

Long-Lasting Cognitive Deficits Resulting from Adolescent Nicotine Exposure in Rats

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Adolescence is a developmental period, during which the brain and particularly medial prefrontal cortical (mPFC) regions thereof have not fully matured. Because epidemiological data have suggested that adolescent nicotine use may result in disturbances in cognitive function in adulthood, we investigated the long-term effects of adolescent nicotine exposure in rats. Male Wistar rats were exposed to either nicotine (three times daily, 0.4 mg/kg s.c.) or saline for 10 days during (postnatal day (PND) 34–43) or following (PND 60–69) adolescence. After 5 weeks during adulthood, separate groups of animals were tested in operant paradigms taxing attention and distinct measures of impulsivity. Visuospatial attention and impulsive action were tested in the five-choice serial reaction time task, whereas impulsive choice was assessed in the delayed reward task. Our data show that adolescent, but not postadolescent, nicotine exposure affects cognitive performance in adulthood and results in diminished attentional performance and increments in impulsive action, while leaving impulsive choice intact. This altered cognitive performance appeared to be associated with enhanced releasability of dopamine in the mPFC. Together, these data suggest that adolescence is a time window during which the brain is vulnerable to long-lasting cognitive disturbances resulting from nicotine exposure.

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INTRODUCTION

Adolescence is a critical developmental period during which the human brain continues to develop until approximately the age of 20, with synaptic pruning and myelination of frontal cortical regions maturing in the later stages of adolescence (for review, see Casey *et al*, 2005). Because the adolescent brain is still plastic, exposure to addictive substances such as nicotine inhaled from tobacco smoke may greatly impact the development of frontal cortical regions and thus lead to changes in brain function that persist in adulthood (for review, see Slotkin, 2004). It is well established that frontostriatal brain regions are essential in executive functions, such as attentional processing and inhibitory response control as well as decision-making processes (for reviews, see Arnsten and Li, 2005; Miller and Cohen, 2001). The late development of these brain regions involved in impulse control might cause adolescents to be

more risk-taking and to experiment with drugs, such as nicotine. Indeed, most adult smokers start their habit before the age of 19 and several retrospective and prospective studies have suggested that early onset of smoking behavior may predict cigarette smoking and nicotine dependence in adulthood (Brown *et al*, 1996; Chassin *et al*, 1996; Dappen *et al*, 1996). In the Netherlands, 71% of adolescents have tried a cigarette at least once (Van Andel *et al*, 2003), and approximately 40% smoke at least monthly at the age of 19. These figures are comparable to other European countries (Godeau *et al*, 2004), and even higher compared to the United States, where 25% of adolescents report cigarette use in the past 30 days (Johnson *et al*, 2006). Together, these numbers stress the importance to study the potentially hazardous long-term effects of nicotine on the developing adolescent brain.

To date, clinical studies assessing changes in cognitive functioning following nicotine exposure in adolescents have largely focused on its short-term effects. Moreover, in these studies, levels of smoking were often determined by self-report measures and did not control for presmoking levels of cognitive performance. Nevertheless, in adolescent smokers disturbances in working memory processes and attention have been reported (Jacobsen *et al*, 2005) as well as reduced attention-associated prefrontal cortical

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blood-oxygen level dependent responses (Musso *et al*, 2007). Furthermore, epidemiological data suggest long-lasting behavioral disturbances and mental health problems in adulthood following adolescent nicotine use (for review, see Mathers *et al*, 2006). However, the difficulty to control gene-environment interactions in the human population hampers a straightforward interpretation of adolescent nicotine exposure on subsequent behavioral changes.

Animal models have greatly contributed to our understanding of the adolescent brain and are well suited to explore putative causal relationships between early drug exposure during adolescence and cognitive functioning (in the absence of nicotine) in adulthood. In rats, typical adolescent-like behaviors include increments in peer-directed behavior, risk-taking behavior, indexed by elevated exploratory behavior and novelty-seeking, and altered sensitivity to drugs of abuse. These behaviors are generally observed in the period that ranges from approximately postnatal day (PND) 28–50 (for review, see Spear, 2000).

The long-term effects of adolescent nicotine exposure on cognitive performance in rats have not been studied before. Previous work in rats has shown that adolescent nicotine, followed by 2–5 weeks of abstinence, has long-term effects on behavior in both the emotional and motivational domain, exemplified by an increase in novelty-induced activity, elevated anxiety, higher levels of passive avoidance, and enhanced vulnerability to nicotine self-administration (Adriani *et al*, 2006, 2003; Slawewski *et al*, 2005; Trauth *et al*, 2000).

