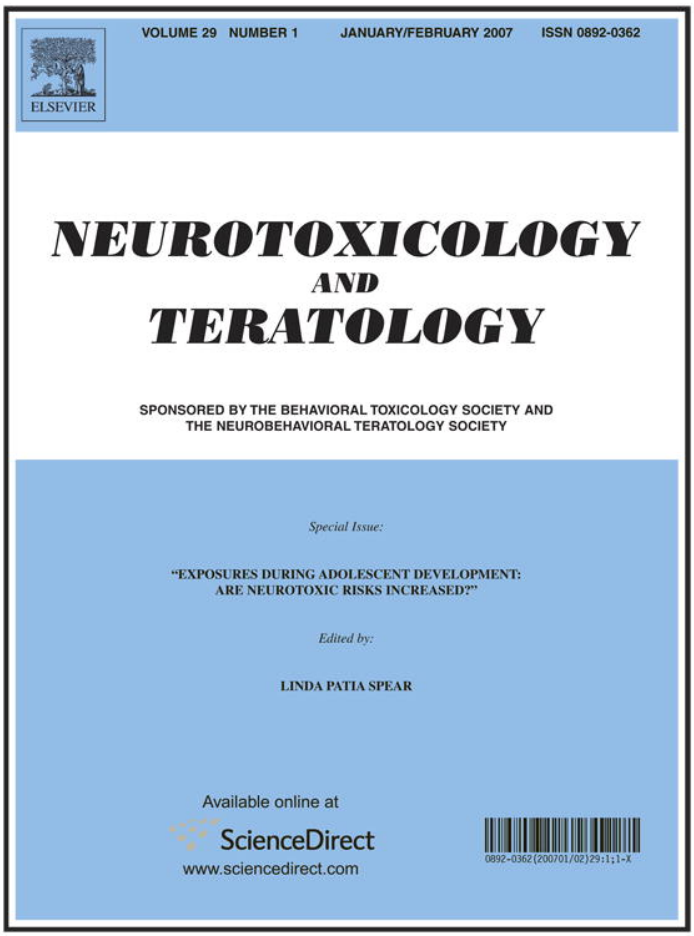

Available online at www.sciencedirect.com
ScienceDirect
Neurotoxicology and Teratology 29 (2007) 17–22
NEUROTOXICOLOGY AND TERATOLOGY
www.elsevier.com/locate/neutera

Adolescent nicotine exposure produces less affective measures of withdrawal relative to adult nicotine exposure in male rats

Laura E. O'Dell *, Oscar V. Torres, Luis A. Natividad, Hugo A. Tejeda

Department of Psychology, The University of Texas at El Paso, 500 W. University Avenue, El Paso, TX 79902, USA

Received 1 June 2006; received in revised form 3 October 2006; accepted 4 November 2006
Available online 14 November 2006

Abstract

Vulnerability to nicotine addiction is significantly increased in individuals who begin smoking during adolescence; however, the underlying mechanisms of this phenomenon remain unclear. This study examined the motivational effects of nicotine withdrawal in adolescent (PND 27–42) and adult (PND 60–75) rats using the conditioned place aversion paradigm. Male Wistar rats were tested for their initial preference for either of two distinct compartments of our conditioning apparatus. Rats were then implanted with subcutaneous (sc) pumps that produce equivalent blood plasma levels of nicotine for 14 days. Conditioning was conducted over the last 8 days of nicotine exposure. Rats received the nicotinic antagonist mecamylamine (1.5 or 3.0 mg/kg, sc) to precipitate withdrawal in their initially preferred compartment, and on alternate days they received saline in their non-preferred compartment. Following conditioning, rats were re-tested for their preference for each compartment. A subsequent study was conducted to examine potential developmental differences in learning place aversion produced by another aversive stimulus, lithium chloride (LiCl). Rats received LiCl (0, 10, 30, or 100 mg/kg, sc) in their initially preferred side using similar conditioning procedures. Adults displayed robust place aversion produced by nicotine withdrawal. This effect was lower in adolescent rats even in a group of young rats that received 7 additional days of nicotine exposure prior to conditioning. This developmental difference was specific to nicotine withdrawal since there were no differences between adolescents and adults in learning place aversion with LiCl. Our findings demonstrating reduced effects of nicotine withdrawal constitute a powerful basis for the increased vulnerability to nicotine dependence during adolescence.

