Early adolescent nicotine exposure affects later-life cocaine reward in mice

Mai Alajajia, Matthew f. Lazenkaa, Dena Kota a, Laura E. Wise a, Rabha M. Younis a, F. Ivy Carroll b, Amir Levine c, Dana E. Selley a, Laura J. Sim-Selley a, M. Imad Damaja, *

a Department of Pharmacology and Toxicology and Institute for Drug and Alcohol Studies, Medical Campus, Virginia Commonwealth University, Richmond, VA 23298-0613, USA
b Center for Drug Discovery, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709-2194, USA
c Columbia University, Department of Psychiatry, New York, NY 10032, USA

Article info

Article history:
Received 12 September 2015
Received in revised form
18 January 2016
Accepted 21 January 2016
Available online 22 January 2016

Keywords:
Nicotine
Cocaine
Adolescence
Mice
Reward
DeltaFosB

Abstract

Adolescence represents a unique developmental period associated with increased risk-taking behavior and experimentation with drugs of abuse, in particular nicotine. We hypothesized that exposure to nicotine during early adolescence might increase the risk for drug reward in adulthood. To test this hypothesis, male ICR mice were treated with a subchronic regimen of nicotine or saline during adolescence, and their preference for cocaine, morphine and amphetamine was examined using the conditioned place preference (CPP) test in adulthood. Long-term behavioral changes induced by nicotine suggested a possible role of altered gene transcription. Thus, immunoblot for \( \Delta FosB \), a member of the Fos family of transcription factors, was conducted in the nucleus accumbens of these mice. Mice treated with nicotine during early but not late adolescence showed an increase in CPP for cocaine, morphine and amphetamine later in adulthood. This effect was not seen in mice pretreated with a subchronic regimen of nicotine as adults, suggesting that exposure to nicotine specifically during early adolescence increases the rewarding effects of other drugs in adulthood. However, adolescent nicotine exposure did not alter highly palatable food conditioning in mice. The enhancement of cocaine CPP by nicotine was strain-dependent and was blocked by pretreatment with nicotinic antagonists. In addition, nicotine exposure during early adolescence induced \( \Delta FosB \) expression to a greater extent than identical nicotine exposure in adulthood, and enhanced cocaine-induced locomotor sensitization later in adulthood. These results suggest that nicotine exposure during early adolescence increases drug-induced reward in adulthood through mechanisms that may involve the induction of \( \Delta FosB \).

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1. Introduction

Adolescent drug use is highly predictive of susceptibility to drug abuse and addiction later in life. Adults with substance abuse disorders typically initiate drug use as adolescents, and the earlier the age drug use begins, the greater the likelihood of developing addition and with worse outcomes. For example, initiation of nicotine and tobacco product use typically begins during adolescence, at the average age of 13, and an estimated nine out of 10 adult smokers began smoking before age 18 (Kota et al., 2009; Dickson et al., 2011). In addition, tobacco and nicotine use during adolescence has been linked to use of tobacco, alcohol, cocaine and other illicit drugs in adulthood. For example, individuals who smoke cigarettes before the age of 15 are estimated to be 80 times more likely to use illegal drugs such as cocaine (Lai et al., 2000). Given the widespread use of tobacco and nicotine products among adolescents, there is a critical need to understand the relationship between adolescent use of nicotine and the future risk of addiction. While both human and animal studies are necessary to address this important issue, controlled animal studies are needed to fully understand the possible mechanisms for this age vulnerability.

Studies in rodents have also shown that adolescent nicotine exposure affects nicotine and cocaine reward and sensitivity later in adolescence (McQuown et al., 2007; Dao et al., 2011). We showed that mice exposed to nicotine for one week during early...
adolescence exhibited increased rewarding effects of nicotine in the conditioned place preference (CPP) test in adulthood (Kota et al., 2009). Nicotine administration during adolescence also alters the effects of psychomotor stimulants in adulthood. Nicotine exposure during adolescence has been reported to increase cocaine reward in adult rodents (Dickson et al., 2011, 2014; McMillen et al., 2005). Nicotine treatment during adolescence also enhanced the locomotor effects of psychomotor stimulants in adult animals (Collins and Izenwasser, 2004). However, neither the behavioral and age specificity of this increase in psychostimulant reward by nicotine nor the role of nicotinic receptor subtypes involved have been clearly identified.

In addition, the molecular and genetic mechanism(s) underlying these observations are not well understood. Although drugs of abuse have different initial pharmacological targets, most enhance mechanisms underlying enhancement of reward.