The aim of the present study was to assess long-term effects of nicotine exposure specifically during the adolescent period on executive cognitive functions in rats. To this end, different behavioral paradigms were used to assess visuospatial attention as well as distinct forms of impulsivity, namely the 5-choice serial reaction time task (5-CSRTT) and the delayed reward paradigm that measure aspects of impulsive action and impulsive choice, respectively (for recent review, see Winstanley *et al*, 2006a). Whereas acute and (sub)chronic nicotine challenges have been shown to increase impulsivity and might result in improved visuospatial attention in the 5-CSRTT (Day *et al*, 2007; Grottick and Higgins, 2000; Hahn *et al*, 2002; Van Gaalen *et al*, 2006a), the present study focused on the long-term effects of adolescent nicotine exposure on 5-CSRTT performance in adulthood. Moreover, to explore putative long-lasting neuroplasticity in frontostriatal brain regions resulting from adolescent nicotine exposure, we also measured the electrically evoked release of (radiolabeled) dopamine in slices of the medial prefrontal cortex (mPFC) and nucleus accumbens (Acb) in separate groups of rats.

MATERIALS AND METHODS

Animals

Timed pregnant Wistar female rats arrived at 5 days of gestation (Harlan, Horst, the Netherlands) and were housed individually in Macrolon cages under standard conditions and a reversed day-night cycle (lights on from 1900 to 0700 hours). Upon delivery, litters were culled to eight pups per mother and preferably consisted of male rats only, but were occasionally matched with female rats. At PND 21, animals

were weaned and housed two per cage. Only male rats were used in these experiments. During the course of behavioral testing, animals were food restricted to maintain 85–90% of their free-feeding weight. Water was available *ad libitum*. All experiments were approved by the animal ethical committee of the Vrije Universiteit, Amsterdam, The Netherlands.

Nicotine Exposure

Two groups of 48 animals and a third group of 32 rats were injected subcutaneously with either nicotine (0.4 mg/kg, calculated as a base ((–)Nicotine hydrogen tartrate salt; Sigma, St Louis, MO, USA)) or saline three times a day (at 1000, 1300, and 1500 hours) for 10 days. Nicotine was administered to half of the animals during adolescence (PND 34–43), whereas the other animals were exposed during adulthood (PND 60–69). The saline controls were littermates of the nicotine-exposed animals in both age groups. Treatment of adult and adolescent animals occurred simultaneously and 5 weeks later, when groups had reached adulthood, behavioral tests commenced ($n = 12$ per group). The first group of 48 animals was trained in the 5-CSRTT, and the second group in the delayed reward task (DRT). A third group of rats was decapitated 5 weeks following nicotine exposure and was used to study neurotransmitter release ($n = 8$ per group). All behavioral testing was carried out in the dark period under red light conditions.

5-CSRTT

Experiments were conducted in rat operant chambers with stainless steel grid floors (MED-NPW-5L; Med Associates Inc., St Albans, VT, USA) as described elsewhere (Van Gaalen *et al*, 2006a). On-line control of all operant chambers and data collection were performed using MED-PC version IV (Med Associates Inc.). Five sessions were scheduled per week from Monday until Friday, one session per day.

A more detailed description of training in the 5-CSRTT has been reported previously (Van Gaalen *et al*, 2006a). In short, rats were trained to detect and respond to a 1 s visual stimulus in either one of five apertures, during presentation of the stimulus or during the 2 s limited hold period, to obtain a food reward (45 mg, Formula P; Research Diets Inc., New Brunswick, NJ, USA). Each session terminated after 100 trials or 30 min, whichever occurred first. Initially the duration of this stimulus was 32 s and was gradually decreased to 1 s over sessions until animals reached stable baseline performance (accuracy > 80% correct choice and < 20% errors of omission). Incorrect, premature responses (responses during the 5 s intertrial interval (ITI)) and errors of omission did not lead to the delivery of a food reward and resulted in a 5 s time-out period during which the house light was extinguished. Responses during the time-out period resulted in a new time-out period. Perseverative responses, ie repeated responding into an aperture following correct choice and before pellet collection were measured but did not have any programmed consequences. The following behavioral measures were recorded: (1) accuracy, ((number correct trials/(correct + incorrect trials)) × 100); (2) latency of correct responses; (3) percentage of premature responses ((mean number premature

responses during the ITI/mean number (correct + incorrect + perseverative + premature responses) \times 100); (4) perseverative responses after correct choice; (5) percentage of omissions ((number omissions/(correct + incorrect responses + omissions)) \times 100), and (6) feeder latency.