Keywords: Development; Dependence; Mecamylamine; Rat; Adolescence; Place aversion

1. Introduction

The wide spread use of tobacco among adolescents is a major concern in light of findings that nicotine dependence and smoking related-diseases are more prevalent among smokers who begin at an early age [3,7]. In general, it seems that smoking behavior is motivated by both the positive reinforcing effects of nicotine and avoidance of the negative effects associated with nicotine withdrawal. Studies using animal models indicate that enhanced reinforcing effects of nicotine may contribute to tobacco use during adolescence [2,8,13,25]. Recent work demonstrates that the physical signs of nicotine withdrawal are lower in adolescent relative to adult rats [15,16]. These findings suggest that there are likely different mechanisms that drive smoking behavior in adolescent and adult smokers. In addition, adolescent smokers may be less likely to quit smoking because they do not experience the negative aspects of nicotine withdrawal that might otherwise signal dependence and the need to quit smoking in adults. Previous work focused on the physical signs of nicotine withdrawal, and potential differences between these age groups with regard to the affective properties of nicotine withdrawal have not been thoroughly studied. Thus, the present study examined place aversion produced by nicotine withdrawal in adolescent and adult rats. The advantage of using a rat model to study nicotine dependence is that we are able to eliminate the influence of social factors that also contribute to smoking behavior in adolescent humans, such as peer influence, parental smoking rates, and enhanced propensity for risk-taking and novelty-seeking behaviors.

The prototypic age range for adolescence in rats conservatively ranges from postnatal day (PND) 28–45 [22]. Although it is difficult to define an exact time frame of adolescence, most

* Corresponding author. Tel.: +1 915 747 6557; fax: +1 915 747 6553.
E-mail address: lodell@utep.edu (L.E. O'Dell).

0892-0362/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ntt.2006.11.003

researchers agree that this phase of development is a period of transition that reflects a period during which age-specific behavioral discontinuities from younger and older animals are most evident. Most behavioral and physiological systems reach maximal maturation by PND 60, and are considered adults beyond this age. The present study tested adolescent rats that were between 21 to 42 days of age and adult rats between 60 to 75 days of age.

The conditioned place aversion paradigm involves repeated pairing of distinct environmental cues with aversive drug effects such that when given a choice, animals avoid the drug-paired cues relative to neutral cues. In the present study, nicotine withdrawal was precipitated in nicotine-dependent rats in the presence of distinct environmental cues using administration of either of two doses of the non-competitive nicotinic receptor antagonist mecamylamine [24,26]. On alternating days, rats were exposed to an adjacent and distinct compartment following saline administration. Following several pairings, rats were given free access to the withdrawal-paired compartment and the neutral side. Previous work demonstrates that nicotine-dependent adult rats reliably display a place aversion for a compartment where they experienced nicotine withdrawal [12,23,26]. Further, a place aversion study using mecamylamine to precipitate withdrawal demonstrated that mecamylamine dose-dependently produces place aversion in some, but not all strains of nicotine-dependent rats [24], suggesting that place aversion procedures are sensitive to genetic variations and dose manipulations.

We hypothesized that adolescent rats would display less sensitivity to nicotine withdrawal, and therefore less place aversion relative to adult rats. This is based on our finding that adolescent rats do not display elevated intracranial self-stimulation (ICSS) reward thresholds following mecamylamine administration relative to saline controls [16]. ICSS involves allowing a rat to self-administer small amounts of electrical current into reward-related structures. A shift to responding for higher current levels is hypothesized to reflect a *decrease* in brain reward function. Our finding that withdrawal from nicotine produces an increase in current intensity thresholds in adult rats that is not evident in adolescents suggests that adolescents are less sensitive to the negative affective properties of nicotine withdrawal relative to their adult counterparts. The present study extends this work by utilizing conditioning procedures that involve associations with environmental cues that are important in the maintenance of nicotine dependence [5].

