeneous variance between males and females, and among the different ages. Interactions of treatment with the other factors triggered subdivisions, each of which was then tested with lower-order tests. As permitted by the interaction terms, individual differences from controls were identified post-hoc with Fisher's Protected Least Significant Difference Test. However, where treatment effects were not interactive with other variables, we report only the main treatment effects without performing lower-order analyses of individual values. Significance was assumed at the level of  $p < 0.05$ .

Data were compiled as means and standard errors. For ready visualization of treatment effects across the different studies and ages, the results are given as the percent change from control values; Table 1 provides the corresponding control values, normalized across all studies. Statistical procedures were always conducted on the original data, with log transforms because of heterogeneous variance as noted above, and with treatment comparisons made only against the contemporaneous control group.

## 3. Results

### 3.1. Adolescent nicotine infusion

Nicotine infusions given to adolescents had effects on cardiac norepinephrine that were highly dependent on both age and sex (Fig. 1). Neither males nor females showed any significant changes during the period of nicotine treatment, nor did withdrawal trigger a change. However, beginning nearly a month after the termination of nicotine exposure, males exhibited a significant increase in cardiac norepinephrine ( $p < 0.02$  for the period from 75 to 105 days of age) that peaked a month later and then declined. Subsequently, a long-term deficit emerged that was still present at six months of age. In contrast, females showed no significant overall change in cardiac norepinephrine throughout this period.

### 3.2. Adult nicotine infusion

A very different pattern was obtained in adults given nicotine (Fig. 2). For males, there was a small, but significant elevation during the period of nicotine treatment, and then withdrawal elicited a sharp spike. Values declined to normal ten days later and remained within control limits throughout the ensuing two months, out to 180 days of age. At that point, the values for females were subnormal, but this effect should be interpreted with caution, since the overall ANOVA did not find a significant treatment effect or

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