During acquisition one animal from the adolescent nicotine group unexpectedly died, whereas one animal from the adolescent saline group persistently displayed high levels of omissions (>60%). Therefore these animals were excluded from all analyses.

Delayed Reward

Experiments were conducted in the same operant chambers as described for the 5-CSRTT. A more detailed description of training in the DRT has been previously reported (Van Gaalen *et al*, 2006b). In short, rats needed to start a trial by nose poking in the middle illuminated aperture (initiation period). This dimmed the cue light and illuminated the apertures immediately adjacent to the middle aperture (choice phase). When a rat failed to respond within the initiation period, the cue light was extinguished, and an ITI started. The choice phase lasted 10 s or until a nose poke was made. The latter resulted in the delivery of either a small reward (one pellet) or large reward (four pellets) depending on the aperture. The aperture associated with small and large reward was always fixed for each animal, but counterbalanced across rats. After the choice phase, an ITI started until the next trial started. Nose poking in any of the nonilluminated apertures was recorded, but had no consequences. A session was divided into five blocks of 12 trials, each starting with two forced trials, during which, after initiating the trial either the aperture associated with small or large reward was illuminated in a counterbalanced fashion. For the next 10 trials, the animal had a free choice. The delay for the large reward increased per block of 10 trials from 0, 2, 4, 8, 12 s to eventually 0, 5, 10, 20, 40 s.

Neurotransmitter Release

An additional cohort of 32 rats was exposed to nicotine or saline as described above. After 5 weeks, comparable to the time window of training in the behavioral paradigms, animals were decapitated and the Acb and mPFC (infralimbic and prelimbic cortex) were rapidly dissected from the brain. In replicate experiments, brain tissue was sliced ($0.3 \times 0.3 \times 2$ mm) and for each brain region the slices of two animals were pooled. Subsequently, brain slices were incubated and superfused as described before (Schoffeleer *et al*, 1988). Briefly, slices were incubated for 15 min in Krebs–Ringer bicarbonate containing 5 μ Ci [3 H] dopamine (Radiochemical Centre, Amersham, Buckinghamshire, UK) in an atmosphere of 95% O₂–5% CO₂ at 37°C. As the Acb and mPFC have a dense noradrenergic innervation, 3 μ M desipramine (Sigma) was added to the medium of these brain structures to prevent accumulation of [3 H] dopamine in noradrenergic nerve terminals. After labeling, slices were rapidly washed and transferred to each of the 24 chambers of a superfusion apparatus (approximately 4 mg tissue in 0.2 ml volume) and superfused (0.2 ml/min) with medium gassed with 95% O₂–5% CO₂ at 37°C. In each experiment, neurotransmitter release from brain slices of all four groups

(nicotine and saline pretreatment during or following adolescence) was studied simultaneously in 24 parallel superfusion chambers. The superfusate was collected as 10-min samples after 40 min of superfusion ($t = 40$ min). Neurotransmitter release was induced by exposing the slices to electrical biphasic block pulses (Acb: 1 Hz, 2 ms at 30 mA, mPFC: 1 Hz, 2 ms at 20 mA, for 10 min at $t = 50$ min). The radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1 N HCl. The radioactivity in superfusion fractions and tissue extracts was determined by liquid scintillation counting. The efflux of radioactivity during each collection was expressed as percentage of the amount of radioactivity in the slices at the beginning of the respective collection period. The electrically evoked release of neurotransmitter was calculated by subtracting the spontaneous efflux of radioactivity from the total overflow of radioactivity during stimulation and the next 10 min. A linear decline from the 10-min interval before that 20–30 min after the start of stimulation was assumed for calculation of the spontaneous efflux of radioactivity. The release evoked was expressed as percentage of the content of radioactivity of the slices at the start of the stimulation period.

Statistical Analyses

Data obtained in the 5-CSRTT were subjected to repeated measures analysis of variance (ANOVA) with exposure regimen (age and drug) as between-subjects variables and session as within-subjects variables using the Statistical Package for the Social Sciences version 11 (SPSS Inc., Chicago, IL, USA). Data obtained in the DRT were subjected to repeated measures ANOVA with exposure regimen (age and drug) as between-subjects variables and delay to large reinforcer (DRT) as within-subjects variables. Neurotransmitter release in pooled brain structures from nicotine treated rats was calculated as percentage change in neurotransmitter release from saline-treated rats and analyzed using univariate ANOVA with exposure regimen (age and drug) as between-subjects variable. The homogeneity of variance across groups was determined using Mauchly's tests for equal variances and in case of violation of homogeneity, corrected and therefore more conservative Huynh–Feldt probability values were used for subsequent analyses. In case of statistically significant main effects, further *post hoc* comparisons were conducted using Student–Newman–Keuls Tests. The level of probability for statistically significant effects was set at 0.05.