2. Materials and methods

2.1. Subjects

Male ICR mice were obtained from Harlan Laboratories (Indianapolis, IN) and male C57BL/6J (B6) and DBA/2J (D2) mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were treated during early (PND 28-34) or late (PND 47-59) adolescence, as defined in the literature (Spear, 2000). Possible litter effects were controlled by including mice from different litters in each test group. Mice were housed 4 per cage in a humidity and temperature controlled vivarium on a 12-h light/dark cycle with food and water ad libitum and were acclimated for 7 days prior to experiments. Animals were maintained in an AAALAC approved facility, and procedures were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

(+)-Nicotine hydrogen tartrate salt [(-)-1-methyl-2(3-pyridyl)pyrrolidine (+)-bitartrate salt, methyllycaconitine citrate salt (MLA), mcamylamine hydrochloride [2-(methylamino)isocamphane hydrochloride] and dihydro-β-erythroidine (DHβE) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). The α7 nicotinic agonist PHA-543613 (N-3R)-1-Azabicyclo[2.2.2]oct-3-yl-furo[2,3-c]pyridine-5-carboxamide hydrochloride, morphine sulfate, α-ampethamine and cocaine HCl were provided by the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). All compounds were injected subcutaneously (s.c.) except for cocaine, which was injected intraperitoneally (i.p.) at a volume of 10 ml/kg body weight. Control groups received saline injections at the same volume and by the same route of administration. All drugs were dissolved in sterile saline (0.9% sodium chloride) and prepared fresh before each experiment. All doses are expressed as the free base of the drug.

2.3. Drug exposure protocol

Nicotine (0.1, 0.5 and 1 mg/kg) or saline was administered to early adolescent (PND 28) mice s.c. twice daily (09:00 and 16:00) for either 1 (acute) or 7 (repeated) days. Mice were then housed in their home cages and allowed to reach adulthood (>PND 70), at which point they were evaluated as described below. For the studies with the α7 nicotinic agonist, PHA-543613 (8 mg/kg, s.c.) or saline was given to early adolescent (PND 28) mice s.c. twice daily (09:00 and 16:00) for 7 days. Mice were then housed in their home cages and allowed to reach adulthood (>PND 70), at which point they were tested.

Control studies to examine nicotine treatment in late adolescent and adults were performed using a separate group of mice. Nicotine (0.5 mg/kg) or saline was administered to late adolescent (PND50) and adult (PND 70) mice s.c. twice daily (09:00 and 16:00) for 7 days. Mice were then housed in their home cages for 35 days and then tested in behavioral and molecular experiments.

For the strain differences study, nicotine (0.5 mg/kg) or saline was administered to early adolescent (PND 28) C57BL/6J and DBA/2J mice s.c. twice daily (09:00 and 16:00) for 7 days. Mice were then housed in their home cages and allowed to reach adulthood (PND 70), at which point they were tested.

2.4. Conditioned place preference test

Mice were tested for morphine, amphetamine- and cocaine-induced preference using the CPP paradigm. The place conditioning chambers and software were purchased from Med Associates (St. Albans, VT). Place conditioning boxes consisted of two distinct sides (20 cm × 20 cm × 20 cm) separated by a smaller center gray compartment. Openings from the center compartment allowed access to either side of the chamber. An unbiased CPP paradigm was utilized in this study as described in Kota et al. (2007). On day 1, animals were placed in the boxes and allowed to move freely from side to side for 15 min, and time spent in each side was recorded. On days 2–4 (conditioning days), twice per day, mice were injected with saline or drug [morphine (5 mg/kg, s.c.), amphetamine (0.2 mg/kg s.c.) or cocaine (1, 5 or 10 mg/kg i.p.)] and subsequently paired with either the white or black chamber, where they were allowed to roam for 15 min. Vehicle-treated animals were paired with saline in both chambers and drug-treated animals received saline in one chamber and drug in the opposite chamber. Pairing of the drug with either the black or white chamber was randomized within the drug-treated group of mice. Animals in the drug group received drug each day. Injections were counterbalanced so that some mice received drug in the morning, others in the late afternoon. On day 5 (test day), mice did not receive an injection. They were placed into the center chamber for 5 min, the partitions were lifted, and they were allowed to roam freely for 15 min. Data are expressed as preference score (time spent on drug-paired side compared to saline-paired side) expressed as preference score (time spent on drug-paired side compared to saline-paired side).
To determine if adolescent nicotine exposure broadly affects conditioning, food place conditioning was measured using highly palatable food (Kraft Classic Philadelphia Cheesecake, Deerfield, IL) or standard laboratory chow (Harlan, Laboratories; Indianapolis, IN), as we previously described in Sanjakdar et al. (2015). Briefly, mice that were exposed to nicotine or saline during early adolescence were conditioned as adults (PND70) as described with drug CPP with the following exceptions. Immediately after establishing baseline preference (Day 1), mice were allowed to consume the highly palatable food (or standard chow) for the next 4–6 h in the home cage. Next, the highly palatable food (or standard chow) was paired with one large chamber during which the standard chow was paired with the other large chamber during daily 40 min sessions that occurred for the next 6 days.

