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Increased anxiety-like behavior in adult rats exposed to nicotine as adolescents

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Abstract

Objective: Twenty percent of adolescents between 12 and 18 years old are regular smokers. Recently developed animal models demonstrate that adolescent nicotine exposure produces behavioral and electrophysiological changes, which persist into adulthood. The purpose of this study was to further define the behavioral effects of nicotine exposure during adolescence. *Methods*: Male 31–36-day-old adolescent rats were administered 5.0 mg/kg/day nicotine using transdermal Nicoderm CQ patches (SmithKline Beecham). During nicotine exposure, motor activity was assessed. Behavior in both standard open field and modified open field was examined 2–3 weeks after exposure ended. *Results*: Nicotine exposure significantly enhanced motor activity in nicotine-exposed rats compared with controls, demonstrating the acute stimulatory effects of transdermal nicotine. Two to three weeks after nicotine exposure ended, significantly lower levels of exploratory activity were observed relative to controls in the standard open field. Rats exposed to nicotine during adolescence also retreated to the perimeter of the open field more quickly than control rats. In a modified open field, nicotine exposure reduced approaches to food, contact with food and food intake. *Conclusions*: Taken together, these data suggest that adolescent nicotine exposure may induce an anxiogenic profile, which persists beyond acute nicotine withdrawal. Given the hypothesized role of stress and anxiety in the maintenance of smoking, it could be speculated that anxiety associated with smoking abstinence may play an important role in continued adolescent tobacco use.

Keywords: Adolescent; Nicotine; Anxiety; Exploratory activity

1. Introduction

Over the last 25 years, an average of 6–8% of the annual health care expenditures in the United States has been directed towards smoking-related illness (Warner et al., 1999; Warner, 2000). Despite continued efforts directed at reducing adolescent tobacco use, nearly 20% of adolescents between 12 and 17 years old continue to regularly use tobacco products (National Household Drug Abuse Survey, 1999). This is of considerable interest because Chen and Millar (1998) have reported that adolescent initiation of smoking increases adult daily cigarette consumption and decreases the probability of successfully abstaining from smoking. In this light, it is important to examine the potential effects of adolescent nicotine exposure on adult neurobehavioral function.

A number of recent animal studies confirm that adolescent nicotine produces lasting neurobehavioral alterations (Slawecki and Ehlers, 2002; Trauth et al., 2000a,b,c,d, 2001). Adult rats exposed to nicotine during adolescence have decreased locomotor activity and increased passive avoidance behavior relative to age-matched controls (Slawecki and Ehlers, 2002; Trauth et al., 2000d). Exposure to nicotine during adolescence also results in long-term changes in adult neurophysiological activity. In adult rats exposed to nicotine during adolescence, decreased slow wave power in the cortical electroencephalogram (EEG) and increased amplitude of the N1 component of the cortical auditory event-related potential have been observed (Slawecki and Ehlers, 2002). Neuroanatomical studies indicate that adolescent nicotine exposure produces cortical and hippocampal cell loss, increased nicotinic receptor binding in the cortex and hippocampus and reduced hemicholinium and paroxetine binding in the hippocampus (Trauth et al., 2000a,b,c; Xu et al., 2001). Taken together, these data

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clearly indicate that adolescent nicotine exposure has lasting neurobehavioral effects.

