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ORIGINAL INVESTIGATION

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Differential behavioral effects of nicotine exposure in adolescent and adult rats

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Abstract *Rationale:* Although the detrimental effects of nicotine in early brain development and the addictive properties in adulthood are well known, little is known about the neurobiological effects of nicotine in adolescence. An important question is whether adolescents and adults differ in the development of nicotine sensitization and drug-cue conditioning. *Objective:* To examine the behavioral effects of multiple, repeated injections of nicotine on both sensitization and drug-cue conditioning in the adolescent rat, and to compare this profile with the adult rat. *Methods:* Sixteen male adolescent (28 day) and 16 young adult (70 day) rats were given injections of either saline or nicotine and tested for motor activity for 90 min for ten consecutive days. Following 4 days of no testing, animals were given a sham injection and placed in the testing apparatus for 90 min. A dose–response curve for nicotine was also generated using two additional groups of ten adolescent and ten adult male rats. *Results:* Adolescent rats, unlike adults, did not exhibit signs of nicotine-cue conditioning, and displayed less robust sensitization to the locomotor effects of nicotine than adults. Dose–response testing revealed differences in adolescent responsivity to nicotine in measures of rearing, but not ambulation. Initial exposure to nicotine resulted in increased sensitivity to the motor-activating effects of nicotine but less sensitivity to the depressant effects of nicotine in rearing in adolescents. *Conclusions:* Adolescent animals display different long-term neuroadaptive responses to nicotine than adult animals, possibly related to immature or still-developing plasticity mechanisms in the prefrontal cortex.

Keywords Adolescence · Drug-cue conditioning · Nicotine · Rats

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Introduction

According to the Federal Interagency Forum on Child and Family Statistics in 2001, the CDC reported there were approximately 46 million smokers in the United States; of this 46 million, 4.1 million were adolescents, comprising 18% of all teenagers, generating a major health risk for this age group (2000; Gilpin et al. 1999; Chassin et al. 1990). In light of the fact that early intervention in preventing nicotine use may significantly reduce drug abuse in this country, much research has focused on the social factors influencing smoking during adolescence. However, the neurobiology of nicotine use has been largely ignored during this important developmental period.

Studies have indicated that adolescents respond to nicotine differently than adults. Adolescents smoke with less regularity, are less likely to smoke daily, and smoke fewer cigarettes per day (Colby et al. 2000). Yet, the symptoms of nicotine dependence in adolescents can develop before the onset of daily smoking, with some adolescents reporting symptoms of dependence within days or weeks of monthly smoking (DiFranza et al. 2000). Despite the high prevalence of nicotine abuse by teens, the neurobiology of nicotine addiction in adolescence remains relatively unexplored. Only a few attempts in animal models have been made to examine the effects of nicotine on the substrate of the adolescent brain (Abreu-Villaca et al. 2003; Slawecki and Ehlers 2002, 2003; Slawecki et al. 2003; Slotkin 2002; Trauth et al. 1999, 2000a,b, 2001) as well as to define

adolescent-specific behavioral alterations caused by nicotine (Adriani et al. 2002; Cheeta et al. 2001; Faraday et al. 2001; Klein 2001; Levin et al. 2003; Trauth et al. 2000b; Vastola et al. 2002). Recent studies in animal models of adolescence have described the influence of nicotine on nicotinic cholinergic receptor expression and indicated that alterations in the expression of genes involved in cell differentiation and apoptosis occur following nicotine administration (Slotkin 2002; Trauth et al. 1999, 2000a; Xu et al. 2002). Another study demonstrated that mice exposed to nicotine during adolescence exhibit decreased sensitivity to cocaine in adulthood (Kelley and Middaugh 1999). While these recent studies are a good start, there is clearly a large gap in our understanding of the neurobiological effects of nicotine on its most rapidly growing group of users.

A critical aspect of drug addiction is the effect of conditioned cues on drug-seeking behavior. The presence of cues in the environment can trigger cravings by providing strong reminders of drug and affective state even when not actively taking the drug (Childress et al. 1988; Wallace 1989). Displaying drug cues to an addicted individual has been shown to activate discrete regions of the brain, producing the subjective state of craving (Childress et al. 1999; Ragozzino et al. 1999; Schroeder et al. 2001). Conditioned effects are likely to be a major factor in the high rates of relapse in drug abuse. The association of cues, drug state, and affective state requires mechanisms of learning and plasticity (Hakan and Ksir 1988; Reid et al. 1996, 1998). In addition to the initiation of smoking, sensitization to nicotine, and the long-term effects of nicotine use, the conditioned effects of nicotine are a critical area of research that has not been investigated in animal models of adolescence.