2.5. Cocaine locomotor sensitization

For this study, only early adolescent mice (PND 24-30) were pretreated with saline or nicotine (0.5 mg/kg) injections for 7 days twice daily as describe above. Briefly, once the mice had reached PND 70, a 13-day cocaine sensitization protocol began. All of the behavioral procedures were performed during the light phase of the light/dark cycle between 10:00 am and 2:00 pm. Three days before starting the experiments, mice received a daily i.p. saline injection and were subjected to the locomotor activity chambers (30 min) to minimize stress induced by experimenter handling, the injection procedures, and exposure to the novel environment. The activity count of the last day was considered as the baseline activity of mice. Mice were randomly divided into three groups: saline—saline, saline—cocaine, and cocaine—cocaine (groups represent the acquisition day drug followed by the challenge day drug). Mice were then given another injection of either saline or cocaine 20 mg/kg (i.p.), depending on the assigned group, and placed in the chambers again for a 30 min acquisition period. This procedure was repeated on days 2–5. Days 6–12 were considered a drug free week in which the animals were not given injections or exposure to the chambers. On day 13, mice were tested again in the same manner as described for days 1–5, but mice in the cocaine group received a challenge dose of cocaine of 5 mg/kg (i.p.). Counts were recorded as number of photocell interrupts after a 30 min test period.

2.6. Plasma nicotine and cotinine levels

PND 28 male B6 and DBA mice were exposed to nicotine for 7 days as detailed above. Fifteen minutes after the last injection, mice were decapitated and trunk blood was collected and immediately centrifuged for 10 min. Blood plasma was stored for 7 days at −80 °C. Plasma nicotine and cotinine levels were measured using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) as previously described (AliSharari et al., 2013). At least five animals were used per group.

2.7. Immunoblotting

Adolescent and adult mice received nicotine or saline twice daily for 7 days as described above and were then sacrificed for immunoblot studies (Zacharioiu et al., 2006; Lazenka et al., 2014). Tissue from the nucleus accumbens (NAc) was homogenized in 20 mM HEPES buffer (pH 7.8) with 0.4 M NaCl, 20.0% glycerol, 5.0 mM MgCl2, 0.5 mM EDTA, 0.1 mM EGTA, and 1% NP-40 (EMSA buffer) containing 500 μM dithiothreitol and Hal™ protease inhibitor cocktail. Samples were loaded in 10% Tris–HCl gels and separated by electrophoresis. Gels were transferred onto nitrocellulose paper, blocked and incubated in FosB (1:1000; Cell Signaling Technology, Beverly, MA, USA) and α-tubulin loading control (1:5000; Upstate, Temecula, CA, USA) antibodies. Blots were washed and incubated with Alexa 680 goat anti-rabbit IgG (1:12,000) and Alexa 800 goat anti-mouse IgG (1:12,000) for 45 min at room temperature. Fluorescent intensity was visualized using the Odyssey LI-COR infrared scanner. LI-COR software version 2.1 was used to measure integrated intensity between treatments for the band of interest, with subtraction of the background (average of intensities 3 border widths above and below the band).

2.8. Data analysis

For all data, graphs and statistical analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software; San Diego, CA). All CPP results were expressed as mean preference scores ± standard error of the mean. Preference scores were measured in seconds, and indicate time spent in the drug-paired side during post-conditioning – time spent in the drug-paired side pre-conditioning (baseline). Statistical analyses of all behavioral and ΔFosB studies were performed with mixed-factor (one or two-way) ANOVA and Bonferroni post hoc analyses were used to determine significant differences between groups (p < 0.05).

3. Results

3.1. Early-adolescent nicotine exposure enhances cocaine-induced CPP in adulthood

To assess the effect of early-adolescent nicotine exposure on cocaine-mediated reward-like effects in adulthood, ICR mice were pretreated with nicotine (s.c.) during early adolescence (PND 28), and then allowed to mature to adulthood (PND 70) before CPP testing. In the first study, the effect of a one-day exposure to either saline or nicotine (0.1 or 0.5 mg/kg, two injections at 8 am and 5 pm) in PND 28 mice was investigated. At PND 70, these mice were then conditioned with cocaine or saline in the CPP test. As shown in Fig. 1A, mice developed a significant preference [F (1, 36) = 104, p < 0.0001] for the chamber paired with cocaine (10 mg/kg) as compared to saline-conditioned mice. However, two-way ANOVA analysis revealed no significant effect of one-day adolescent nicotine pretreatment [adolescent pretreatment × adult CPP treatment; F (2, 36) = 0.12, p = 0.8906] on cocaine preference.