What is striking from initial studies of adolescent nicotine exposure in rodent models is the seeming contradiction between neurophysiological and behavioral effects of nicotine exposure. Neurophysiological indices suggest increased cortical arousal (Slawecki and Ehlers, 2002). For example, decreases in slow wave activity are typically associated with shifts away from sleep and towards states of increased arousal (Cape and Jones, 2000; Ehlers et al., 1997; Maloney et al., 1997). Similarly, increases in cortical N1 amplitude are associated with increased arousal and attention (Alho et al., 1994; Coull, 1998; Hansen and Hillyard, 1980; Robledo et al., 1998). However, it is difficult to reconcile increased cortical arousal with the robust decreases in motor activity observed following adolescent nicotine exposure (Slawecki and Ehlers, 2002; Trauth et al., 2000d). In order to potentially resolve this discrepancy, it was hypothesized that decreased activity in rats exposed to nicotine during adolescence results from an increase in anxiety-like behavior (Slawecki and Ehlers, 2002). It has been reported that anxiogenic agents such as corticotropin-releasing factor (CRF) can decrease motor activity under some circumstances (Liang and Lee, 1988). Further, enhanced anxiety would be consistent with the negative affect associated with nicotine withdrawal in abstinent smokers (Brown et al., 2002; Kenford et al., 2002; Killen et al., 2001; Lennox and Taylor, 1994; O'Loughlin et al., 2002) and in animal models (Epping-Jordan et al., 1998; Helton et al., 1993; Watkins et al., 2000). It is these negative withdrawal symptoms that may in part contribute to failed smoking cessation (Brown et al., 2002; Kenford et al., 2002; Killen et al., 2001; Lennox and Taylor, 1994; O'Loughlin et al., 2002).

The primary focus of this study was to assess anxiety-like behavior following adolescent nicotine exposure. Two common behavioral paradigms were used. In the first test (i.e., the standard open field), exploratory behavior was assessed as an index of anxiety (Blokland et al., 2002; Bowman et al., 2002; Mechan et al., 2002; Sarbadhikari et al., 1996). In the second test (i.e., a modified open field), anxiety-like behavior was indexed in a conflict situation by examining behaviors directed towards food placed in the center of an open field in food-restricted rats (Britton et al., 1982; Britton and Thatcher-Britton, 1981; Rex et al., 1998). Increased anxiety in this test is indexed by decreased food-directed behavior. It was hypothesized that in the standard open field, rats exposed to nicotine during adolescence would display decreased exploratory behavior. In the modified open field (aka, novelty-induced feeding suppression test), it was hypothesized that rats exposed to nicotine during adolescence would display less food-directed behaviors relative to age-matched controls. The open-field paradigms were chosen because the apparatus and testing environments were relatively similar to those used in prior studies, which have assessed motor activity and neurophysiological function after adolescent nicotine exposure (Slawecki and Ehlers, 2002). As a result, data from these models may be more readily interpreted in regards to our previous work. A secondary goal of this study was to further characterize our transdermal adolescent nicotine exposure model. As such, motor activity during nicotine exposure was assessed.

2. Materials and methods

2.1. Subjects

Twenty-eight male Sprague–Dawley rats obtained from Harlan Sprague–Dawley (Indianapolis, IN) were used for this study. Upon receipt, rats were 28 days old and averaged 95 ± 1 g. During nicotine exposure, rats were housed four per cage in standard cages [25 cm (w) × 20 cm (h) × 45 cm (l)]. However, rats were separated from each other with dividers to prevent the removal of the nicotine patch by a cage mate. When nicotine exposure ended, rats were housed two per cage. Pair housing was maintained for the remainder of the experiment. A 12 h light/dark cycle (lights on at 6 am) and ad libitum feeding were maintained throughout the study, except as noted below in the modified open-field test. Animal care was in accordance with NIH and institutional guidelines (Institute for Laboratory Animal Resources, 1996).

2.2. Adolescent transdermal nicotine exposure

The procedure for transdermal nicotine exposure has previously been described in detail (Slawecki and Ehlers, 2002). Briefly, on postnatal day 30, all rats were anesthetized with halothane (5% in air for induction, 1–2% in air for maintenance). A patch of fur on the back was then thoroughly shaved, depilated with Nair depilatory lotion and cleansed with water. Starting on postnatal day 31, a portion of the nicotine patch (Nicoderm CQ Step3, 7 mg/day; SmithKline Beecham, Pittsburgh, PA) delivering 5.0 mg/kg/day nicotine was applied to the shaved region. This dose of nicotine produces blood nicotine levels averaging 90 ng/ml (Slawecki and Ehlers, 2002). Pieces of flexible fabric Band-Aid and waterproof tape were put on top of the nicotine patch to improve its adherence to the rat. Nicotine was administered to 12 rats. Rats in the control group ($n = 16$) were shaved and depilated, but only the Band-Aid and waterproof tape was placed on the back. This application procedure was repeated for 5 consecutive days (i.e., postnatal days 31–36).

