Persistent and delayed behavioral changes after nicotine treatment in adolescent rats

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Abstract

Despite the increasing use of tobacco by adolescents, few animal studies have addressed the neurobehavioral consequences of nicotine exposure during this period. We administered nicotine to adolescent rats via continuous infusion on postnatal days (PN) 30 through 47.5, using a dosage regimen that maintains plasma levels similar to those found in smokers or in users of the transdermal nicotine patch. Behavior in a novel open field and learning a passive avoidance task were assessed during nicotine treatment and for 2 weeks post-treatment. On PN44, during nicotine exposure, female rats showed decreased grooming, an effect not seen in males; this effect is opposite to the effects of nicotine in adult rats. Two weeks after cessation of nicotine administration, females showed deficits in locomotor activity and rearing, whereas males again were unaffected; the behavioral deficits appeared at the same age at which gender-selective brain cell damage emerges. In contrast, nicotine exposure enhanced passive avoidance, with the effect intensifying and persisting throughout the post-treatment period. These results reinforce the concept that developmental vulnerability to nicotine extends into adolescence, with patterns of drug effects different from those in earlier or later periods. The correlation of neurochemical with behavioral effects strengthens the connection between adolescent nicotine exposure and persistent functional changes that may influence drug habituation, learning and memory.

Theme: Neural basis of behavior

Topic: Drugs of abuse: amphetamine and other stimulants

Keywords: Adolescence; Nicotine; Open field activity; Passive avoidance

1. Introduction

Recent estimates indicate that over one-third of U.S. high school students are using tobacco products and that nearly 3000 children under the age of 18 begin smoking every day [20]. Early initiation of smoking leads to higher daily consumption of cigarettes and a reduced probability of quitting [4,22,24]. Most life-long smokers begin their habit as adolescents [20,22] and it is therefore somewhat surprising that relatively little attention has been paid to establishing animal models of adolescent nicotine exposure. Recently, we found that adolescent rats given nicotine show gender-selective, persistent changes in nicotinic receptor expression, accompanied by brain cell loss [35], hippocampal damage that appears after several weeks’ delay [34,35], and persistent changes in cholinergic synaptic activity, particularly in the hippocampus [33]. Initial behavioral studies with adolescent nicotine exposure also found long-term changes in the learning related to reward tasks [14] but there have been no systematic evaluations of behavioral performance in the period during and immediately after adolescent nicotine administration.

Accordingly, the current study examines the effects of adolescent nicotine exposure on two sets of behaviors known to be affected by nicotine administration in adults: open field behaviors (locomotion, rearing, grooming) [3,5,13,36] and passive avoidance [23,29,37]. Nicotinic cholinergic pathways are essential in the acquisition of passive avoidance behaviors [19] and hippocampal cholinergic pathways are specifically involved [1]. We used an established minipump infusion model that produces and

Abbreviations: ANOVA, analysis of variance; PN, postnatal day
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maintains plasma nicotine levels within the range seen in
typical smokers or in users of the nicotine transdermal
patch and that, in the adolescent brain produces the
biochemical changes already described [33–35].

2. Methods

2.1. Animal treatments

All studies 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 prom-
ulgated by the National Institutes of Health. Litters of
Sprague–Dawley rats (Zivic Laboratories, Pittsburgh, PA)
were shipped with their dams by climate-controlled truck
(tot transit time less than 12 h). At weaning, postnatal
day (PN) 21, animals were housed individually and
allowed free access to food and water. Drug treatments
were administered by osmotic minipump infusions begin-
ning on PN30. Each animal was anesthetized lightly with
ether, a 3×4 cm area on the back was shaved and an
incision made to permit s.c. insertion of osmotic
minipumps (type 1002, Alza Corp., Palo Alto, CA). 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 per day [27,34,35]. 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 bacteriostatic
water and sodium bitartrate equivalent to the nicotine
bitartrate concentration. It should be noted that the pump,
marketed as a two week infusion device, actually takes
approximately 17.5 days to be exhausted completely
(information supplied by the manufacturer) and thus the
nicotine infusion terminates on PN47.5. This paradigm
demonstrably activates central nicotinic receptors
[17,21,28,34] and produces plasma nicotine levels similar
to those in typical smokers, approximately 25 ng/ml [35].
Cotinine levels are 6–8-fold higher [35], also as found in
human smokers; cotinine is also a potential developmental
neurotoxin [2]. This regimen produces a small (10%) reduc-
tion in body weight during treatment, an effect which
disappears immediately after termination of the infusion
[34], with little or no effect on brain region weight [34,35].
Unlike injected nicotine, the infusion of nicotine via
osmotic minipumps does not cause any overt signs of
hypoxia/ischemia, such as blanching of the skin or
cyanosis [26,27].