The possibility exists that adolescent rats may be less able to associate environmental cues with nicotine withdrawal relative to adults. To address this potential developmental difference in learning, a second study compared the degree to which an aversive stimulus (LiCl injections) produces place aversion in adolescent and adult rats. Various doses of LiCl were used in order to maximize the possibility that developmental differences in learning could be detected with our conditioning parameters.

2. Methods

Male Wistar rats (Harlan, Inc.) were group-housed under a 12-h/12-h light/dark cycle with food and water available *ad libitum*. Testing was conducted during the light phase of the subjects' light/dark cycle. A different set of naïve rats was used for each experiment. Animals were acclimated to handling for 4–5 days prior to and following the surgical procedures. The adolescent rats were received in the laboratory past their weaning period (PND 21), and were tested during the adolescent period of development (i.e., PND 27–42). Adult rats were received in the laboratory after PND 50, such that testing occurred during the adult period of development (i.e., PND 60–75). The PND of the adolescent and adult rats during each experimental manipulation is noted in Table 1. An additional group of adolescent rats ($n=5$) was included that was exposed to nicotine for 7 additional days prior to conditioning. The rationale for including these rats was to examine whether adolescent rats require more days of nicotine exposure to produce dependence. These animals were born in our laboratory from fully out-bred rats from Harlan, Inc. because we needed to prepare them with minipumps on PND 21 and the rats could not be shipped prior to weaning. In general, we have found that there are no behavioral differences between out-bred Wistar rats that are shipped to our laboratory versus those that are born in our facility. All procedures were conducted in adherence to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The University of Texas at El Paso.

The conditioning apparatus consisted of two adjacent chambers. One compartment had a metal bar floor with chlorophyll bedding beneath it, and the other compartment had a perforated Plexiglas floor with pine bedding beneath it. Both compartments were equally illuminated and constructed from stainless steel sheets with one-way mirrors on the front walls to allow for behavioral observations. Conditioning was conducted using a biased procedure whereby rats were first allowed to shuttle freely between two distinct compartments for 15 min. On the preference tests the animals were all placed initially in the same side of the conditioning apparatus (metal floor side). The rats were conditioned in their initially preferred compartment that was defined as the side they spent more than 50% of their time during the pretest. Animals that spent more than 65% of their time on either side were eliminated, such that the initial bias of the rats was between 50 and 65%. The 65% elimination criterion was based on preliminary work demonstrating that it is difficult to reverse initial preferences that are stronger than 65% with nicotine withdrawal. This criterion is consistent with other

Table 1
Postnatal day of adolescent and adult rats in Experiment 1

Experimental manipulation:	Adolescent PND	Adult PND
Rats were acclimated to handling	23–26	56–59
Pre-test for preference	27	60
Mini-pump implantation	28*	61
Conditioning	34–41	67–74
Post-test for preference	42	75

*An additional group of adolescent rats were implanted with nicotine minipumps on PND 21 to examine whether a longer period of nicotine exposure would facilitate place aversion in adolescent rats.

laboratories examining place aversion produced by nicotine withdrawal [23]. The initial preference of the rats was not statistically different across the treatment conditions.