Since one-day exposure to nicotine did not affect cocaine CPP, ICR mice were treated with three different doses of nicotine (0.1, 0.5, or 1.0 mg/kg) for 7–days during adolescence (PND28–35) and tested for cocaine CPP at PND 70. As shown in Fig. 1B, one-week exposure to nicotine during early adolescence significantly increased the preference for cocaine in adulthood [two-way ANOVA adolescent pretreatment × adult CPP treatment; F (7, 42) = 30.49, p < 0.0001] in a nicotine treatment dose-related manner as compared to mice that received saline during adolescence. In addition to the first group that was treated with nicotine and then cocaine 10 mg/kg, another group of early-adolescent mice (PND 28) received 0.5 mg/kg nicotine (2 × 1× day for 7 days) and animals were tested at PND70 with various doses of cocaine (1, 3, and 10 mg/kg) in the CPP procedure. Pretreatment with 0.5 mg/kg nicotine during adolescence produced a leftward shift in the cocaine dose–response curve (Fig. 1C), and subsequent doses of 5 and 10 mg/kg cocaine evoked a significant CPP response in adulthood as compared to saline control mice [F (2, 40) = 14.33, p < 0.001]. The role of nicotinic receptor subtypes in enhancing the effect of cocaine was investigated using three nicotinic antagonists and a selective agonist. Studies were first conducted by pretreating adolescent ICR mice with the nonelective nicotinic antagonist...
mecamylamine followed 10 min later by nicotine administration to determine whether blocking nicotinic receptors prevents nicotine-induced enhancement of cocaine preference in adulthood. Fig. 2A shows that pretreatment of early adolescent mice with mecamylamine (2 mg/kg s.c.) and nicotine (0.5 mg/kg, s.c. 2 ×/day for 7 days) prevented the nicotine-induced enhancement of cocaine CPP in adulthood \( F(4,28) = 4.169, p = 0.009 \). These results suggest that the activation of nicotine receptors (nAChRs) is required for nicotine-induced enhancement of cocaine in the CPP test. Furthermore, administration of DHβE (2 mg/kg), a selective antagonist for β2* nAChR subtypes, before daily nicotine pretreatment in adolescence, also blocked nicotine-induced enhancement of cocaine preference in the CPP test. Furthermore, administration of DHβE (2 mg/kg), a selective antagonist for β2* nAChR subtypes, before daily nicotine pretreatment in adolescence, also blocked nicotine-induced enhancement of cocaine CPP in adulthood \( F(4,28) = 4.169, p = 0.009 \). These results suggest that the activation of nicotine receptors (nAChRs) is required for nicotine-induced enhancement of cocaine in the CPP test. Furthermore, administration of DHβE (2 mg/kg), a selective antagonist for β2* nAChR subtypes, before daily nicotine pretreatment in adolescence, also blocked nicotine-induced enhancement of cocaine CPP in adulthood \( F(4,28) = 4.169, p = 0.009 \). These results suggest that the activation of nicotine receptors (nAChRs) is required for nicotine-induced enhancement of cocaine CPP in adulthood.

To determine whether enhancement of cocaine preference is specific to nicotine treatment during early adolescence, late adolescent (PND 50) and adult (PND 70) ICR mice were administered nicotine (0.1 or 0.5 mg/kg) for 7 days, and then tested for cocaine-induced CPP after the same drug-free period (36 days) as early adolescents (Fig. 3A and B). All mice conditioned with cocaine in the CPP test exhibited a significant preference for the cocaine-paired side, as shown by one-way ANOVA \( F(2, 27) = 124.9; p < 0.0001 \) (Fig. 3A). In contrast to data from mice that received nicotine during early adolescence (Fig. 1B), mice treated with nicotine during late adolescence did not demonstrate any
significant differences from mice pretreated with saline when assessed for cocaine CPP in adulthood \[F(5, 27) = 0.57; p > 0.05\] (Fig. 3A). Similarly, mice that received either nicotine or saline during adulthood displayed similar preference for cocaine \[F(5, 27) = 34; p > 0.05\] (Fig. 3B).

### 3.2. Early-adolescent nicotine exposure enhances amphetamine- and morphine-induced CPP in adult mice

Studies were then conducted to determine whether nicotine treatment during early adolescence affects preference for other abused drugs in adulthood. Early-adolescent ICR mice (PND 28) were pretreated with nicotine (0.5 mg/kg, s.c.) or saline twice daily with injections approximately 6 h apart (9:00 am and 3:00 pm) for 7 days. Mice were then housed in their home cages and allowed to mature until they reached adulthood (>PND 70), at which point they were evaluated in the CPP test as described in Materials and Methods. Data are expressed as mean ± S.E.M. of n = 6 mice/group. "p < 0.05 vs saline control group. dihydro-β-erythroidine = DHβE.