In adult rats it is well established that repeated exposure to nicotine results in behavioral sensitization (Clarke and Kumar 1983; Walter and Kuschinsky 1989). Previous work in our laboratory has also shown that adult rats show conditioned increases in locomotor activity in environments previously paired with repeated nicotine exposure (Schroeder et al. 2001). The purpose of the following experiments was to examine the behavioral effects of multiple, repeated injections of nicotine on both sensitization and drug-cue conditioning in the adolescent rat, and to compare this profile with effects in the adult rat.

Materials and methods

Subjects and handling

A total of 52 male Sprague-Dawley rats (Harlan, Madison, WI) were used in this study. Of these rats, 26 were tested at approximately 70 days of age (adult), and 26 were tested between 28 days and 42 days (adolescent). Adolescent and adult rats were tested simultaneously in parallel. Rats were housed in age-matched pairs in clear plastic cages in an animal colony. Food and water were available at all times. Lighting in the animal colony was on a 12-h light/dark cycle, with lights on at 0700–1900 hours. Animals arrived in the laboratory 3 days before initiation of testing and were gently handled daily in order to minimize stress during testing. All animal care was in strict accordance with IACUC guidelines.

Behavioral testing

All testing was performed in clear, polycarbonate activity cages (48×26×20 cm, San Diego Instruments, San Diego, CA, USA) in a testing room separate from the animal colony. Four infrared photobeams spaced at 9-cm intervals along the bottom length of the cages recorded both horizontal activity (any beam break along the bottom of the cage) and ambulation (consecutive breaks of adjacent beams). Eight photobeams spaced at 2.5-cm intervals along the top width of the cages and 16 cm from the bottom of the cages recorded rearing (vertical moment). The dependent variables thus recorded were total horizontal movement, ambulation, and rearing. Since horizontal movement and ambulation were always correlated in these experiments, only ambulation and rearing are shown for the purposes of simplicity. The activity cages were different from the home cages, containing wire mesh placed over aspen chips (instead of cobb) to provide a different olfactory cue. A PC attached to the system collected data in 10-min intervals over a period of 90 min. Testing was always conducted between 1000 hours and 1500 hours.

Experiment 1: nicotine-induced sensitization and conditioning

This experiment assessed the effects of daily nicotine injection on general motor activity in adults and adolescents and also examined the conditioned locomotor response to nicotine-associated cues following the end of treatment. For ten consecutive days, male adolescents (PN28, n=16) and young adults (PN70, n=16) were placed in the activity testing chamber immediately following a nicotine injection [n=8 adolescent, n=8 adult, 0.4 mg/ml/kg s.c. nicotine hydrogen tartrate salt (Sigma, St. Louis, MO, USA), dissolved in saline and adjusted to pH 7.2 with NaOH] or a saline injection (n=8 adolescent, n=8 adult, 1 ml/kg, s.c.). Activity was recorded for 90 min, after which animals were returned to their home cages. Four days after the 10-day treatment, all animals were given a mock injection and placed in the testing apparatus for 90 min.

Experiment 2: nicotine dose–response

This experiment examined potential differences in the response to nicotine between adolescent and adult male rats. Adolescent male rats (n=10) and young adult male rats (n=10) were tested on alternate days using a randomized, within-subjects design (different groups from experiment 1). Before beginning the dose–response testing, animals were habituated to the activity chamber and to receiving injections. Three days of exposure to the chamber preceded testing with the different nicotine doses. On the first day of habituation, rats were weighed and placed in the chamber for 1 h. Data from this session was used to determine the motor response to novelty in adolescents versus adults. Following this, rats were removed and injected with saline (1 ml/kg, s.c.), then returned to the cage for 90 min while activity was recorded. The data from this saline trial also served as the control for the initial nicotine injection. Animals were then returned to their home cage. On the second day of habituation (48 h later) animals were acclimated to the drug's initial effects. Animals were exposed to the chamber for 1 h, removed, injected with nicotine (0.1 mg/kg s.c.), and returned to the chamber for 90 min. This also provided an opportunity to assess the initial response to a low dose of nicotine in all the rats (it is well established that nicotine initially can induce depressant effects to which tolerance rapidly develops). On day 3, and thereafter (48 h later), the rats were habituated to the activity chamber for 1 h before testing, removed, and injected (s.c.) with either saline, 0.01, 0.04, 0.1, or 0.4 mg/kg nicotine (pH 7.4) in a randomized design. Activity was recorded for 90 min immediately following the administration of nicotine. Testing was repeated on alternate days until each animal had received each dose of nicotine. Data from the initial exposure to the testing chamber, and the first exposure to

nicotine were generated from the first 2 days of testing for each group.