2.3. Locomotor activity assessment

Motor activity during nicotine administration was assessed using Digiscan Dmicro Animal Activity Monitors

and MicroPro V1.30i software (AccuScan Instruments, Columbus, OH). Each activity monitor consisted of a single pair of sensors, which monitored the animal's activity along the length of the cage via 16 infrared light beams. Sensors [46 cm (l) × 30.5 cm (w) × 13 cm (h)] were mounted 4 cm above the floor of the test chamber. Ambulatory activity and episodes of movement (i.e., movement number) were assessed. Assessments of locomotor activity were performed 1 h after nicotine patch application on the first 4 days of nicotine exposure. Motor activity was also assessed 10 days after nicotine exposure ended. Activity sessions lasted for 10 min.

2.4. Standard open-field assessment

Assessment of exploratory behavior in the open field was performed without prior exposure to the open field apparatus. The open field was 76 cm (w) × 76 cm (l) × 50 cm (h). The floor of the open field was demarcated into 25 equally sized squares (16 perimeter squares and 9 center squares). During tests, the open field was illuminated by a single white light (50 lx) situated 3.5–4 ft above the floor of the apparatus. On the test day, all subjects were weighed and transferred to a dimly lit anteroom. They were provided at least 15 min to habituate to this anteroom. At the start of the test, the rat was placed in the center of the open field. Rats were permitted to freely explore the apparatus for 5 min. The latency to move to the perimeter of the maze, the number of perimeter square entries and the number of center square entries were recorded during the test. At the conclusion of the test, the rat was returned to its home cage. The apparatus was cleaned with alcohol and water prior to assessing the next subject. Testing was carried out 17–19 days after the cessation of nicotine exposure. Tests were run between 9 am and 12 pm. On the test day, an individual selected nicotine and control rats to be run in an alternating fashion. A separate individual, who was blind to treatment group, scored behavior in the open field.

2.5. Modified open-field assessment

Assessment of anxiety-like behavior in the modified open field was performed without prior exposure to the apparatus. The test apparatus was constructed from a standard 32 gal trash can. A single 5 g food pellet was fixed in place at the center of the apparatus prior to each test. The apparatus was illuminated by a single white light (50 lx) located 3.5–4 ft above the floor of the apparatus. Twenty four hours prior to the test, all subjects were food deprived. On the test day, all subjects were weighed and transferred to a dimly lit anteroom. They were provided at least 15 min to habituate to this anteroom. To start each test, a rat was placed in the center of the apparatus. Rats were given 5 min to freely explore the apparatus. The number of food contacts, the time of contact with food and the amount of food eaten were recorded during each test. The average time spent in contact with food during each approach was also assessed (i.e., total food contact time/number of food approaches). At the conclusion of the test, the rat was returned to its home cage. The apparatus was cleaned with alcohol and water prior to assessing the next subject. Testing in the modified open field took place 24–25 days after nicotine exposure had ended. Tests were run between 9 am and 12 pm. On the test day, an individual selected nicotine and control rats to be run in an alternating fashion. A separate individual, who was blind to treatment group, scored behavior in the modified open field.

2.6. Statistical analysis

Statistical analyses were performed using Systat for the Macintosh (Systat). Two-way mixed ANOVA were used to assess differences in body weight during nicotine exposure (Group × Day) and differences in motor activity during nicotine exposure (Group × Day). Group was assessed as a between-subject measure. Day was assessed as a within-subject repeated measure. Greenhouse–Giesser-adjusted *P*-values are reported for all repeated-measure assessments. Activity data of one rat from the nicotine group was lost due to a computer error. As a result, activity during nicotine exposure was assessed in 11 nicotine rats and 16 control rats. For assessment of activity 10 days after nicotine exposure ended, 12 nicotine rats and 16 control rats were assessed.