<table>
<thead>
<tr>
<th>Adolescent treatment</th>
<th>CPP treatment</th>
<th>Preference score (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline/Saline</td>
<td>Saline</td>
<td>13 ± 9</td>
</tr>
<tr>
<td>Saline/Saline</td>
<td>Cocaine</td>
<td>154 ± 35*</td>
</tr>
<tr>
<td>DHβE/Saline</td>
<td>Saline</td>
<td>15 ± 17</td>
</tr>
<tr>
<td>DHβE/Saline</td>
<td>Cocaine</td>
<td>156 ± 43*</td>
</tr>
<tr>
<td>MLA/Saline</td>
<td>Saline</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>MLA/Saline</td>
<td>Cocaine</td>
<td>167 ± 21*</td>
</tr>
<tr>
<td>Mecamylamine/Saline</td>
<td>Saline</td>
<td>15 ± 31</td>
</tr>
<tr>
<td>Mecamylamine/Saline</td>
<td>Cocaine</td>
<td>162 ± 25*</td>
</tr>
</tbody>
</table>

Fig. 3. The influence of adolescent period of nicotine exposure on cocaine condition preference in adulthood. (A) Late adolescent (PND 50) mice were injected s.c. with either saline or nicotine (0.1 or 0.5 mg/kg) twice a day for 7 days, and were assessed for cocaine CPP test on PND 92. (B) Adult (PND 70) mice were injected s.c. with either saline or nicotine (0.1 or 0.5 mg/kg) two times per day for 7 days, and were assessed for the cocaine CPP test on PND 112. The legend represents the pretreatment group during early adolescence. Results are expressed as mean ± SEM of n = 8/group. "p < 0.05 from respective saline control.
significant preference for the drug-paired side as compared to their respective saline controls. Interestingly, mice that received nicotine during adolescence displayed a significantly enhanced preference to amphetamine compared to mice that received saline during adolescence \(F(3, 26) = 17.18, p < 0.05\). A similar increase in morphine-induced CPP was also observed (Fig. 4B). Indeed, mice that received nicotine during adolescence displayed a significantly enhanced preference to morphine compared to mice that received saline during adolescence \(F(3, 23) = 17.44, p < 0.05\).

### 3.3. Enhancement of cocaine CPP by adolescent nicotine exposure is strain-dependent

The effect of genotype on nicotine enhancement of cocaine CPP was investigated by treating B6 and D2 mice, well known and used inbred strains in drug abuse studies, with nicotine during early adolescence (0.5 mg/kg twice per day for 7 days). Two-way ANOVA \(F_{\text{strain}}(1, 47) = 208.7, p < 0.0001\) showed that cocaine CPP tested in adulthood was significantly enhanced in B6 mice treated with nicotine during adolescence as compared to mice that had received saline (Fig. 5). In contrast, cocaine preference in D2 mice that received nicotine during adolescence did not significantly differ from their saline pretreated controls (Fig. 5). In addition, similar plasma levels of nicotine were found in the blood of B6 and D2 mice treated with nicotine for 7 days (Table 2). Plasma nicotine levels were measured. Data are expressed as mean ± S.E.M. of n = 5 mice/group. *p < 0.05 vs C57 mice.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Nicotine levels (ng/ml)</th>
<th>Cotinine levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>45.7 ± 3.6</td>
<td>30.5 ± 6.2</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>41.7 ± 6.1</td>
<td>43.4 ± 2.8*</td>
</tr>
</tbody>
</table>

Taken together, the results of behavioral studies demonstrate that repeated nicotine administration in early, but not late, adolescence enhances the potency of cocaine to induce CPP in adulthood, and that this effect is both \(\beta2^*\) nAChR-dependent and strain-specific.

### 3.4. Adolescent nicotine exposure did not alter highly palatable food conditioning

To assess whether the effect of adolescent nicotine treatment is specific for drug place conditioning, drug conditioning for highly palatable food was examined. Fig. 6 shows that a significant preference was observed for highly palatable food compared to standard chow \(F_{(1,24)} = 31.85; p < 0.0001\) in ICR mice. However, no effect of adolescent nicotine pretreatment was observed on palatable food conditioning (p > 0.05). These data suggest that adolescent nicotine does not generally alter reward-related behaviors using these food treatment conditions.
demonstrate that early adolescent nicotine exposure enhances the saline pretreated animals (p < 0.05) as compared to pretreated with nicotine during adolescence showed increased molecular changes occur. Studies were therefore conducted to determine whether other cocaine dependence related-behaviors are similarly affected, ICR mice were treated as described above and assessed for cocaine-induced locomotor sensitization. Results shown in Fig. 7 demonstrate that nicotine treatment during early adolescence also enhances cocaine-induced behavioral sensitization in adulthood. A day before the acquisition period, the three different groups (saline, saline—cocaine, and cocaine—cocaine) did not differ in their baseline locomotor activity [F(5,25) = 2.02; p = 0.109] (Fig. 7B). On challenge day, one group received saline i.p., while the other two groups received an injection of cocaine (5 mg/kg, i.p.). Both saline and nicotine pretreated mice that received cocaine during acquisition displayed enhanced locomotor activity as compared to those mice treated with saline during acquisition [F(5,25) = 33.57; p < 0.0001] (Fig. 7C). However, mice that were pretreated with nicotine during adolescence showed increased sensitization to cocaine—induced locomotor activity as compared to saline pretreated animals (p < 0.05) (Fig. 7C). These results demonstrate that early adolescent nicotine exposure enhances the induction of locomotor sensitization to cocaine.