Data analysis

Behavioral data from the activity cages were analyzed using the StatView software program (SAS Institute, Cary, NC, USA). For the 10-day nicotine treatment, a three-factor, between-within analysis of variance (ANOVA) was carried out on the motor activity data (ambulation and rearing) with treatment and age as between-subjects factors, and days as the within-subjects factor. For the conditioning (test) days, a three-factor, between-within ANOVA was carried out with treatment and age as between-subjects factors, and time (interval) as the within-subjects factor. For behavior in a novel environment (first exposure to the activity cages) and first exposure to nicotine, a two-factor, between-within ANOVA was carried out with age as the between-subjects factors, and time as the within-subjects factor. For the dose–response testing, a two-way ANOVA was performed, with age as the between-subjects factor and dose as the within-subjects factor.

Results

Experiment 1: nicotine sensitization and conditioning in adolescent and adult rats

Nicotine sensitization

During the 10 days of exposure to the drug-paired environment, both adolescent and adult rats receiving nicotine displayed increased ambulation and rearing consistent with the known stimulatory effects of nicotine (Fig. 1). There was an overall effect of drug treatment for all parameters. Ambulation [$F(1,28)=74.9$, $P<0.01$] was significantly increased by nicotine. Although a significant age effect was present for the measure of ambulation [$F(1,28)=5.1$, $P=0.03$], no treatment×age interactions were present for ambulation, which suggests that adolescents make more gross locomotor movements in a horizontal plane than adults, regardless of drug treatment.

Rearing was also significantly increased by nicotine [$F(1,28)=49.7$, $P<0.01$]. A significant drug×age effect was found [$F(1,28)=5.2$, $P=0.03$], indicating that nicotine increased rearing in adults to a greater degree than adolescents. A significant age effect [$F(1,28)=21.8$, $P<0.01$] was also present, but in contrast, vertical movements made by adolescents were fewer in number than by adults. As seen in Fig. 1, nicotine robustly increased rear-ing in adults, whereas much smaller increases and overall number of rears were seen in the adolescent rats.

Unlike adult animals, which showed a strong progressive increase in amounts of ambulation and rearing consistent with the sensitizing effects of nicotine, adolescent rats showed considerably less sensitization to nicotine. For example, although no significant day×treatment×age interaction was present for ambulation, a significant linear trend across days×treatment×age was present for adult-nicotine treated rats ($t=6.4$, $P<0.01$) but no other groups, suggesting a lack of sensitization in measures of ambulation. It can be observed from Fig. 1 that the rearing re-