One-way ANOVA were used to assess group differences in body weight and behavior during open field and modified open field testing. Standard open field variables assessed included total square entries, center square entries, perimeter square entries and latency to enter the perimeter at the start of the test. Modified open field test variables assessed included amount of food eaten, approaches to food, contact time with food and average time/food approach. Standard open field data from one control subject could not be assessed due to a recording error. As a result, 15 control and 12 nicotine rats were compared in this test. In the modified open field test, 16 control rats and 12 nicotine rats were assessed.

3. Results

3.1. Behavioral effects of adolescent nicotine exposure

3.1.1. Body weight

There were no significant differences in body weight between nicotine and control groups immediately prior to nicotine exposure (Nicotine = 122 ± 1 g, Control = 120 ± 2 g). Body weight of the nicotine rats was significantly [$F(1,26) = 6.57$, $P = .01$] lower than control rats after the first day nicotine exposure (Nicotine = 127 ± 2 g,

Control = 134 ± 2 g) but not on any other day of exposure. When nicotine exposure ended, body weight did not differ between groups (Nicotine = 155 ± 2 g, Control = 160 ± 2 g).

3.1.2. Motor activity

Statistical analyses of motor activity revealed significant effects of Group [Ambulatory activity: $F(1,25) = 10.03$, $P = .004$, Movement number: $F(1,25) = 9.87$, $P = .004$], Day [Ambulatory activity: $F(4,100) = 10.20$, $P < .0001$, Movement number: $F(4,100) = 18.28$, $P < .0001$], and a Group × Day interaction [Ambulatory activity: $F(4,100) = 3.53$, $P = .016$, Movement number: $F(4,100) = 5.27$, $P = .002$]. Overall, group effects were the result of ambulatory activity and movement number being greater in nicotine rats relative to controls (Table 1). Between-group analyses of day-by-day group differences revealed that ambulatory activity was significantly greater in nicotine rats relative to controls on day 1 [$F(1,25) = 12.47$, $P = .002$], day 3 [$F(1,25) = 10.90$, $P = .003$] and day 4 [$F(1,25) = 6.90$, $P = .015$] of nicotine exposure. Movement number in nicotine rats was significantly increased relative to controls on day 1 [$F(1,25) = 16.42$, $P < .0001$], day 2 [$F(1,25) = 9.31$, $P = .005$] and day 3 [$F(1,25) = 11.10$, $P = .003$] of nicotine exposure.

3.2. Protracted behavioral effects of adolescent nicotine exposure

3.2.1. Motor activity

Assessment of motor activity 10 days after nicotine exposure ended revealed significant differences between control and nicotine rats (Table 1). Ambulatory activity was significantly [$F(1,25) = 10.50$, $P = .003$] reduced in nicotine rats relative to control rats. Movement number was also significantly [$F(1,25) = 10.80$, $P = .003$] lower in nicotine-exposed rats compared with controls.

3.2.2. Standard open field

Behavior in the standard open field was assessed 17–19 days after nicotine exposure ended. At this time, there were no differences in body weight between nicotine rats (292 ± 5 g) and control rats (295 ± 5 g). Nicotine rats made significantly fewer perimeter square entries [$F(1,25) = 13.85$, $P = .001$] and total square entries [$F(1,25) = 14.92$, $P = .001$] compared with controls (Fig. 1). Center square crossings tended to be lower in nicotine rats relative to control rats, but the differences were not statistically significant. The latency to retreat from the center of the open field at the start of the test was also significantly reduced [$F(1,25) = 4.63$, $P = .042$] in nicotine rats compared with control rats (Fig. 1). Rearing, grooming, urination and defecation occurred with very low frequency and thus were not assessed statistically. There were no apparent differences in these measures between groups.