ΔFosB induction is greater in mice treated repeatedly with nicotine during early-adolescence than mice that received nicotine as adults. The longevity of the enhanced behavioral responses to cocaine after adolescent treatment with nicotine suggests that persistent molecular changes occur. Studies were therefore conducted to measure expression of ΔFosB, a stable transcription factor that has been implicated in the rewarding effect of drugs of abuse.

Adult ICR mice (PND 70) were treated with nicotine (0.5 mg/kg, 2 injections each day) for 7 days, and the NAc was collected 24 h after the last nicotine injection (PND77). Immunoblot showed that ΔFosB levels in the NAc increased by approximately 1.4-fold [p < 0.005] compared to levels in saline-treated control mice (Fig. 8). Early-adolescent mice (PND 28) were similarly treated with saline or nicotine for 7 days, and the NAc was collected 24 h later (PND 35). Results showed that repeated nicotine exposure during early adolescence significantly increased ΔFosB by approximately 4-fold [p < 0.005] compared to saline-treated controls (Fig. 8). Therefore, while repeated administration of nicotine induced ΔFosB expression in both adolescent and adult mice, a greater induction was measured in mice treated during early adolescence.

3.5. Effects of adolescent nicotine exposure on locomotor sensitization to cocaine

The studies above showed that nicotine exposure during adolescence enhanced cocaine reward in adulthood. In order to determine whether other cocaine dependence related-behaviors are similarly affected, ICR mice were treated as described above and assessed for cocaine-induced locomotor sensitization. Results shown in Fig. 7 demonstrate that nicotine treatment during early adolescence also enhances cocaine-induced behavioral sensitization in adulthood. A day before the acquisition period, the three different groups (saline, saline—cocaine, and cocaine—cocaine) did not differ in their baseline locomotor activity [F(5,25) = 2.02; p = 0.109] (Fig. 7B). On challenge day, one group received saline i.p., while the other two groups received an injection of cocaine (5 mg/kg, i.p.). Both saline and nicotine pretreated mice that received cocaine during acquisition displayed enhanced locomotor activity as compared to those mice treated with saline during acquisition [F(5,25) = 33.57; p < 0.0001] (Fig. 7C). However, mice that were pretreated with nicotine during adolescence showed increased sensitization to cocaine—induced locomotor activity as compared to saline pretreated animals (p < 0.05) (Fig. 7C). These results demonstrate that early adolescent nicotine exposure enhances the induction of locomotor sensitization to cocaine.

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4. Discussion

In the present study, we have shown that repeated, but not short-term administration of nicotine during early adolescence enhanced cocaine CPP in adult mice. These behavioral changes are long -lasting and dependent upon the age at which nicotine is administered. Furthermore, the effects of nicotine are mediated by β2 nAChR subtypes and are strain-dependent. In addition, nicotine’s effects in enhancing CPP extended to other drugs of abuse (amphetamine and morphine) but not to CPP induced by highly palatable food. Furthermore, exposure to nicotine during early adolescence enhanced locomotor sensitization to cocaine during adulthood. Finally, our initial molecular studies showed that ΔFosB accumulation in the NAc might be an important player in these maladaptive events after exposure to nicotine in adolescence because ΔFosB is induced to a greater extent by nicotine in early adolescence than in adulthood.

Repeated but not acute pretreatment with nicotine in early adolescence increased cocaine-induced CPP in a dose-related manner during adulthood, suggesting a sustained effect of nicotine exposure. Thus, enhanced cocaine conditioned reward as a result of adolescent exposure to nicotine may lead to neuro-adaptations that predispose individuals to consume more cocaine. Importantly, the enhancement of cocaine CPP by nicotine depends on the age of exposure to the drug. Indeed, an increased behavioral response to cocaine was observed when nicotine exposure occurred during early adolescence (PND 28—34), but not during late adolescence or adulthood. These findings indicate that early adolescence is a critical period of vulnerability for nicotine exposure. Relevant to our findings, Belluzzi et al. (2004) demonstrated that early-adolescent rats displayed a preference for an environment paired with a single injection of nicotine, whereas late-adolescent and adult animals did not. Similarly, Dao et al. (2011) showed that early but not late adolescent pretreatment with nicotine enhanced subsequent cocaine self-administration in rats. Theses similarities in findings across species and behavioral tests provide strong support for early adolescence as a critical period mediating the increase in sensitivity to nicotine.