RESULTS

Baseline Performance in the 5-CSRTT

Stable baseline performance, during which rats always completed all 100 trials, was reached in the four different groups within 28–30 sessions after the start of training. Under these conditions, the groups differed in terms of accuracy and premature responding. They did not show differences in acquisition of the task; differences became apparent after nine sessions on a stimulus duration of 1 s. As displayed in Figures 1a and b, under stable baseline responding over five consecutive sessions (session:

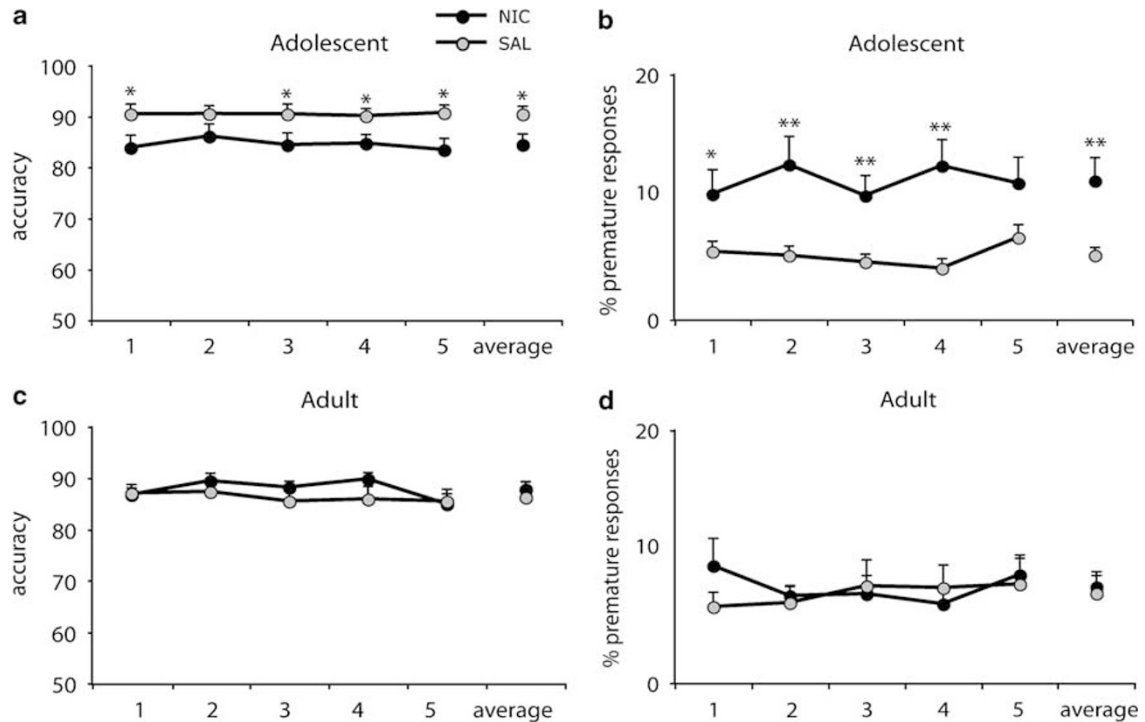


Figure 1 Performance in the five-choice serial reaction time task (5-CSRTT) over five baseline sessions, including the average of these five sessions. Accuracy (a, c) calculated as the percentage of correct responses over total correct and incorrect responses. Impulsive action (b, d) calculated as the percentage of responses during the intertrial interval (ITI) (premature responses) over total responses (correct, incorrect, premature, and perseverative responses). Depicted are means \pm SEM of nicotine- (●) and saline (○)-pretreated adolescent (a, b) and adult (c, d) animals. * $p < 0.05$ compared to nicotine-pretreated rats and ** $p < 0.01$ compared to saline-pretreated rats.