2.2. Behavioral testing

Behavior in a novel open field was tested during the
light cycle on PN44, PN50, or PN60; a different set of
animals was used at each age. Each animal was videotaped
for a 5-min period in a circular field (93-cm diameter×45-
cm height) divided into 10-cm squares. Scoring included
locomotion (squares crossed), rears and grooms. The
apparatus was cleaned in between animals with dilute
Lysol and dried. The day after open field testing (PN45,
PN51, or PN61), the animals were trained for passive
avoidance using the Gemini Avoidance System (San Diego
Instruments, San Diego, CA). The test apparatus contained
two chambers, each 21×25.5×16.5 (height) cm. Subjects
were placed in the lighted chamber and allowed up to 10
min to enter the darkened chamber, whereupon the door
closed and they received a mild foot shock (0.75 mA, 2 s).
Twenty-four hours later the animals were tested in the
apparatus and allowed up to 10 min to cross into the dark
chamber. For both types of behavioral tests, animals were
selected in a random order and were tested alternating
control and nicotine, male and female animals.

2.3. Data analysis

Data are presented as means and standard errors.
Treatment effects were analyzed initially using a repeated
measures ANOVA across all three open field indices
(locomotion, rearing and grooming), or across the two
passive avoidance measures (training, retention) with three
factors (treatment, age, gender); data were square-root- or
log-transformed because of heterogeneous variance. When
the initial analysis indicated an interaction of treatment
with the other variables, appropriate lower-order ANOVAs
were conducted, followed by post-hoc evaluations of
individual differences using Fisher’s Protected Least Sig-
ificant Difference; post-hoc tests were not conducted
where there were only main treatment effects (i.e. without
interactions with age or gender), in which case only the
main effect is reported. Significance was assumed at the
level of P<0.05 for main effects; for interactions, values
of P<0.1 were examined for lower-order main effects after
subdivision of the interactive variables [30].

3. Results

Global statistical analyses (Table 1) across all three
open field behaviors indicated significant main effects of
gender and significant differences among the measures
(main effect of measure). In terms of the effects of nicotine
administration, there were age-related treatment effects
that were distinct for each of the divers measures (inter-
action of treatment×age×measure). In addition, the ac-
tions of nicotine were gender-selective, as indicated by an
interaction of treatment×age×gender×measure. In light of
the interaction of treatment×measure×other variables, we
next subdivided the results into the three separate open
field measurements (locomotor, rearing, grooming). The
interactions of treatment×age and of treatment×age×
gender were still present for two of the three measures
(locomotor activity and grooming but not rearing). Therefore, results for open field activities are presented for each of the measures with subdivision by gender and age.

In female rats (Fig. 1, left panels), adolescent nicotine administration did not alter locomotor activity or rearing during the period of nicotine administration (PN44), nor was activity affected on PN50, immediately after the cessation of nicotine exposure. However, on PN60, nearly 2 weeks after discontinuing nicotine, locomotor activity was significantly reduced; rearing showed the same effect, and although the result did not achieve statistical significance due to greater variability for this measure, the overall effect of nicotine was not separable across the two measures (no interaction of treatment×measure). Grooming showed a completely different pattern, with significant reductions during drug administration and no significant effects thereafter. Male adolescent rats given the same nicotine treatment failed to show any significant differences in the three open field measures (Fig. 1, right panels).

Across both of the passive avoidance measures (training, testing), the global analysis indicated main effects of age and measure (Table 1). Nicotine treatment showed an effect that was selective for individual measures (treatment×measure interaction), as well as age and gender (interaction of treatment×age×gender×measure). However, when these were subdivided into training and testing, it was evident that nicotine did not affect training but rather influenced only the results of the subsequent testing measurement (main effect of treatment). Furthermore, the interaction of treatment with other variables could no longer be detected with the lower order tests. Accordingly, results for passive avoidance are presented individually for the two measures but without separation by gender.

During the training session for passive avoidance, adolescent nicotine exposure did not alter the latency to enter the dark compartment in which the animals received the foot shock (Fig. 2), regardless of whether the training session was conducted during the period of drug treatment (PN45), shortly after the cessation of exposure (PN51) or after 2 weeks (PN61). When these animals were retested for retention, however, the nicotine-treated animals performed better overall (main effect of treatment); the effect was not lost during the post-treatment period but rather intensified, superimposed on a general increase in latency with age (main effect of age).

### Table 1

Global statistical analyses

<table>
<thead>
<tr>
<th></th>
<th>All open field activities</th>
<th>Locomotor</th>
<th>Rearing</th>
<th>Grooming</th>
<th>Passive avoidance training and testing</th>
<th>Training</th>
<th>Testing</th>
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<tr>
<td>Treatment</td>
<td>NS</td>
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<td>P&lt;0.01</td>
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<tr>
<td>Gender</td>
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<td>P&lt;0.01</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.03</td>
<td>P&lt;0.0001</td>
<td>NS</td>
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<tr>
<td>Measure</td>
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<tr>
<td>Treatment×age</td>
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<tr>
<td>Treatment×measure</td>
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<tr>
<td>Age×gender</td>
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<tr>
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<tr>
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<td>P&lt;0.09</td>
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### 4. Discussion