In Experiment 1, rats (n=5–7 per group) were anesthetized with isoflurane gas and prepared with subcutaneous osmotic pumps (Alzet, Inc., model ML2) that delivered saline or nicotine (4.7 mg/kg/day in adolescents and 3.2 mg/kg/day in adults; expressed as nicotine base) for 14 days. Conditioning was delayed for 6 days after pump implantation to ensure that nicotine dependence was established. The length of the delay is based on work demonstrating that at least 6 days of nicotine exposure is necessary to observe nicotine withdrawal in adult rats [26]. The rationale for using a higher dose of nicotine in adolescents is based on work from our laboratory [16] and others [25] demonstrating that a 1.5 fold higher concentration of nicotine is needed to produce equivalent blood plasma levels in adolescent and adult rats with nicotine minipumps. Our previous work demonstrated that a nicotine dose of 4.7 mg/kg/day in adolescents results in 76.2±7 ng/ml of plasma nicotine and a nicotine dose of 3.2 mg/kg/day in adults results in 65.4±9 ng/ml of plasma nicotine 7 days after the rats were prepared with minipumps [16]. Adolescent rats gain weight at a faster rate than adults, such that one might expect that adolescents might have lower daily levels of nicotine relative to adults. However, our previous work determining equivalent blood plasma levels of nicotine was conducted 7 days after the rats were prepared with minipumps and this is when the rats began conditioning in the present study [16]. Thus, we expect that these age groups had equivalent blood levels of nicotine when the conditioning phase of the experiment began. An additional group of adolescent rats (n=5) were included that received 7 additional days of nicotine exposure prior to conditioning. These rats were included to address whether adolescent rats require a longer exposure time to nicotine to produce dependence relative to adult rats that require at least 7 days [26]. In light of the small size of the rats on PND 21, these rats were implanted with a smaller model of minipumps (Alzet model 2001) that was replaced 7 days later with the same size pump as all other groups of rats. The dose of nicotine was adjusted for the lower volume in the small minipumps so that the adolescents still received 4.7 mg/kg/day. Conditioning was conducted over 8 days. On one day, rats were confined to their initially preferred side for 30 min following mecamylamine administration in separate groups of rats (1.5 or 3.0 mg/kg, sc) to precipitate withdrawal. On alternate days, rats were confined to their initially non-preferred side for 30 min following saline administration (sc). After conditioning, rats were re-tested for their preference for each compartment. Adolescents received the preference test on PND 42 and adults were tested on PND 75.

Experiment 2 examined potential developmental differences in learning by comparing place aversion produced by LiCl in adolescent and adult rats. LiCl is an aversive stimulus that causes skin irritation, diarrhea and discomfort to rats [17]. Conditioning was conducted in a similar manner and at the same ages that were used in Experiment 1. Conditioning was delayed until PND 34 so that the adolescent rats were tested at the same ages as Experiment 1. Adolescent and adult rats (n=5–8 per group) received LiCl (0, 10, 30, or 100 mg/kg, sc) in their initially preferred side. The doses of LiCl were chosen based on previous literature [18]. Three doses of LiCl were used in order to maximize the possibility that developmental differences could be observed with our conditioning parameters.

3. Results

3.1. Place aversion produced by nicotine withdrawal

Overall, the results revealed that adult rats displayed place aversion produced by nicotine withdrawal; however, this effect was lower in adolescent rats. The difference in the amount of time adult and adolescent rats spent in their initially preferred compartment after conditioning minus before conditioning was compared, such that negative values reflect a place aversion (Fig. 1). Our initial analysis focused on adolescent and adult rats that were prepared with saline or nicotine minipumps and received the 1.5 mg/kg dose of mecamylamine during conditioning. This analysis revealed a significant overall group difference with adult rats displaying a significant difference in the amount of time spent before and after conditioning relative to adult control rats (F[4,29]=2.7, p<0.05). However, there was no difference in the amount of time spent before and after conditioning in either group of adolescent rats that were exposed to nicotine for 7 or 14 days prior to conditioning. There were also no differences in time spent following conditioning in either age group of control rats that were prepared with saline pumps and