Table 1 Behavioral Parameters in the 5-CSRTT Averaged over five Baseline Sessions

5-CSRTT parameter	Adolescent		Adult	
	Saline	Nicotine	Saline	Nicotine
Correct responses	84.2 \pm 2.3	68.4 \pm 3.5 *	74.5 \pm 2.9	77.3 \pm 2.6
Incorrect responses	8.0 \pm 1.4	12.9 \pm 1.9	12.0 \pm 1.7	10.6 \pm 1.3
% Omissions	8.7 \pm 1.9	14.8 \pm 3.4	13.0 \pm 2.9	11.0 \pm 2.3
Premature responses	5.8 \pm 0.9	14.2 \pm 3.8 *	8.1 \pm 2.1	8.3 \pm 1.3
Perseverative responses	9.8 \pm 2.2	5.1 \pm 1.0	5.5 \pm 1.0	7.6 \pm 1.5
Time-out responses	21.2 \pm 2.7	43.0 \pm 4.7**	33.2 \pm 4.0	29.6 \pm 3.6
Correct response latency (s)	0.30 \pm 0.01	0.33 \pm 0.02	0.33 \pm 0.01	0.32 \pm 0.01
Feeder latency (s)	1.17 \pm 0.16	0.93 \pm 0.07	1.16 \pm 0.19	1.06 \pm 0.09

Abbreviation: 5-CSRTT, 5-choice serial reaction time task. Mean \pm SEM is depicted. * $p < 0.05$ and ** $p < 0.005$ compared to their saline control.

$F(1,42) = 2.23$, NS), previous nicotine exposure differentially affected accuracy in adulthood depending on the age of exposure (age: $F < 1$; drug: $F(1,42) = 2.32$, NS; age \times drug: $F(1,42) = 5.98$, $p = 0.02$). In particular, nicotine exposure during, but not following adolescence had long-term detrimental effects on accuracy of performance. In-depth analyses indicated that reduced accuracy in the adolescent nicotine group resulted from a reduction in correct detection of the stimulus, rather than from an increase in incorrect responses (Table 1).

In addition to changes in visuospatial attentional performance resulting from adolescent nicotine exposure, impulsive action (measured as the percentage of premature responses, ie responses during the ITI) was also differentially affected by nicotine when administered during adolescence versus adulthood (Figures 1c and d; session and age: both $F_s < 1$; drug: $F(1,42) = 5.77$, $p = 0.02$; age \times drug: $F(1,42) = 4.09$, $p = 0.049$). Further comparisons revealed a twofold increase in the percentage of premature responding when animals were exposed to nicotine during adolescence. In contrast, nicotine exposure during adulthood had no long-term effects on premature responding.

Other behavioral parameters in the 5-CSRTT (depicted in Table 1), including the percentage of omissions, correct response latencies, and feeder latencies were neither changed by periadolescent nor adult exposure with nicotine, indicating that there were no differences in motivation or motor activity. Nonetheless, in addition to impulsive action, there was an increase in time-out responses following adolescent nicotine exposure (age: $F < 1$; drug: $F(1,42) = 5.68$, $p = 0.02$; age \times drug: $F(1,42) = 11.05$, $p = 0.002$).

Performance in the DRT

To study whether adolescent nicotine exposure would increase other forms of impulsivity, a different group of animals was trained in the DRT measuring impulsive choice. Stable baseline responding was achieved in the DRT after approximately 27 sessions, with no differences in acquisition between periadolescent and adult rats exposed to nicotine. As shown in Figure 2, in all groups the choice

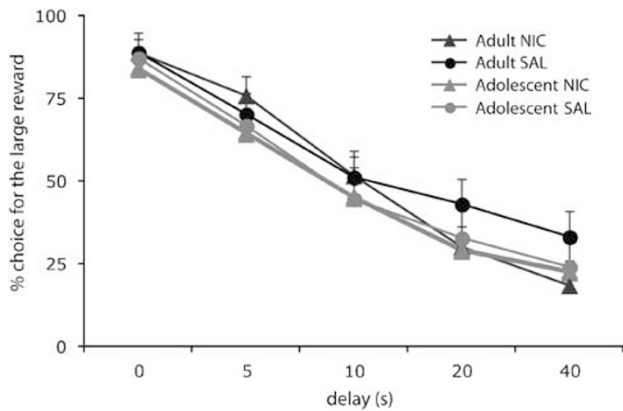


Figure 2 The effects of nicotine pretreatment on the delayed reward task. Depicted is the mean \pm SEM percentage choice for the large reinforcer as a function of delay, calculated over five baseline sessions.

for the large reinforcer significantly declined with increasing delays, indicating that all groups showed a hyperbolic delay discounting curve (delay: $F(1,44) = 193.19$, $p < 0.001$). Moreover, preference for the large reinforcer as a function of the delay was not differentially affected by periadolescent or adult nicotine exposure (delay \times age: $F < 1$; delay \times drug: $F(1,44) = 1.60$, NS; delay \times age \times drug: $F(1,44) = 1.09$, NS). Again, there were no differences in motivation between the groups, indicated by similar numbers of choice omissions and omissions to start a trial (data not shown).