The current study demonstrates that the neurochemical changes evoked by adolescent nicotine exposure [33–35] are indeed accompanied by both immediate and delayed behavioral alterations. Interestingly, we observed gender-selective effects in behaviors in an open field, with females affected to a greater extent than males: during nicotine exposure, females showed reduced grooming, and at PN60, 2 weeks after cessation of nicotine exposure, these animals also displayed reduced locomotion and rearing. This result closely resembles the temporal pattern and gender selectivity identified previously for changes in gene expression and biomarkers in the hippocampus, in which female rats exhibited increases in apoptosis-related genes, such as c-fos and p53 [35] and changes in membrane protein cell size markers [34], along with generalized suppression of cholinergic synaptic activity [33]. Future studies will need to determine if the late emergence of behavioral deficits is directly related to these neurochemical changes. In any case, the current results stand in direct contrast to the effects of nicotine seen in adult rats. Acute or chronic nicotine treatment of adults causes an increase in grooming.
Fig. 1. Effects of adolescent nicotine treatment (Rx) on open field activity. Data represent means and standard errors obtained from 10–12 rats in each group for each age and gender. ANOVA across all ages appears within the panels and asterisks denote individual values for which the nicotine group differs from the corresponding controls; individual tests were not conducted in the absence of a treatment x age interaction.

that is not gender-selective [3,13], rather than the decrease specific to females as seen here in adolescents; during withdrawal from nicotine, locomotor activity remains above or near control levels in adult females [5,36], whereas it was reduced in the adolescent. Our results thus provide some of the first behavioral information that
identifies adolescence as a unique, critical period for effects of nicotine on brain function.

The apparent lack of effect of adolescent nicotine exposure on open field activities in males was somewhat surprising, given our previous finding that, in some brain regions, nicotinic receptor upregulation persists longer in adolescent males than in females [34]. Indeed, males also show suppression of hippocampal cholinergic tone that persists after the cessation of nicotine exposure [33]. Male rats may simply be less susceptible to nicotine-induced alterations in locomotor behavior, as adult males also do not display differences under basal conditions, but do so when subjected to an acute challenge [9]. Accordingly, situations that require an integrated response or learning may be necessary to uncover the effects of adolescent nicotine exposure in males [6,14]. In the current study, we evaluated this possibility using passive avoidance, and in this case, the learning task evoked changes in both males and females. Unlike the results with open field activity, adolescent nicotine exposure actually enhanced passive avoidance 24 h after the training session. The temporal pattern of the effect was particularly interesting, as the improvement was minimal during nicotine administration but intensified in the post-treatment period; indeed, a preliminary report indicates that improved cognitive performance may persist for weeks or months after adolescent nicotine treatment [15]. In adults, cognitive tasks, including passive avoidance performance, show improvements with nicotine administration, but unlike the situation seen here, the peak effects are seen during treatment [12,16,23,25,29,32,37]. Again, these discrepancies may reside in the unique neurochemical effects of nicotine on the adolescent brain [33–35]. Studies with hippocampal slices from adults given chronic nicotine treatment demonstrate facilitated induction of long-term potentiation, an event associated with enhanced learning and memory [7,11]. However, nicotine enhances hippocampal learning through both stimulation and desensitization [8], and the adolescent brain shows a different temporal and regional pattern of nicotinic receptor upregulation from that seen in the adult [34], as well as displaying reductions in hippocampal cholinergic tone that persist into the post-treatment period [33]. It is thus likely that the differing patterns of neural activity and receptor expression of the adolescent brain produce distinct behavioral outcomes. Alternatively, the differences in passive avoidance behavior may again rest on the structural damage (cell loss, changes in cell size) evoked by adolescent nicotine exposure [34,35], or through unique effects on other neurotransmitter systems and our preliminary results (manuscript in preparation) indicate both immediate and delayed changes in synaptic activity as monitored by neurotransmitter turnover; again, these include long-term effects on hippocampal noradrenergic systems that are selective for the female gender. Regardless of the mechanisms underlying the behavioral differences between adults and adolescents given nicotine, it should be emphasized that whereas improved retention was observed with the passive avoidance paradigm, the same is not likely to be true for other learning or memory tasks, as nicotine is known to exert dual actions, improving performance in some tasks while impairing it in others [10,16,25,31,32].

The current findings indicate that the behavioral changes elicited by adolescent nicotine exposure differ from those seen in the adult and may emerge well after the cessation of treatment. Along with earlier work demonstrating effects on learning and memory [15] and on reward-directed behaviors lasting into adulthood [14], our results indicate that the vulnerability of the developing brain to nicotine extends into adolescence. Given the strong link of smoking initiation during adolescence with increased addiction liability and poorer cessation rates, these behavioral studies indicate the need for greater concern about the long-term consequences of adolescent tobacco use.

Acknowledgements

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References

[1] F. Anglade, G. Chapouthier, D. Galey, Intraseptal injection of scopolamine increases the effect of systemic diazepam on passive