Our results are consistent with previous behavioral studies that have shown differences in responses to cocaine in rodents treated with nicotine during adolescence compared with animals that receive nicotine as adults. In adolescent mice and rats, pre-exposure to nicotine during adolescence enhanced cocaine-mediated locomotor responses, CPP and self-administration of cocaine (Collins and Izenwasser, 2004; McMillen et al., 2005; McQuown et al., 2007; Dao et al., 2011; Dickson et al., 2014). In contrast to these findings, (Kelley and Rowan, 2004) found that mice demonstrated a decrease in cocaine-induced CPP after adolescent nicotine exposure. This difference could be due to the nicotine dose administered (a high dose of 3 mg/kg vs. our low dose of 0.5 mg/kg), the length of nicotine exposure (25 days vs. our 7 days), or the age of animals during CPP testing (PND 142 vs. our PND 72). Regardless, the preponderance of evidence in the literature indicates enhancement rather than suppression of cocaine abuse-related effects by adolescent nicotine exposure.

We have also shown that the enhancement of the drug reward in the CPP test by prior adolescent nicotine exposure was also observed with amphetamine and morphine. These results are in agreement with previous reports showing that prior treatment with nicotine during early adolescence in rodents sensitized the behavioral effects of amphetamine and nicotine in adulthood (Collins et al., 2004; Adriana et al., 2003; Kota et al., 2009; Dao et al., 2011). While various mechanisms could mediate this cross-
sensitization between nicotine and other drugs of abuse, it is likely that changes in the dopaminergic system affected by nicotine exposure during the early developmental period, play an critical role. Importantly, since we found no effect on conditioning for highly palatable food, this suggests that adolescent nicotine exposure exhibits some behavioral specificity toward drug-induced reward.

The protracted behavioral response suggests the involvement of persistent neuroadaptations underlying the enhancement of cocaine-rewarding effects. This idea is consistent with the recent study by Doura et al. (2010) showing that adolescent rats subjected to chronic nicotine exhibited age-specific persistent gene expression changes in the ventral tegmental area. Indeed, over 500 adolescent-specific genes showed no initial response to chronic nicotine at the end of the 2-week treatment period but showed significant up- or down-regulation 30 days after the cessation of the drug.

We also showed that nicotine-pretreated adolescent C57BL/6J mice displayed a significantly higher level of cocaine-induced preference as adults compared with saline-pretreated mice. In contrast, nicotine failed to enhance the CPP response to cocaine in DBA/2J mice. This is unlikely to be attributable to a pharmacokinetic difference because nicotine blood levels were similar in the two strains of mice after chronic exposure to the drug during adolescence. However, differences in the expression and function of different targets and pathways of nicotine between the two strains could play an important role. Interestingly, these two strains also differ in their responses to nicotine in the CPP test, with DBA/2J mice much less sensitive to the rewarding effects of nicotine compared to C57BL/6J (Jackson et al., 2009).

The enhancement of cocaine-induced behaviors by adolescent nicotine treatment was mediated by nAChRs because it was blocked by co-administration of mecamylamine, a nonselective nicotinic receptor antagonist. In addition, co-administration of the selective z4β2* nAChR antagonist but not the z7 antagonist MLA during adolescence blocked nicotine-induced enhancement of...
adolescent rats increased mRNA expression of repeated administration of low doses of nicotine (0.4 mg/kg i.p.) in presented as et al., 2012). Moreover, exposure to nicotine in mice and rats during (Trauth et al., 1999; Azam et al., 2007; Levin et al., 2007; Counotte later and levels of saline or nicotine (0.5 mg/kg) twice daily for seven days. Animals were sacri- decreased 24 h after nicotine exposure in adolescents and adult mice. Early adolescent (PND 28) or adult (PND 70) male ICR mice were injected s.c. with saline or nicotine (0.5 mg/kg) twice daily for seven days. Animals were sacrificed 24 h later and levels of ∆FosB in the NAc were measured by immunoblot. Results are presented as ∆FosB normalized to α-tubulin and data are expressed as a percentage of saline control (4 animals per group). *p < 0.05 from respective saline and †p < 0.05 from adolescents also treated with nicotine.