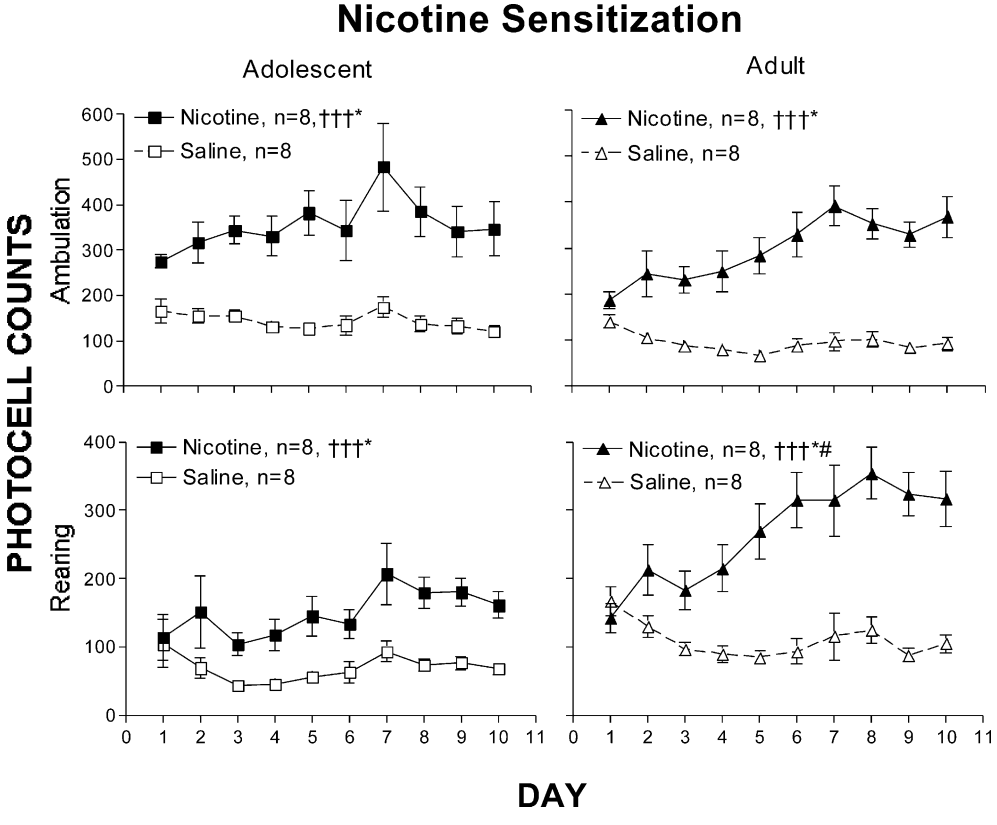

Fig. 1 Effect of repeated nicotine administration on ambulation and rearing in adolescent and adult rats. *$P<0.001$, age effect; $^{\dagger\dagger\dagger}P<0.001$ age×day interaction; $^{\#}P<0.001$ day×treatment×age interaction

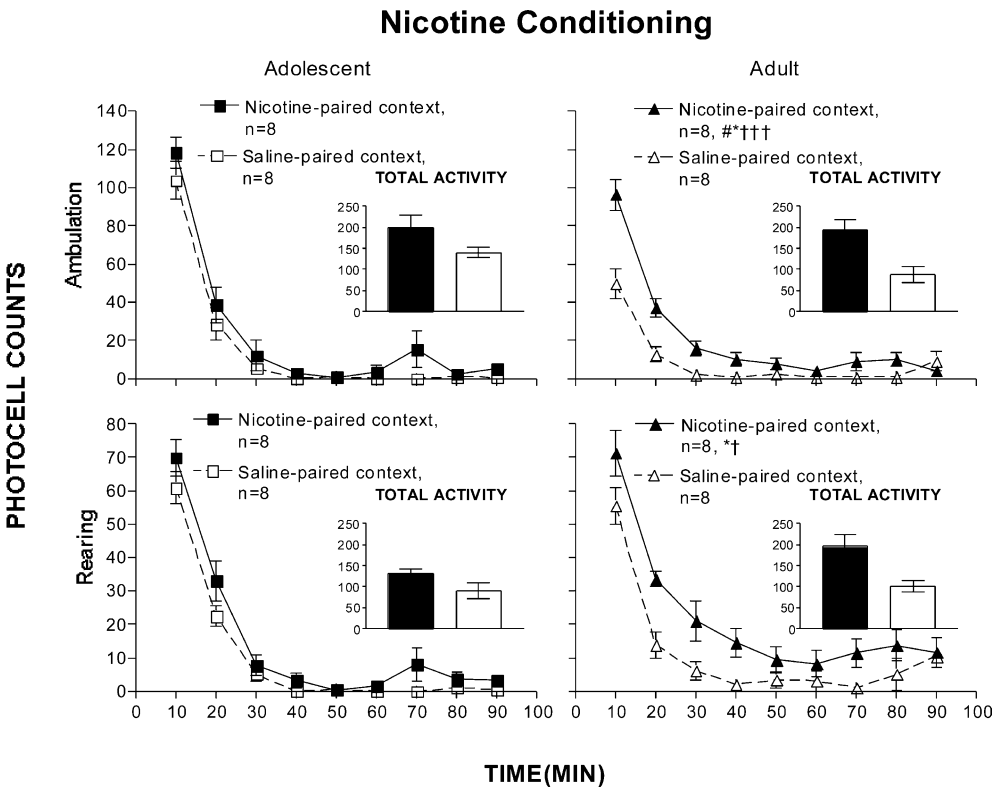

Fig. 2 Drug-cue conditioning in adolescent and adult rats. Although adolescent animals tend to rear less than adult animals, levels of rearing on this day were equivalent between groups. $^{\#}P<0.05$, age×pretreatment×interval interaction; $^{\dagger\dagger\dagger}P<0.001$, pretreatment×interval interaction; $^{\dagger}P<0.01$, treatment×interval interaction; $^{*}P<0.001$, age effect

sponse to nicotine progressively increased over days in both groups, as a significant day×treatment interaction was present [$F(9,252)=6.4$, $P<0.01$]. Although no significant day×treatment×age interaction was present for rearing, an analysis of linear trend across days×treatment×age indicated significant linear trends for rearing were present in both nicotine-treated adult ($t=6.4$, $P<0.01$) and nicotine-treated adolescent rats ($t=4.4$, $P<0.01$), but not in the saline-treated animals. However, the progressive increase was greatly attenuated in the young rats. Thus, the overall profile for sensitization was such that adolescent rats were less sensitive to the sensitizing effects of nicotine than with adults.