In Vitro Neurotransmitter Release

The electrically evoked ^3H -dopamine release from mPFC slices in saline-treated animals amounted to $6.4 \pm 0.3\%$ of total tissue tritium for adolescent and $7.5 \pm 0.8\%$ of total tissue tritium for adult nicotine-exposed animals. As shown in Figure 3a, nicotine exposure differentially affected evoked ^3H -dopamine release in adulthood. In periadolescent nicotine-exposed animals, electrically evoked ^3H -dopamine release was increased approximately 1.4-fold, whereas in adult nicotine-exposed animals electrically evoked ^3H -dopamine release was not changed in this brain region (age: $F(1,15) = 1.30$, NS; drug: $F < 1$; age \times drug: $F(1,15) = 12.70$, $p = 0.004$). The electrically evoked ^3H -dopamine release from Acb slices in saline-treated animals amounted to $3.4 \pm 0.1\%$ of total tissue tritium for adolescent and $3.1 \pm 0.2\%$ of total tissue tritium for adult-exposed animals. No long-term changes were found in electrically evoked ^3H -dopamine release in the Acb as a result of periadolescent or adult nicotine exposure (Figure 3b; age: $F(1,15) = 1.03$, NS; drug and age \times drug: both F 's < 1).

DISCUSSION

The present findings demonstrate that periadolescent, but not postadolescent nicotine exposure, has long-lasting effects on cognitive functioning in rats. Specifically, the present data point to deficits in visuospatial attention and increased impulsivity that is prominent after more than 5 weeks of abstinence. Deficits in impulse control were observed on impulsive action as measured in the 5-CSRTT,

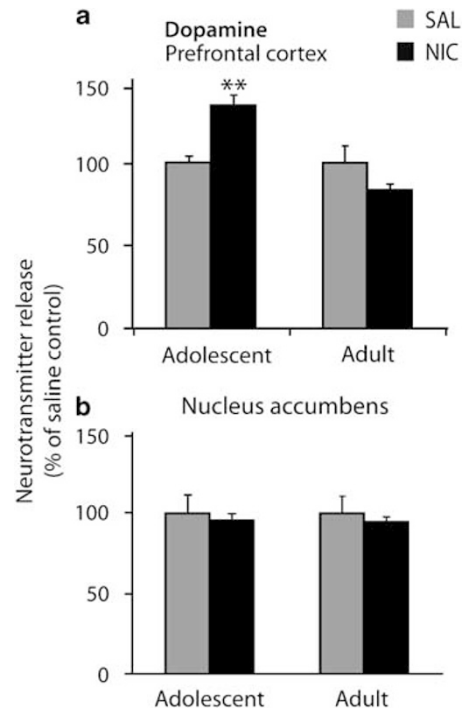


Figure 3 Electrically evoked dopamine release. Dopamine release in the medial prefrontal cortex (a) and nucleus accumbens (b) of animals pretreated with nicotine (■) or saline (□) during adolescence or adulthood. Data of nicotine-pretreated animals are expressed as the percentage of saline-pretreated animals. Depicted are means \pm SEM of four experiments. ** $p < 0.005$ compared to their respective saline controls.

but not on a different form of impulsivity, ie impulsive choice as measured in a delayed reward paradigm. In separate groups of animals, neurochemical analyses demonstrated long-term hyperresponsiveness of dopaminergic nerve terminals in the mPFC following exposure to nicotine during, but not following, adolescence. Overall, these data suggest that adolescence is a unique time window during which the brain is more vulnerable and prone to develop long-lasting changes in cognitive functions resulting from nicotine exposure.

Adolescent Nicotine Exposure and Impulsivity

Smoking appears to be closely related to several forms of impulsivity in humans. For example, adult smoking has been associated with both impulsive choice (Bickel *et al*, 1999; Mitchell, 1999) as well as deficits in inhibitory control when compared to nonsmokers (Mitchell, 1999; Skinner *et al*, 2004; Spinella, 2002). However, whether impulsivity in adult smokers results from nicotine exposure, or alternatively, is a preexisting vulnerability trait predisposing individuals to initiate and maintain smoking is as yet unclear. Recent preclinical data would support the latter view and suggest that trait impulsivity may predict the vulnerability to initiate and maintain nicotine seeking (Diergaarde *et al*, 2008).