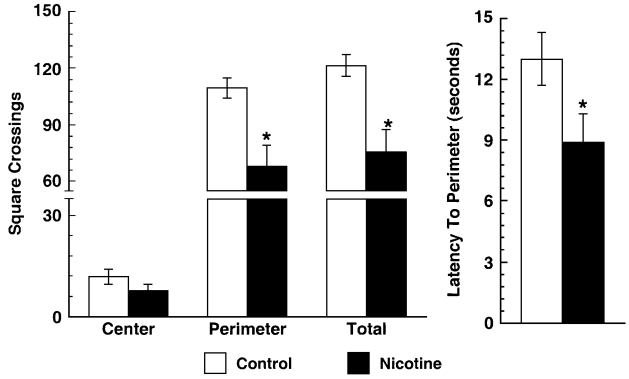

Fig. 1. Effects of adolescent nicotine exposure on square entries and latency to move to the perimeter of the standard open field. Open bars represent the age-matched control group ($n = 15$). Filled bars represent rats exposed to nicotine during adolescence ($n = 11$). Asterisks depict statistically significant differences between groups.

3.2.3. Modified open-field behavior

Behavior in the modified open field was assessed 24–25 days after nicotine exposure ended (Fig. 2). On the test day, there were no differences in body weight between nicotine (288 ± 4 g) and control (295 ± 3 g) rats. Relative to control rats, nicotine rats made significantly fewer approaches to the food placed in the center of the apparatus [$F(1,26) = 13.32$, $P = .001$], spent significantly less time in contact with food [$F(1,26) = 12.14$, $P = .001$] and consumed significantly less food relative to controls [$F(1,26) = 6.44$, $P = .017$]. The average amount of time spent in contact with food during each food approach was also significantly

Table 1
Ambulatory activity and movement number (mean ± S.E.M.) in nicotine ($n = 11$ or 12) and control ($n = 16$) rats

		Pre-nicotine	Nicotine, Day 1	Nicotine, Day 2	Nicotine, Day 3	Nicotine, Day 4	Post-nicotine, 10 days
Ambulatory activity	Control	369 ± 16	444 ± 45	494 ± 51	395 ± 31	368 ± 36	326 ± 16
	Nicotine	386 ± 30	675 ± 50*	599 ± 65	614 ± 71*	531 ± 58*	246 ± 21*
Movement number	Control	62 ± 3	67 ± 3	61 ± 4	48 ± 3	51 ± 4	45 ± 2
	Nicotine	69 ± 4	92 ± 6*	81 ± 5*	72 ± 8*	57 ± 8	34 ± 3*

Activity is presented from the day prior to nicotine exposure (Pre-nicotine), during nicotine patch application (Nicotine, days 1–4) and 10 days after cessation of nicotine exposure (Post-nicotine, 10 days).

* Significant differences compared with the control group.

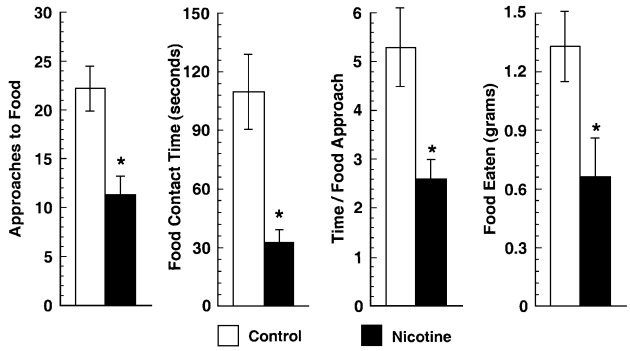

Fig. 2. Effects of adolescent nicotine exposure on food approaches, food contacts, food consumed and average time spent in contact with food during each approach in the modified open field. Open bars represent the age-matched control group ($n=16$). Filled bars represent rats exposed to nicotine during adolescence ($n=12$). Asterisks depict statistically significant differences between groups.

lower [$F(1,26)=7.81$, $P<.01$] in nicotine rats compared with controls rats.