Cue conditioning

Prior nicotine treatment did not result in the expected conditioned motor response in adolescents, although it was present in adults, as shown in Fig. 2. Differences in activity between adolescent rats exposed to the nicotine or saline-paired environment were smaller than those of their adult counterparts on the conditioning day, suggesting attenuated conditioning. Upon analysis, additional patterns became apparent. As expected, adults tended to show increased general activity in the nicotine-paired environment compared with saline controls, as shown previously (Schroeder et al. 2001); however, this increase was not displayed by the adolescents. Indeed, a significant three-way interaction of interval×treatment×age was present for ambulation [$F(8,224)=2.0$, $P=0.05$], indicating a significant conditioning response in the adults but not in the adolescents. For rearing, no significant interval×treatment×age nor treatment×age interactions were found; however, inspection of Fig. 2 indicates a trend in the same direction as ambulation.

Experiment 2: nicotine dose–response

Response to a novel environment

Response to a novel environment was examined by the analysis of data from the first hour of exposure to the activity chamber on the first day of testing. Adolescent and adult animals displayed different profiles of motor activity when exposed to a novel environment, as seen in Fig. 3.

Although there was no significant effect of age on ambulation, there was a time×age interaction [$F(5,90)=14.0$, $P<0.01$]. Adolescent rats showed higher levels of ambulatory activity during the first 10 min of recording in the novel environment than the adult animals. Following this initial burst of activity, adolescent animals exhibited a rapid decline in levels of ambulation, and were no longer crossing the chamber by 30 min of exposure. Adult animals showed lower initial levels of activity, which declined more slowly.

For rearing activity in the novel environment, significant effects were found for age [$F(1,18)=19.6$, $P<0.01$] and a time×age interaction was also present [$F(5,90)=2.2$, $P=0.05$]. Adolescent animals reared approximately half as

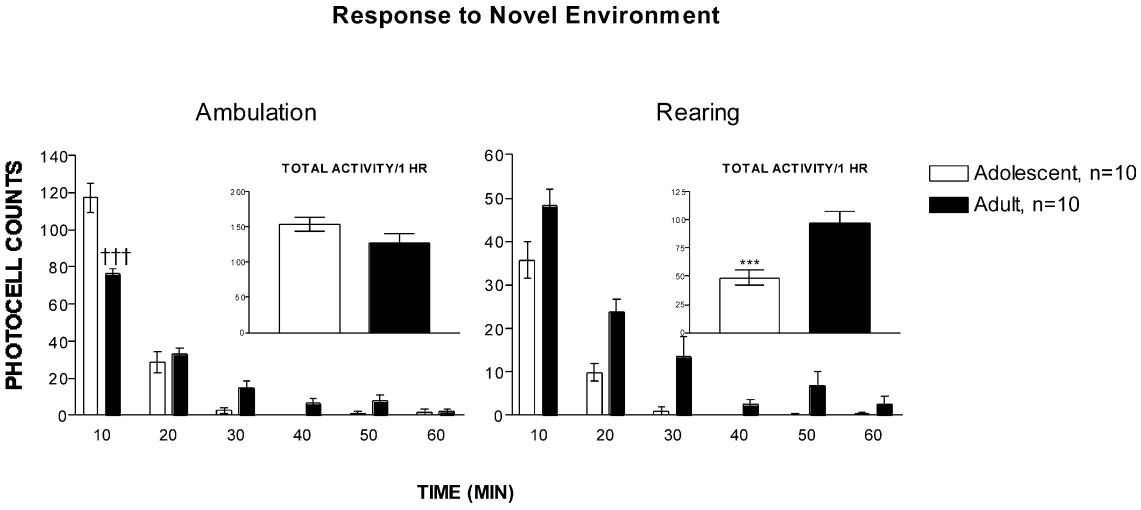

Fig. 3 Response to a novel environment in adolescent and adult rats. $^{\dagger\dagger\dagger}P<0.001$, age×time interaction; $^{***}P<0.001$ age effect