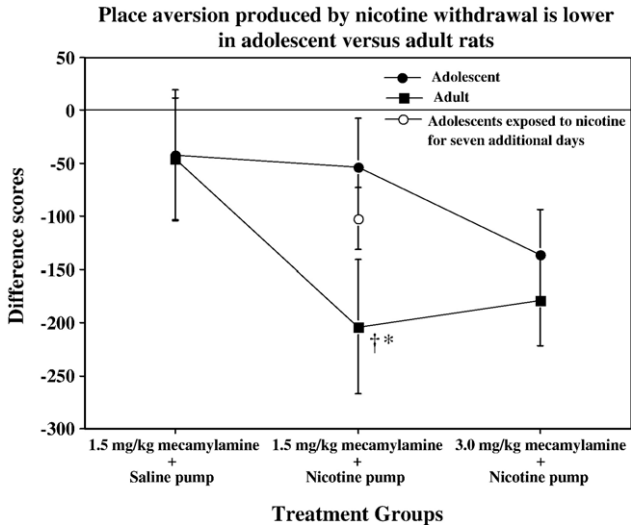

Fig. 1. The data reflect the difference (±SEM) between time spent (seconds) in the initially preferred side after conditioning *minus* time spent in that same compartment prior to conditioning, such that negative values reflect a place aversion. During conditioning, adult and adolescent rats prepared with saline or nicotine mini-pumps received repeated mecamylamine injections to precipitate withdrawal in their initially preferred compartment. Adult rats displayed a significant decrease in the amount of time they spent in the initially preferred compartment after conditioning (i.e., a place aversion). However, adolescents displayed fewer changes in time spent before and after conditioning even in adolescents exposed to nicotine for 7 additional days. The asterisk (*) reflects a significant difference relative to adult saline controls, and the dagger (†) represents a significant difference relative to adolescents receiving the same dose of mecamylamine (p<0.05).

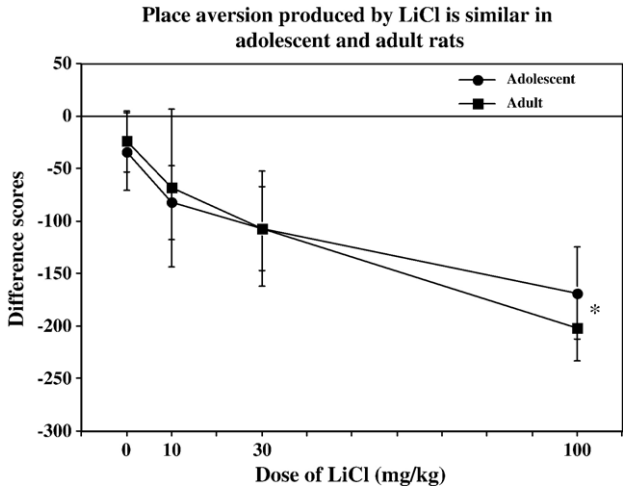

Fig. 2. The data reflect the difference (±SEM) between time spent (seconds) in the initially preferred side after conditioning *minus* time spent in that same compartment prior to conditioning, such that negative values reflect a place aversion. During conditioning, adult and adolescent rats received repeated LiCl injections (0, 10, 30, or 100 mg/kg, sc) in their initially preferred compartment. Both adult and adolescent rats displayed a decrease in the amount of time they spent in the initially preferred compartment after conditioning with LiCl (i.e., a place aversion). The asterisk (*) denotes a main effect of treatment that reflects a place aversion produced by LiCl ($p<0.01$) that is not different across age groups.

received mecamylamine during conditioning ($F[1,10]=0.005$, $p<0.94$), suggesting that mecamylamine was not aversive in the absence of nicotine. An analysis of adolescent and adult rats prepared with nicotine minipumps that received either the 1.5 or 3.0 mg/kg dose of mecamylamine revealed a significant overall group difference with adult rats exhibiting a larger difference in time spent before and after conditioning relative to adolescents ($F[3,25]=3.2$, $p<0.04$). Specifically, adults receiving the 1.5 mg/kg dose of mecamylamine displayed significantly less time in their initially preferred side after conditioning relative to adolescent rats receiving the same dose of mecamylamine (Fisher's LSD post hoc test, $p<0.01$). This developmental difference was not observed in adolescent and adult rats receiving the 3.0 mg/kg dose of mecamylamine.

3.2. Place aversion produced by LiCl

The difference in the amount of time adult and adolescent rats spent in their initially preferred compartment after minus before conditioning was compared (i.e., difference scores; see Fig. 2). The overall analysis revealed a main effect of treatment ($F[3,47]=5.5$, $p<0.01$), with rats receiving the highest dose of LiCl displaying a significant decrease in time spent in the initially preferred compartment after conditioning (Fisher's LSD post hoc test, $p<0.05$). The magnitude of place aversion produced by LiCl was not different across age groups ($F[1,47]=0.01$, $p=1.0$).