To date, human evidence describing the long-term effects (in absence of nicotine) of adolescent smoking on impulsivity in adulthood is limited. It has been demonstrated recently though, that the inability to abstain from smoking in adolescents is associated with elevated levels of impulsive

action in the continuous performance task as well as increased impulsive choice in a real-time delay discounting paradigm (Krishnan-Sarin *et al*, 2007), which is comparable to our rat delayed reward paradigm. Nevertheless, the long-lasting effects of smoking on measures of impulsivity in both abstinent as well as nonabstinent adolescent smokers and nonsmoking adolescents have not been evaluated as yet. Similarly, adolescent smokers show higher rates of delay discounting compared to nonsmokers. However, reported length of smoking history did not correlate with delay discounting, suggesting that adolescent nicotine exposure has no long-term effects on delay discounting, which is comparable to our findings (Reynolds, 2004).

Adolescent Nicotine Exposure and Attention

Another important observation in the present study is that exposure to nicotine during, but not following adolescence, causes long-term decrements in attentional performance. These disturbances in cognitive performance appear not to be caused by deficits in motivation and motor behavior, as other behavioral parameters in the 5-CSRTT, such as percentage omissions, feeder latencies, and correct response latencies are not affected by adolescent nicotine treatment. Interestingly, previous studies have reported an inverse correlation between attentional performance and premature responding in the 5-CSRTT (Dalley *et al*, 2008; Puumala *et al*, 1996). Therefore, the observed deficits in attentional performance and impulsive action might be associated and derived from a similar neurobiological construct.

Clinical evidence for long-term effects of adolescent smoking on executive functions such as attentional performance is scarce. However, our findings are in line with recent observations indicating short-term impairments in auditory attention in adolescent male smokers (Jacobsen *et al*, 2005, 2007). In addition, in a longitudinal study it was shown that similar cognitive deficits in adolescent smokers persist after controlling for presmoking levels of cognitive performance (Fried *et al*, 2006). Most of these deficits showed reversal after cessation of smoking, suggesting that they were transient and/or associated with nicotine withdrawal. In this latter study, decrements on performance on an arithmetic task persisted, indicating that adolescent nicotine exposure has some specific, longer-lasting effects in humans. The present data further extend these observations by demonstrating long-lasting disturbances on visuospatial attention that persist into adulthood.

Adolescent Nicotine Exposure and Dopamine Release

In terms of neurobiological changes, to date only few studies have addressed the long-term effects of nicotine exposure during adolescence. A recent study showed that adolescent nicotine exposure causes cortical cholinergic hypoactivity, which persists into adulthood (Slotkin *et al*, 2007). Our neurochemical data show long-lasting increments in depolarization-induced dopamine release in slices of the mPFC, but not of the Acb, in animals exposed to nicotine during, but not following, adolescence. Whether these changes in dopamine releasability are causally related to the observed decrements in visuospatial attention and impulsive action remains to be established. However, dopamine transmission has been

shown to be involved in modulating attentional performance in the 5-CSRTT. For instance, several studies have demonstrated that under similar test conditions amphetamine impairs visuospatial attention (Cole and Robbins, 1987; Harrison *et al*, 1997; Pattij *et al*, 2007), an effect that could be mimicked by the selective dopamine reuptake inhibitor GBR 12909 (Van Gaalen *et al*, 2006a). Also, dopamine may modulate working memory in an inverted U-shaped fashion, meaning that both too little or too much dopaminergic input impair working memory (Robbins, 2005). In addition, evidence has been presented that dopamine in the mPFC may modulate visuospatial attention in the 5-CSRTT in a similar manner (Granon *et al*, 2000).

