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ORIGINAL INVESTIGATION

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Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction

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Abstract *Rationale:* Enhanced reinforcing effects of nicotine during adolescence appear to contribute to the rapid development of dependence in this age group. However, the contribution of nicotine withdrawal to dependence in adolescents is unclear. *Objective:* We compared motivational and somatic signs of nicotine withdrawal in adolescent and adult rats. *Materials and methods:* In experiment 1, motivational signs of nicotine withdrawal were compared using intracranial self-stimulation procedures after administration of mecamylamine (1.5 mg/kg, i.p.) in adolescent and adult rats made dependent on nicotine (9 mg/kg/day). Somatic signs of withdrawal were compared in two experiments using various doses of nicotine (adolescent doses: 0, 1.6, 3.2, 4.7 mg/kg/day; adult doses: 0, 1, 2.1, 3.2 mg/kg/day, expressed as nicotine base) to produce dependence and one dose of mecamylamine (1.5 mg/kg, i.p.) to precipitate withdrawal (experiment 2) and in a subsequent experiment, using various doses of mecamylamine (0, 0.75, 1.5, 3.0 mg/kg, i.p.) to precipitate withdrawal and a dose of nicotine (adolescent dose: 4.7 mg/kg/day; adult dose: 3.2 mg/kg/day) that produced equivalent nicotine blood levels in these age groups (experiment 3). *Results:* Adolescents did not display the decreases in brain reward function observed in adults experiencing withdrawal, and displayed fewer somatic signs of nicotine withdrawal relative to adults regardless of the dosing procedure used. *Conclusion:* The negative effects of nicotine withdrawal are lower during adolescence relative to later periods of development. Both the enhanced rewarding effects and the diminished nicotine withdrawal likely contribute to the rapid development of nicotine use during adolescence.

Keywords Adolescence · Development · Nicotine · Dependence · Withdrawal · Rat

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Introduction

The incidence of smoking behavior among adolescents is of concern in light of epidemiological studies demonstrating that nicotine addiction is higher among smokers who begin at an early age (Breslau and Paterson 1996; Taioli and Wynder 1991). In addition, young smokers are at greater risk of developing various tobacco-related diseases that are the highest cause of preventable deaths (see DiFranza and Wellman 2003). In spite of the widely appreciated magnitude of this problem, there is still a critical gap in the knowledge base regarding the mechanisms that drive adolescent smoking behavior.

The adolescent period of development in rats has been widely used to compare physiological and behavioral effects at various stages of mammalian development. Although it is difficult to define an exact time frame of adolescence, this stage of development reflects a period during which age-specific behavioral discontinuities from younger and older animals are most evident. When assessing the boundaries of the adolescent period in rats, most researchers agree that the prototypic age range for adolescence conservatively ranges from postnatal day 28–42 (see Spear 2000). Most behavioral and physiolog-

ical systems reach maximal maturation by postnatal day 60 in rats and are considered adults beyond this age (de Graaf-Peters and Hadders-Algra 2006; Herlenius and Lagercrantz 2004; McDonald and Johnston 1990). Therefore, the present study considered adolescent rats to be before 45 days of age and adult rats to be beyond 60 days of age.

Much work has demonstrated that adolescent rats display hypersensitivity to the reinforcing effects of nicotine relative to adult animals. For example, adolescent female rats display increased nicotine intravenous self-administration (IVSA) that is nearly double that of adults (Levin et al. 2003). Additionally, adolescent rats that initiate nicotine IVSA during adolescence display higher nicotine intake as adults relative to rats that initiate IVSA as adults. Nicotine IVSA is also increased in rats that receive nicotine injections during adolescence relative to rats that receive these injections as adults (Adriani et al. 2003b). Furthermore, a preference for drinking a nicotine solution rather than water was observed in early [postnatal day (PND) 23–45], but not middle (PND 37–48) or late (PND 50–61; Adriani et al. 2002) adolescent mice. Place preference studies also demonstrate that a single injection of nicotine produces place preference in adolescent but not adult rats even after four additional conditioning trials (Belluzzi et al. 2004). In addition, place preference is produced by administration of relatively low doses of nicotine in adolescent but not adult rats (Torrella et al. 2004; Vastola et al. 2002). Collectively, these studies suggest that the rewarding effects of nicotine are enhanced in adolescent relative to adult rats.

The motivational and somatic effects of nicotine withdrawal have been widely studied in adult rats using repeated administration of nicotine via subcutaneous osmotic mini-pumps (Kenny and Markou 2001; Malin 1994, 2001). The emergence of the nicotine withdrawal syndrome in rats is observed after the cessation of nicotine administration (i.e., spontaneous withdrawal) or nicotinic receptor antagonist administration (i.e., precipitated withdrawal). Most studies examining precipitated withdrawal use a protocol whereby rats are first prepared with subcutaneous osmotic pumps that deliver nicotine for at least 6–7 days, then withdrawal can be induced by administration of the non-competitive nicotine receptor antagonist mecamylamine. This compound has been demonstrated to produce robust somatic signs of withdrawal in nicotine-dependent adult rats (Malin 2001; Watkins et al. 2000). The somatic signs of nicotine withdrawal in rats include abdominal constrictions, facial fasciculation, writhes, gasps, eye blinks, and ptosis (Malin 2001; Suzuki et al. 1996; Watkins et al. 2000).