4. Discussion

The main finding of this report is that adolescent nicotine exposure produces less of the negative affective properties of nicotine withdrawal relative to nicotine exposure during the adult stage of development. Adult rats displayed a robust place aversion for an environment in which they previously experienced nicotine withdrawal. Place aversion was evident at lower doses of mecamylamine to precipitate withdrawal in adult rats relative to adolescents. Moreover, adolescents given 7 additional days of nicotine exposure still did not display place aversion produced by nicotine withdrawal. This suggests that adolescent rats are less sensitive to the affective properties of nicotine withdrawal relative to adults. There was a lack of developmental differences in adolescent and adult rats receiving the highest dose of mecamylamine. However, this may be due to a floor effect because adult rats receiving the highest dose of mecamylamine to precipitate withdrawal displayed a place aversion of approximately −200 s, which was the largest observed magnitude of place aversion produced by high doses of LiCl. Lower place aversion produced by nicotine in adolescent rats does not appear to be due to differences in learning because young rats displayed a similar place aversion to LiCl as their adult counterparts. Collectively, these findings suggest that adolescents are less sensitive to the negative affective properties of nicotine withdrawal relative to their adult counterparts.

The present findings are consistent with our previous work demonstrating that adolescent rats display less *physical* signs of nicotine withdrawal relative to adults [15,16]. Specifically, adolescent rats display fewer overt physical signs of precipitated withdrawal than adults across a range mecamylamine doses to produce withdrawal and a range of nicotine doses to produce dependence. The latter finding is important since adolescent rats require 1.5-fold higher levels of nicotine in their pumps to produce equivalent blood levels of nicotine as adult rats [see Refs. 16,25].

Our findings are in line with previous research examining the affective properties of nicotine withdrawal during adolescence. First, the present study extends our previous work examining the motivational/affective measures of nicotine withdrawal using ICSS procedures. Specifically, nicotine produces an increase in current intensity thresholds in adult rats that is not evident in adolescents, suggesting that young rats do not display the decreases in brain reward function during nicotine withdrawal that adult rats experience [16]. The present study further extends the results of the ICSS study because it utilized a paradigm involving environmental associations with the affective properties of nicotine [5]. Second, the present study is consistent with the finding that adult rats display taste aversion for a solution previously paired with nicotine; however, adolescent rats do not display this taste aversion [27]. Lastly, adolescent rats are less sensitive to the anxiety-inducing effects of withdrawal relative to adults [28]. Taken together, these studies suggest that adolescence is a period of development whereby exposure to nicotine produces less of the aversive properties of nicotine relative to nicotine exposure during the later stages of adult development.

The mechanisms mediating lower sensitivity to nicotine withdrawal during adolescence are presently unclear. However, they do not appear to involve differences in learning since adolescent rats display place aversion produced by LiCl that is

similar to adults. The observed behavioral effects may be due to differences in neurochemical mechanisms that mediate nicotine withdrawal. Previous work has demonstrated that dopamine levels are decreased and acetylcholine levels are increased in the nucleus accumbens of adult rats experiencing nicotine withdrawal [6,10,19]. Therefore, the possibility exists that adolescents are less sensitive to nicotine withdrawal due to a lack of changes and/or development of these systems mediating nicotine withdrawal. Lastly, adolescent rats may display less sensitivity to nicotine withdrawal due to a decrease in nicotinic receptor function [4]. However, a lack of nicotine receptors does *not* appear to explain developmental sensitivity because nicotine exposure during adolescence is associated with an up-regulation of nicotinic receptor number [20] and function [1].

Adolescent smoking is a major concern since nicotine dependence rates in adults are higher among smokers who begin during adolescence. It is well accepted that adult smoking behavior is maintained by a balance between the positive reinforcing effects of nicotine and avoiding the negative consequences of nicotine withdrawal. However, our findings suggest that the negative effects of nicotine withdrawal are lower during adolescence, such that adolescent smoking may be facilitated by a lack of aversive effects associated with nicotine. Without experiencing any negative effects of nicotine to deter smoking behavior, adolescents are more likely to continue smoking. Furthermore, there is a large body of literature demonstrating that adolescent rats are more sensitive to the reinforcing [2,13] and stimulant [8] effects of nicotine. Taken together, it appears that adolescent rats are more sensitive to the positive effects of nicotine reward and less sensitive to the negative effects of nicotine withdrawal. This is consistent with the alcohol literature suggesting that negative effects of alcohol are lower and the reinforcing effects of alcohol are enhanced during adolescence [22]. Therefore, we suggest that the enhanced sensitivity to the reinforcing effects of nicotine and resistance to the negative effects of nicotine and nicotine withdrawal constitute a powerful basis for rapid increases in tobacco smoking behavior during adolescence, which sets the stage for vulnerability to addiction at later stages of development. This work was conducted using male rats, and the possibility exists that female rats may differ in their behavioral responses to nicotine withdrawal. These differences to nicotine withdrawal might explain the enhanced vulnerability to tobacco use, progression to stable intake, and dependence measures in female versus male smokers. Future work will examine sex differences in nicotine withdrawal in adolescent and adult male and female rats.