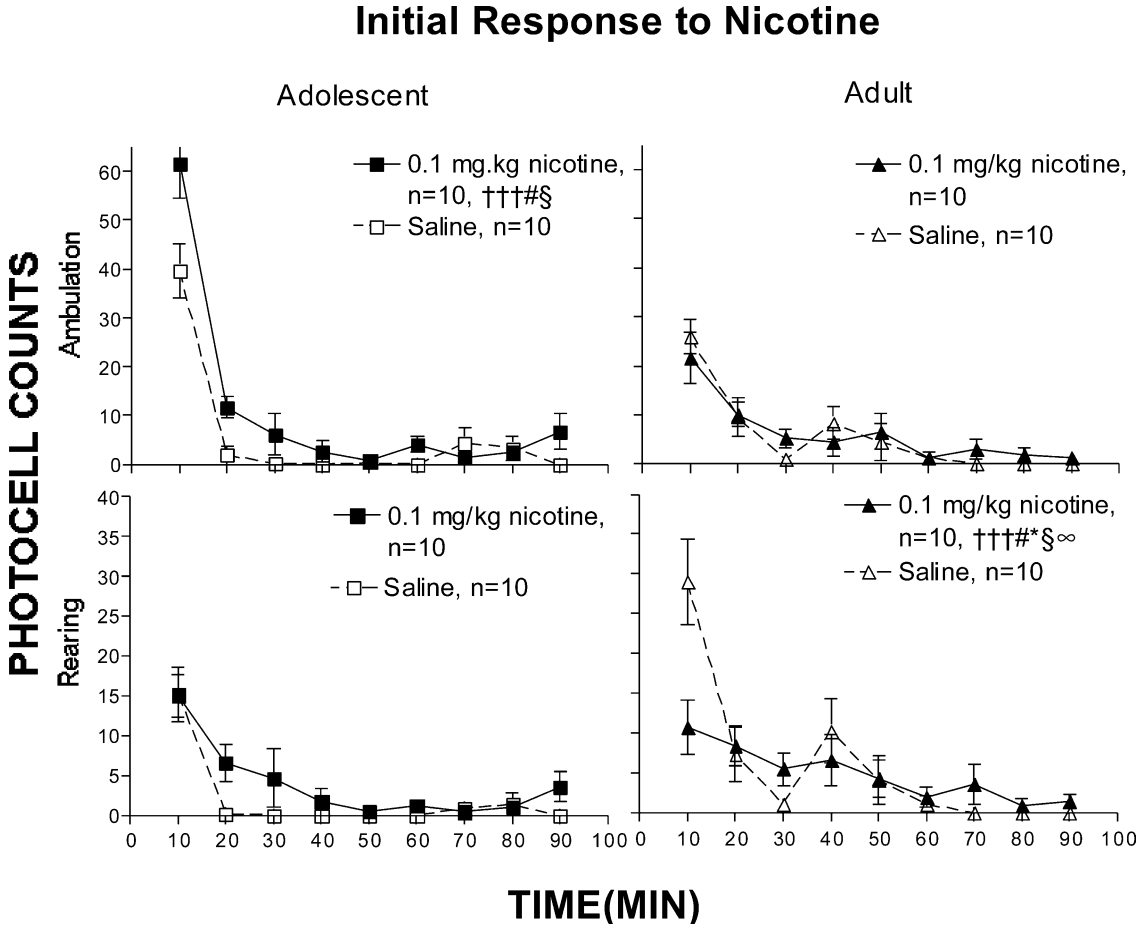

Fig. 4 Adolescent and adult rats' behavioral profiles following a single injection of 0.1 mg/kg nicotine. $^{*}P<0.05$ age effect; $^{\S}P<0.5$ treatment effect; $^{\dagger\dagger\dagger}P<0.001$ time×age interaction; $^{\infty}P<0.01$ treatment×time interaction; $^{\#}P<0.01$ treatment×time×age interaction

much as the adult animals; however, the pattern of a rapid drop in adolescent motor behavior and a more prolonged period of adult activity again held for this measure of activity.

Initial response to nicotine

Another measure of behavior provided by this experimental design was the determination of differential effects

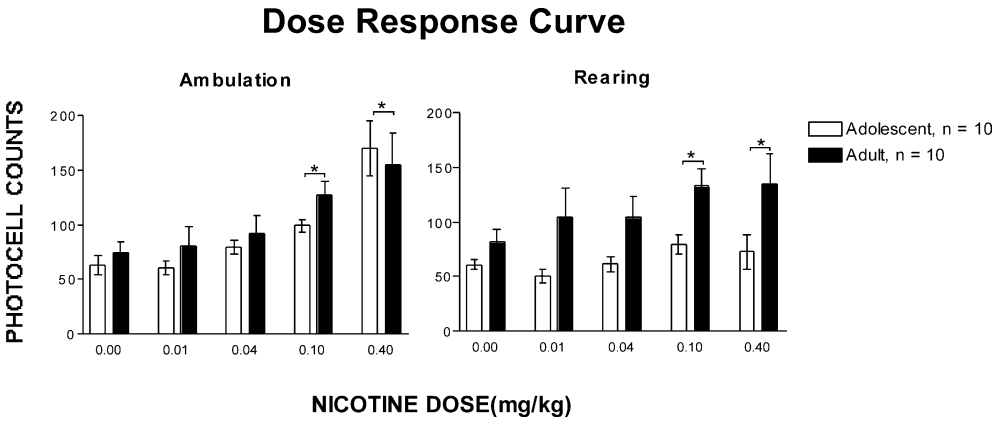

Fig. 5 Nicotine dose–response curves for ambulation and rearing in adolescent and adult rats. *P<0.01 difference from saline