4. Discussion

In the present study, adolescent rats were exposed to nicotine during adolescence using transdermal nicotine patches in order to examine nicotine's lasting effects on adult anxiety-like behavior. Consistent with our previous study (Slawecki and Ehlers, 2002), nicotine administration via transdermal patches produced a transient reduction in weight gain. In the present study, this weight reduction did not last beyond the first day of exposure. The nicotine-induced reduction in motor activity observed 10 days after nicotine exposure ended is also consistent with previous studies (Slawecki and Ehlers, 2002; Trauth et al., 2000d). By monitoring motor activity during nicotine exposure, it was also demonstrated that nicotine reaches physiologically relevant levels within 1 h of patch application. Further, the sustained high levels of activity in nicotine rats during exposure suggest that physiologically relevant levels of nicotine are maintained for the treatment period. Taken together, these data indicate that transdermal patches effectively deliver nicotine to the adolescent rat.

In the standard open field, decreased time and/or entries into the center squares and a decrease in the latency to retreat to the perimeter of the open field at the start of the test have been suggested to serve as indices of enhanced anxiety (Blokland et al., 2002; Bowman et al., 2002; Sarbadhikari et al., 1996; Yilmazer-Hanke et al., 2002). In the modified open field, an anxiety-like profile is characterized by decreased time spent in contact with food, decreased approaches to food and decreased food eaten (Britton et al., 1982; Britton and Thatcher-Britton, 1981; Rex et al., 1998). This general profile of behaviors was observed in rats exposed to nicotine during adolescence. Therefore, it seems reasonable to hypothesize that the protracted neurobehavioral effects of adolescent nicotine exposure (i.e., decreased activity and enhanced cortical arousal) may be partly attributed to enhanced anxiety. Further assessment of anxiety-like behavior using paradigms with more established predictive validity (i.e., the elevated plus maze or light–dark box) will strengthen this hypothesis.

Several previous studies have demonstrated decreased motor activity after adolescent nicotine exposure (Slawecki and Ehlers, 2002; Trauth et al., 2000d). Although the present data suggest a portion of this hypoactivity may represent decreased exploratory activity associated with increased anxiety, it is difficult to attribute the observed hypoactivity solely to enhanced anxiety. Decreased perimeter square crossings in the open field have been suggested to index arousal-related motor behavior and not increased anxiety (Boguszewski and Zagrodzka, 2002). However, overall decreases in activity are less likely to account for other indices of increased anxiety observed in this study (i.e., decreased latency to the perimeter, decreased food contact and decreased feeding). For example, Rex et al. (1998) have suggested that food intake and food contact time in the modified open-field test are not influenced by overall activity levels. Further, decreases in the average amount of time spent in contact with during each approach in rats exposed to nicotine suggest an increased "motivation" to avoid the center of the open field. Therefore, while the influence of activity levels on anxiety-like behaviors cannot be excluded at this time, it is unlikely to account for all of the behaviors observed in this study.

There is sufficient self-reported evidence that the cessation of nicotine use can induce depressive-like symptoms (Markou and Kenny, 2002; O'Loughlin et al., 2002). As a result, it seems reasonable to hypothesize that an increase in depressive-like behavior could partly account for the results of the present study. A prominent feature of depressive-like behavior is anhedonia or lack of interest in "reward" (Markou and Kenny, 2002). In this regard, we have previously reported that adolescent nicotine exposure does not produce lasting decreases in sucrose preference, an animal model of anhedonia (Slawecki and Ehlers, 2002). Further, it has been demonstrated that anhedonic behavior associated with nicotine withdrawal dissipates after 4–5 days of abstinence following adult nicotine exposure in rats (Kenny and Markou, 2001). While it is possible that anhedonia could be more pronounced and longer lasting following adolescent exposure, evidence for such an enhancement has not yet been demonstrated. This hypothesis would be further strengthened by assessment of depressive-like behavior in a paradigm with well-established predictive validity, such as the forced swim test. Therefore, at present, the behavioral profile associated with adolescent nicotine exposure better fits increased anxiety-like behavior as opposed to enhanced depressive-like behavior.