The present findings are significant because they suggest that the mechanisms that drive smoking behavior are different during the adult and adolescent period of development. As an outcome, our findings provide insight into which treatment strategies may not work for adolescent smokers who are at risk of developing diseases caused by long-term tobacco use. For example, the finding that nicotine patches are generally not associated with lowered abstinence rates in adolescent smokers supports the idea that fundamental differences mediate smoking behavior during different stages of development [9,11,14,21]. Our findings suggest that one reason that the nicotine patch does not alter abstinence rates during adolescence is because young smokers do not experience nicotine withdrawal. Future research should be directed towards studying the neural mechanisms that drive smoking behavior during adolescence.

Acknowledgements

This research was supported by start up funds from The University of Texas at El Paso, The National Science Foundation Support of Mentors and Students Program (DUE 04-26266), and the National Institute of Mental Health Career Opportunities in Research Program (MH 019978-08). The authors would like to acknowledge Dr. Donald Moss for his assistance in the preparation of this manuscript.

References

[1] Y. Abreu-Villaca, F.J. Seidler, T.A. Slotkin, Impact of adolescent nicotine exposure on adenylyl cyclase-mediated cell signaling: enzyme induction, neurotransmitter-specific effects, regional selectivities, and the role of withdrawal, Brain Res. 988 (2003) 164–172.
[2] W. Adriani, S. Spijker, V. Deroche-Gamonet, G. Laviola, M. Le Moal, A.B. Smit, P.V. Piazza, Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats, J. Neurosci. 23 (2003) 4712–4716.
[3] N. Breslau, E.L. Peterson, Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences, Am. J. Public Health 86 (1996) 214–220.
[4] A.W. Bruijnzeel, A. Markou, Adaptations in cholinergic transmission in the ventral tegmental area associated with the affective signs of nicotine withdrawal in rats, Neuropharmacology 47 (2004) 572–579.
[5] A.R. Caggiula, E.C. Donny, A.R. White, N. Chaudhri, S. Booth, M.A. Gharib, A. Hoffman, K.A. Perkins, A.F. Sved, Cue dependency of nicotine self-administration and smoking, Pharmacol. Biochem. Behav. 70 (2001) 515–530.
[6] G. DiChiara, Role of dopamine in the behavioural actions of nicotine related to addiction, Eur. J. Pharmacol. 393 (2000) 295–314.
[7] J.R. DiFranza, P.J. Wellman, Preventing cancer by controlling youth tobacco use, Semin. Oncol. Nurs. 19 (2003) 261–267.
[8] M.M. Faraday, B.M. Elliott, J.M. Philips, N.E. Grunberg, Adolescent and adult male rats differ in sensitivity to nicotine's activity effects, Pharmacol. Biochem. Behav. 74 (2003) 917–931.
[9] K. Hanson, S. Allen, S. Jensen, D. Hatsukami, Treatment of adolescent smokers with the nicotine patch, Nicotine Tob. Res. 5 (2003) 515–526.
[10] B.E. Hildebrand, G.G. Nomikos, P. Hertel, B. Schilstrom, T.H. Svensson, Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome, Brain Res. 779 (1998) 214–225.
[11] R.D. Hurt, G.A. Croghan, S.D. Beede, T.D. Wolter, I.T. Croghan, C.A. Patten, Nicotine patch therapy in 101 adolescent smokers: efficacy, withdrawal symptom relief, and carbon monoxide and plasma cotinine levels, Arch. Pediatr. Adolesc. Med. 154 (2000) 31–37.
[12] Y. Ise, M. Narita, H. Nagase, T. Suzuki, Modulation of opioidergic system on mecamylamine-precipitated nicotine-withdrawal aversion in rats, Psychopharmacology 151 (2000) 49–54.
[13] E.D. Levin, A.H. Rezvani, D. Montoya, J.E. Rose, H.S. Swartzwelder, Adolescent-onset nicotine self-administration modeled in female rats, Psychopharmacology 169 (2003) 141–149.
[14] E.T. Moolchan, M.L. Robinson, M. Ernst, J.L. Cadet, W.B. Pickworth, S.J. Heishman, J.R. Schroeder, Safety and efficacy of the nicotine patch and gum for the treatment of adolescent tobacco addiction, Pediatrics 115 (2005) 407–414.
[15] L.E. O'Dell, A.W. Bruijnzeel, S. Ghozland, A. Markou, G.F. Koob, Nicotine withdrawal in adolescent and adult rats, Ann. N.Y. Acad. Sci. 1021 (2004) 167–174.
[16] L.E. O'Dell, A.W. Bruijnzeel, R.T. Smith, L.H. Parsons, M.L. Merves, B.A. Goldberger, H.N. Richardson, G.F. Koob, A. Markou, Diminished nicotine