of initial nicotine exposure in adolescent and adult animals. After a single dose of nicotine, adolescent and adult animals showed behavioral differences in both ambulation and rearing (Fig. 4). For ambulation, there were significant time×age×treatment [$F(8,288)=3.1$, $P<0.01$], as well as significant treatment×age [$F(1,36)=4.9$, $P=0.03$] and time×age [$F(8,288)=13.1$, $P<0.01$] interactions. Effects of age [$F(1,36)=4.4$, $P=0.04$] and treatment [$F(1,36)=7.7$, $P<0.01$] were also significant. Adolescent but not adult rats showed increased ambulatory behavior for the first 20 min of testing following an initial exposure to nicotine relative to a saline control. This suggests that an initial exposure to nicotine has increased activational effects on adolescent relative to adult ambulatory behavior.

For rearing, a significant time×treatment×interval interaction [$F(8,288)=2.499$, $P=0.01$] was present, as well as a trend toward a time×age effect, although no age× treatment effect was present. Adult animals showed a decrease in rearing behavior not displayed by adolescents following the nicotine injection for the first 10 min of activity. No treatment effect was present for an initial dose of nicotine on rearing, although a significant age effect was present [$F(1,36)=8.4$, $P<0.01$], as adolescent rats rear less than adults. The differential activational and depressant actions of nicotine were quickly extinguished in both the adolescent and adult rats.

Nicotine dose–response analysis in adolescent and adult rats

Initial examination of the nicotine dose–response curve for ambulation revealed little difference between adolescent and adult animals (Fig. 5). For both adults and adolescents, nicotine-increased ambulation occurred in a dose-dependent manner [$F(4,72)=21.1$, $P<0.01$] with no age effects or interactions. A significant linear trend of dose was present for both adolescents ($t=5.5$, $P<0.01$) and adults ($t=4.8$, $P<0.01$). For rearing, nicotine also tended to increase rearing in both adults and adolescents, as indicated by a significant dose effect [$F(4,72)=3.5$, $P=0.01$]. A significant linear trend of dose was present in the adult animals ($t=5.0$, $P<0.01$) but not for adolescents; however, there was no interaction of age and nicotine dose. In sum, although there appeared to be no major difference in dose–response sensitivity between adolescents and adults for ambulation, the adolescents tended to show diminished rearing in response to nicotine.

Discussion

The main finding in these studies is that adolescent rats, unlike adults, did not exhibit signs of nicotine-cue conditioning. Moreover, following repeated injections of drug, adolescents displayed less robust sensitization to the locomotor effects of nicotine than adults. Differences were also present in the adolescent and adult response to an initial dose of nicotine, with adolescents showing increased ambulation, and less sensitivity to the depressant effects of nicotine than adults. Although dose–response testing revealed little difference in responsiveness to nicotine in measures of ambulation between adolescents and adults, in measures of rearing, adolescent rats were less sensitive to the activating effects of nicotine. Decreased rearing was also apparent in non-drug motor activity, with adolescents spending more of their time ambulating and less time rearing than adults. This distinction in the pattern of behaviors suggests that adolescent rats may be oriented to different aspects of the environment. The lack of drug-cue conditioning may be related to inattention to environmental cues, a critical element for the development of associative learning. Additionally, as sensitization is less robust in the adolescent, the substrate required for plasticity-related changes that occur with repeated drug environment pairings may be altered in juvenile animals.

In adult animals, an acute dose of nicotine results in initial locomotor depression, followed by behavioral stimulation (Clarke and Kumar 1983; Walter and Kuschinsky 1989). We also found this profile, and noted that adolescents were less sensitive to the first injection of nicotine (although the saline baseline was recorded from the previous day of testing, and was not counterbalanced). Although adolescents showed an increased sensitivity to the initial stimulatory effects of nicotine, the overall profile of sensitization to the drug appeared blunted in comparison with adult animals. Sensitization to chronic

nicotine has been investigated by Faraday et al. (1999, 2001), who also reported differences in behavioral sensitivity to nicotine between adolescents and adults. However, it is difficult to make direct comparisons with this study as implanted minipumps were used to deliver nicotine, and our study utilized repeated injections. In contrast, it has been reported that adolescent rats display decreased locomotor sensitization to cocaine, which has been postulated to be linked to alterations in dopaminergic function (Bolanos et al. 1998; Laviola et al. 1995; Spear and Brake 1983). However, as the level of locomotor sensitization is not predictive of the magnitude of the conditioned effect (Hotsenpiller and Wolf 2002), the decreased sensitization may not solely explain the lack of conditioning exhibited on the testing day.