The behavioral effects associated with periods of protracted abstinence from adolescent nicotine exposure are

similar to those observed during conditions of stress and anxiety (Slawecki and Ehlers, 2002; Trauth et al., 2000d). Intracerebroventricular administration of CRF, a peptide whose central activation is intimately associated with enhanced behavioral indices of stress and anxiety (Heilig et al., 1994; Heilig and Widerlov, 1990), produces a neurophysiological profile, which is remarkably similar to that observed following abstinence from adolescent nicotine exposure. In both cases, decreases in cortical slow wave power in the EEG and increases in amplitude of the cortical N1 component of the auditory event-related potential are observed (Ehlers et al., 1997; Slawecki and Ehlers, 2002). Administration of CRF directly into the amygdala, one of the putative brain regions mediating the anxiogenic effects of CRF (Heilig et al., 1994; Pich et al., 1995; Rassnick et al., 1993), also increases passive avoidance behavior and decreases exploratory activity (Liang and Lee, 1988). This behavioral profile is also observed in adult rats exposed to nicotine during adolescence (Slawecki and Ehlers, 2002; Trauth et al., 2000d). As the behavioral alterations associated with adolescent nicotine exposure are observed several weeks after exposure has ended, it seems likely that these effects are due to long-term neuroadaptations.

Neuroanatomical evidence to date partially supports the hypothesis that adolescent nicotine exposure can impact the neurobiological substrates underlying stress and anxiety. Brain regions, which have been shown to be affected by adolescent nicotine exposure, include the cortex and hippocampus (Slawecki and Ehlers, 2002; Trauth et al., 2000a,c; Xu et al., 2001). Further, cell death and alterations in nicotinic receptor binding have been reported in the cortex and hippocampus following adolescent nicotine exposure (Trauth et al., 2000a,b,c). Studies also indicate that adolescent nicotine exposure produces long-term changes in basal cortical neurophysiological activity (Slawecki and Ehlers, 2002) and cortical responses to intracerebroventricular CRF (Slawecki and Ehlers, 2003). Cortical and hippocampal functions also influence anxiety-related behavior. Several studies have demonstrated that serotonergic and GABAergic systems in the dorsal hippocampus and cortex partially mediate the effects of anxiolytics in animal models (Menard and Treit, 1999; Rex et al., 1997; Serra et al., 1999). Taken together, it is reasonable to suggest that alterations in cortical and/or hippocampal function observed following adolescent nicotine exposure could in part contribute to the anxiety-like behavior observed in the present study. It is important to note that the CRF systems in the amygdala have been shown to play a role in anxiety in a variety of animal models (Heilig et al., 1994; Menard and Treit, 1999; Merali et al., 1998; Pich et al., 1995; Rassnick et al., 1993; Wiersma et al., 1995). Therefore, a role for the amygdala in the increased anxiety associated with adolescent nicotine exposure should be considered. However, to date, there have been no published studies that can confirm or refute such a role.

A recent study has reported that of the more than 60% of adolescents who wished to quit smoking, fewer than 5% were abstinent after 1 year (Burt and Peterson, 1998). This finding is consistent with a significantly lower probability of successful abstinence after adolescent initiation of smoking (Chen and Millar, 1998). This dramatically low level of cessation may indicate an enhanced susceptibility to the development of nicotine dependence when tobacco use is initiated during adolescence, as has been suggested by Chen and Millar (1998). While this has not explicitly been demonstrated by our studies, the possibility of differential adolescent sensitivity to the effects of nicotine is supported by several recent studies in rodents (Trauth et al., 2000b,c). For example, Trauth et al. have reported that the patterns of nicotine-induced cell death differ following adolescent and adult exposure (Trauth et al., 2000c). Reasons for failed attempts at smoking cessation include increased negative affect (i.e., irritability, depression and anxiety) and stressful life events (Brown et al., 2002; Kenford et al., 2002; Killen et al., 2001; Lennox and Taylor, 1994; O'Loughlin et al., 2002). In light of the present data, which suggest enhanced anxiety-like behavior in adult rats exposed to nicotine during adolescence, it could be speculated that anxiety or altered responses to stress that persist beyond acute nicotine withdrawal perpetuate tobacco use throughout adolescence and into adulthood.

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