cocaine-mediated effects. Furthermore, the α7 selective agonist PHA-543613 given during adolescence at a dose of 8 mg/kg, a behaviorally active doses reported in previous studies (Freitas et al., 2013a,b), failed to mimic the effects of nicotine on cocaine CPP. Collectively, these results suggest that activation of α4β2* nAChRs is required for enhancement to occur. The involvement of α4β2* nAChR subtypes is consistent with reports that higher levels of mRNAs for β2 nicotinic subunits and α4β2* high-affinity binding sites are found in adolescents than are found in adult rodents (Trauth et al., 1999; Azam et al., 2007; Levin et al., 2007; Counotte et al., 2012). Moreover, exposure to nicotine in mice and rats during adolescence induces a long-lasting increase in brain α4β2* nAChR levels and function upon reaching adulthood. For example, repeated administration of low dose of nicotine (0.4 mg/kg i.p.) in adolescent rats increased mRNA expression of α5, α6, and β2 nAChR subunits in the ventral midbrain of rats in adulthood (Adriani et al., 2003). These changes in nAChR expression were only associated with adolescent treatments and did not occur in adult animals treated with nicotine for the same duration. A similar nicotine treatment in adolescent mice produced an increase in the α4β2* nAChR activity in various brain regions as compared to adults (Kota et al., 2007).

Our finding that nicotine treatment during adolescence increases cocaine sensitization in adulthood agrees with the results of our CPP studies. We found that a 7-day nicotine pretreatment in early adolescence enhanced locomotor sensitization to cocaine on challenge day as compared to saline pretreated-mice. In rodents, sensitization has been shown to correlate with enhanced predisposition to self-administer psychostimulants (Schenk and Partridge, 2000; Vezina et al., 2002) and reinstatement of extinguished self-administration (De Vries et al., 1998; Suto et al., 2004).

In human studies, adolescents who had previously smoked cigarettes were found to have higher initial “wanting” scores and were more likely to become cocaine-dependent than non-smokers (Lambert et al., 2006). Our findings suggest that nicotine exposure during adolescence enhances long-lasting neuronal alterations in neurochemical systems mediating locomotor sensitization in animals.

Several mechanisms could underlie the protracted behavioral sensitization induced by nicotine during adolescence. Substantial evidence suggests that ∆FosB produces long-term neural adaptations that contribute to drug addiction. Indeed, the induction of ∆FosB in the striatum after chronic exposure to various drugs of abuse persists for at least several weeks (Nestler, 2008). Furthermore, mice overexpressing ∆FosB in NAc exhibited enhanced sensitivity to both acute locomotor activity and rewarding effects of cocaine (Kelz et al., 1999). In addition, ∆FosB overexpression in the NAc increased morphine CPP (Zachariou et al., 2006). These findings suggest that ∆FosB accumulation in the NAc might play a role in nicotine-mediated enhancement of cocaine sensitization and reward. As a first step to investigate this possibility, ∆FosB was measured in the NAc of adolescent and adult mice that received nicotine. Nicotine treatment in adult mice produced a 1.4 fold increase in ∆FosB. The ability of nicotine to induce ∆FosB in the NAc is similar to other drugs of abuse (Perrotti et al., 2008). These results are also consistent with recent behavioral studies showing that nicotine exposure (7 days) in adult mice enhanced cocaine CPP as compared to mice pretreated with saline (Levine et al., 2011; Li H et al., 2014). Interestingly, the 7 days of nicotine pretreatment also enhanced increases in ∆FosB expression produced by subsequent cocaine administration (Li H et al., 2014). Our results also showed an even greater (4-fold) increase in ∆FosB expression after nicotine treatment in adolescents as compared to adults. Considering the longevity of ∆FosB, this mechanism could contribute to the long-lived effect of adolescent nicotine treatment on drug reward in adulthood.

The induction of ∆FosB is long-lived but not permanent because it is degraded and returns to pre-drug levels after 1–2 months (Nestler, 2008). This suggests that the ∆FosB protein itself does not maintain drug dependence, but rather alters the expression of target genes expected to play a role in nicotine and other drug addictions. One possible target is the GluR2 subunit of the AMPA receptor (Kelz et al., 1999). Another putative target gene of ∆FosB in NAc is dynorphin, which is thought to activate kappa-opioid receptors on VTA dopaminergic neurons and inhibit dopaminergic transmission, thereby decreasing reward. Nestler (2008) and Zachariou et al. (2006) have shown that the induction of ∆FosB represses dynorphin gene expression in the NAc, which could contribute to the enhancement of reward mechanisms mediated by ∆FosB.

Overall, our results suggest that nicotine use during early adolescence may convey a greater risk than nicotine use during adulthood. Thus, adolescent smokers may be particularly vulnerable to the risks of drugs of abuse.

Financial disclosures

All authors declare no conflict of interest.

Acknowledgments

This study was supported by Virginia Foundation for Healthy
Youth through the Virginia Youth Tobacco Project to Virginia Commonwealth University and NIH grants DA030404 and DA032246.

References