withdrawal in adolescent rats: implications for vulnerability to addiction, Psychopharmacology 186 (2006) 612–619.
[17] L.A. Parker, Place conditioning in a three- or four-choice apparatus: role of stimulus novelty in drug-induced place conditioning, Behav. Neurosci. 106 (1992) 294–306.
[18] L.A. Parker, R.V. Mcdonald, Reinstatement of both a conditioned place preference and a conditioned place aversion with drug primes, Pharmacol. Biochem. Behav. 66 (2000) 559–561.
[19] P. Rada, K. Jensen, B.G. Hoebel, Effects of nicotine and mecamylamine-induced withdrawal on extracellular dopamine and acetylcholine in the rat nucleus accumbens, Psychopharmacology 157 (2001) 105–110.
[20] T.A. Slotkin, Nicotine and the adolescent brain: insights from an animal model, Neurotoxicol. Teratol. 24 (2002) 369–384.
[21] T.A. Smith, R.F. House, I.T. Croghan, T.R. Gauvin, R.C. Colligan, K.P. Offord, L.C. Gomez-Dahl, R.D. Hurt, Nicotine patch therapy in adolescent smokers, Pediatrics 98 (1996) 659–667.
[22] L.P. Spear, The adolescent brain and age-related behavioral manifestations, Neurosci. Biobehav. Rev. 24 (2000) 417–463.
[23] T. Suzuki, Y. Ise, M. Tsuda, J. Maeda, M. Misawa, Mecamylamine-precipitated nicotine-withdrawal aversion in rats, Eur. J. Pharmacol. 314 (1996) 281–284.
[24] T. Suzuki, Y. Ise, J. Maeda, M. Misawa, Mecamylamine-precipitated nicotine-withdrawal aversion in Lewis and Fischer 344 inbred rat strains, Eur. J. Pharmacol. 369 (1999) 159–162.
[25] J.A. Trauth, F.J. Seidler, T.A. Slotkin, An animal model of adolescent nicotine exposure: effects on gene expression and macromolecular constituents in rat brain regions, Brain Res. 867 (2000) 29–39.
[26] S.S. Watkins, G.F. Koob, A. Markou, Neural mechanisms underlying nicotine addiction: acute positive reinforcement and withdrawal, Nicotine Tob. Res. 2 (2000) 19–37.
[27] C.E. Wilmouth, L.P. Spear, Adolescent and adult rats' aversion to flavors previously paired with nicotine, Ann. N.Y. Acad. Sci. 1021 (2004) 462–464.
[28] C.E. Wilmouth, L.P. Spear, Withdrawal from chronic nicotine in adolescent and adult rats, Soc. Neurosci. Abstr. (2006) (Program Number 827.4).