In the present dose–response study, adolescent and adult rats responded differently to the effects of nicotine on rearing, with adolescents showing no increases in rears in response to an increasing dose of nicotine. This lack of a dose–response relationship may simply be due to a reduced prevalence of rearing in adolescent rats (Spear et al. 1982). However, this is very unlikely, as adolescent rats are clearly capable of showing much higher levels of rearing than those observed in the dose–response study; for example, in Fig. 1, on early days, adolescent rats show 100 rears per hour. It is interesting to note that individual differences in rearing responses in adult animals have been linked to alterations in cholinergic activation in the hippocampus and frontal cortex, as well as ventral and dorsal striatal dopaminergic activity (Canales and Iversen 2000; Fray et al. 1980; Planeta and Marin 2002; Sahakian et al. 1975; Thiel et al. 1998, 1999). Additionally, as the midbrain, cerebral cortex, and hippocampus show age-dependent alterations in nicotinic acetylcholine receptor expression, choline acetyltransferase activity, and dopamine turnover in response to continuous nicotine exposure (Trauth et al. 1999, 2000a, 2001), rearing could provide a behavioral measure of expression of these molecular differences.

Although not directly addressed in the present study, other work has shown that drug-cue conditioning involves brain regions such as the prelimbic, infralimbic, ventrolateral orbital, and anterior cingulate cortex (Schroeder et al. 2000). In adult rats, exposure to the nicotine-associated environment increases c-fos expression, a marker for gene activation, in these areas and several other regions, an effect true for exposure to environments associated with other drugs such as cocaine, morphine, and natural rewards such as chocolate (Neisewander et al. 2000; Schroeder et al. 2000, 2001). Changes in c-fos expression indicate that cue-induced drug effects of nicotine administration can alter gene expression in these areas of the brain involved in normal learning and memory (Schroeder et al. 2000, 2001; Schroeder and Kelley 2002). Moreover, these cue-conditioning induced changes in c-fos expression and behavior have been shown to be entirely due to exposure to the drug-paired environment per se, and not due to non-specific effects of drug treatment (Schiltz 2003, Schroeder and Kelley 2002). Additionally, drug expectancy, or craving, has been shown to result in activation of limbic and prefrontal regions in human imaging studies (Childress et al. 1999; Grant et al. 1996; London et al. 2000).

The lack of drug-cue conditioning in adolescent rats may be due to developmental differences in prefrontal cortex in adults and adolescents. This region has long been known to be one of the last brain regions to myelinate (Nauta 1971) and continues to undergo substantial changes during the adolescent period in humans (Giedd et al. 1999; Seeman 1999; Seeman et al. 1987; Sowell et al. 1999a,b; Thompson et al. 2000), non-human primates (Lewis et al. 1998), and rats (Kolb and Nonneman 1976; Van Eden and Uylings 1985a,b). The prefrontal region is implicated in directing attention, inhibiting inappropriate responses, monitoring behavior with respect to emotional state, and focusing attentional resources (Arnsten 1998; Zahrt et al. 1997). The attention of adolescents may be biased toward activity within the environment without focused attention to cues, due to a less mature prefrontal cortex relative to adults. Contextual cues appear to be critical for the development of drug sensitization effects (Badiani et al. 1995; Reid et al. 1996, 1998). Moreover, this may be one explanation for adolescents showing decreased drug-cue conditioning. Further, differences between adolescent and adult rats in the pharmacokinetics of nicotine cannot be ruled out. Regardless of the explanation for differences in sensitivity, the findings suggest that for certain effects of nicotine, adolescents are less sensitive.

The present findings are significant because they demonstrate a behavioral deficit in nicotine-cue conditioning in the adolescent rat. Additionally, although adolescents displayed differential sensitivity to repeated administration of nicotine compared with adults, these differences may not fully explain the lack of cue conditioning, as a clear dose–response relationship was still present for ambulation. Adolescents were found to consistently rear less than adults throughout testing. As drug-cue conditioning is linked to prefrontal cortical function, these results are suggestive of differences in the adolescent substrate relative to that of the adult, possibly related to immature or still developing plasticity mechanisms in the prefrontal cortex. Additionally, although speculative, decreased rearing in the adolescent may be indicative of decreased attention to cues, which may relate to their failure to display cue conditioning. These findings are an important first step in beginning to examine the neurobiology of nicotine use during this frequently overlooked developmental period.

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