The current findings also indicate that adolescent nicotine exposure selectively increases impulsive action measured in the 5-CSRTT, but not impulsive choice measured in the delayed reward paradigm. These findings are consistent with the notion that the two forms of impulsivity are dissociable on an anatomical and pharmacological level (Pattij and Vanderschuren, 2008; Winstanley *et al*, 2006a). Our neurochemical data revealed that electrically evoked dopamine release was selectively increased in slices of the mPFC and not Acb of periadolescent nicotine-exposed rats. Postadolescent nicotine exposure did not cause any changes in dopamine releasability in the mPFC or Acb. Considering the relationship between our behavioral and neurochemical results, accumulating data have indicated that dopamine signaling is critically involved in modulating both impulsive action and impulsive choice, but probably in different ways. For example, numerous studies indicate that the psychostimulant amphetamine increases impulsive action in the 5-CSRTT (Cole and Robbins, 1987, 1989; Harrison *et al*, 1997; Van Gaalen *et al*, 2006a), but decreases impulsive choice in delayed reward paradigms (Van Gaalen *et al*, 2006b; Wade *et al*, 2000; Winstanley *et al*, 2003). Recent studies have elaborated on these observations and show that primarily dopamine D₂, and to a lesser extent dopamine D₁, receptors appear to mediate the effects of amphetamine on impulsivity (Pattij *et al*, 2007; Van Gaalen *et al*, 2006a). In this respect, our present study shows increased dopamine releasability in the PFC following adolescent nicotine exposure, which is associated with enhanced impulsive action. Our finding that impulsive choice is not associated with such alteration of the stimulus secretion coupling process in dopamine nerve terminals is consistent with the observation that dopamine levels in the mPFC are not altered in rats during performance in the DRT (Winstanley *et al*, 2006b). Nonetheless, as yet little is known about the role of dopamine in the mPFC in impulsive action. Therefore, the causal relationship between enhanced dopamine releasability in the mPFC and impulsive action as observed here remains to be examined. Alternatively, altered—and in particular lowered—functioning of the serotonergic system has been associated with elevated impulsivity in both humans and laboratory animals (for recent reviews, see Chamberlain *et al*, 2006; Pattij and Vanderschuren, 2008). In this regard, it has been demonstrated recently that adolescent nicotine exposure results in long-lasting alterations in cortical serotonin functioning, an effect that was particularly pronounced in male and not female rats (Slotkin *et al*, 2007). Although as yet speculative, it is conceivable that changes in the serotonergic system

may have contributed to the observed increments in impulsive action in adolescent nicotine-treated rats.

The apparent difference in altered dopamine releasability between the mPFC and Acb in response to adolescent nicotine exposure may reflect the different timing of development of these regions, as it has been shown that developing brain regions are more vulnerable to develop long-term changes in sensitivity of neurotransmitter systems (Slotkin, 2004; Slotkin *et al*, 2007). In particular, the mPFC is one of the last regions to develop, with a relatively late wave of neuronal loss, whereas the Acb matures earlier (Markham *et al*, 2007). Moreover, it has been suggested that in addition to a different timing, there might be a different developmental mechanism between mPFC and Acb, as dopamine D1 and D2 receptors in the mPFC undergo overproduction, which subsequently leads to massive pruning during adolescence, whereas Acb dopamine receptors show overproduction at P40, but virtually no pruning occurs (Andersen *et al*, 2000). Also, adolescent nicotine exposure has long-term effects on morphology of a subpopulation of pyramidal neurons in the mPFC, seen as an increase in dendritic length of complex pyramidal cells (Bergstrom *et al*, 2008). These persistent changes induced by adolescent exposure to nicotine may be brought about by the increased induction of plasticity-associated genes, which were found to be specifically upregulated in the mPFC of animals treated with nicotine during adolescence (Schochet *et al*, 2005). However, there are virtually no data on the long-term effect of exogenous substances on the developing mPFC in terms of numbers of synapses or dopaminergic connections. Additional molecular and cellular approaches are needed to further study the putative long-term changes in the prefrontal cortical dopamine system and their role in impulsive action.

Concluding Remarks

The present data demonstrate that nicotine use during adolescence causes long-term disturbances in cognitive functioning and are consistent with a growing body of both clinical and preclinical findings. Collectively, these data strongly suggest that nicotine use during adolescence causes persistent behavioral changes that might contribute to subsequent psychopathologies. In addition, we observe long-term dopamine nerve terminal hyperresponsiveness in the mPFC upon adolescent nicotine exposure only.

Although direct comparisons of preclinical with clinical data should be interpreted with caution, the current findings stress the potential of animal models to study the long-term neurochemical and behavioral effects of adolescent nicotine exposure in more detail. Future studies should address the causal relationship between these phenomena and should provide a more in-depth analysis of the molecular and cellular mechanisms underlying the observed cognitive deficits. Most importantly, our findings imply that particularly adolescent smokers are at risk for developing long-lasting cognitive deficits.

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DISCLOSURES/CONFLICTS OF INTERESTS

The authors do not have any conflicts of interest to disclose.

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