Motivational/affective measures of nicotine withdrawal can be assessed using the intracranial self-stimulation (ICSS) procedure (see Kenny and Markou 2001; Panagis et al. 2000). ICSS involves allowing a rat to self-administer small amounts of electrical current to the brain via an electrode placed in a brain region (i.e., medial forebrain bundle) consisting of efferent projections to several reward-related structures. The stimulation is highly reinforcing and can be easily used to generate a threshold measure of the activity of brain reinforcement circuitry. Withdrawal from nicotine has been shown to produce an increase in current intensity thresholds (Watkins et al. 2000). The shift to responding for higher current levels is hypothesized to reflect a decrease in brain reward function.

Few studies have compared the somatic or motivational effects of nicotine withdrawal in adolescent and adult rats. Work from our laboratory has suggested that the somatic signs of nicotine withdrawal are lower in adolescent relative to adult rats (O'Dell et al. 2004). The possibility also exists that the negative effects of nicotine itself are also experienced to a lesser degree during the adolescent period of development. This suggestion is based on the finding that adult rats display an aversion for flavored solutions paired previously with nicotine, whereas, adolescent animals do not display an aversion for nicotine-paired solutions (Wilmouth and Spear 2004).

Smoking behavior is believed to be due to a balance between the positive effects of nicotine and avoidance of the negative effects of nicotine withdrawal. Although much work suggests that enhanced positive effects of nicotine likely contributes to nicotine dependence in adolescent smokers, the contribution of nicotine withdrawal during this developmental period remains unclear. Therefore, the present study compared motivational and somatic aspects of nicotine withdrawal in adult and adolescent rats that were prepared with osmotic mini-pumps that delivered nicotine for 7–8 days. Nicotine withdrawal was then precipitated after administration of the noncompetitive nicotinic receptor antagonist mecamylamine. Motivational/affective signs of nicotine withdrawal were compared in adult and adolescent rats using measures of brain reward function derived from the ICSS procedure. Somatic signs of nicotine withdrawal were made by behavioral measures of overt somatic signs of withdrawal. Separate studies characterized the dose–response profile of these behaviors in rats receiving various doses of nicotine to produce dependence and various doses of mecamylamine to precipitate withdrawal in these age groups.

Materials and methods

Animals Male Wistar rats (Charles River, Hollister, CA) were group-housed under a 12-h/12-h light/dark cycle (lights off at 0800 hours). Testing was conducted during the dark phase of the subjects' light/dark cycle. Food and water were available ad libitum except during testing. A different set of naïve rats was used for each experiment. Animals were acclimated to handling for 4–5 days before and after the surgical procedures. The adolescent rats were received in the laboratory past their weaning period (PND 22), and were tested during the adolescent period of development (i.e., before PND 40–45 [see Table 1]). The adult rats were received in the laboratory after PND 60, such that testing occurred during the adult period of development (i.e., after PND 70). 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 Scripps Research Institute.

Experiment 1: Intracranial self-stimulation measures of nicotine withdrawal Adult and adolescent rats (n=4–7 per group) were anesthetized with an isofluorane/oxygen mixture (1–3% isoflurane) and were prepared with a stainless steel bipolar electrode (model MS303/2; Plastics One) cut to 11 mm in length, with a diameter of 0.25 mm. The electrode was implanted into the medial forebrain bundle at the level of the posterior lateral hypothalamus according to the following coordinates (adolescents: −0.5 AP, ±1.48 ML, −8.3; adults: −0.5 AP, ±1.7 ML, −8.3 DV). The electrode was anchored to the animals' skull using four stainless steel screws, embedded in dental acrylic (Teets methyl methacrylate denture material; CoOral-Lite, Diamond Springs, CA). The surgical wound was closed with silk sutures and bacitracin was applied topically to the wound.

One week later, the animals were trained in a modified discrete-trial current-threshold procedure developed by Kornetsky and Esposito (1979) and described in Skjei and Markou (2003). At the beginning of each trial, rats received a non-contingent electrical stimulus after which the animals had 7.5 s to turn the wheel manipulandum, one-quarter of a turn, to receive a contingent stimulus identical in all parameters to the noncontingent stimulus, ending the trial. If a response did not occur with 7.5 s, the trial also ended. The intertrial interval ranged from 7.5–12.5 s, averaging 10 s. Any responding during the intertrial interval resulted in a 12.5-s delay before the initiation of the next trial. After the intertrial interval, another trial began with the presentation of a noncontingent electrical stimulus. Stimulus intensities were presented in alternating descending and ascending series with a step size of 5 μA. Animals were offered three trials at each current intensity with a starting current of the first descending series set at 30–40 μA above the subject's baseline threshold level estimated at the end of the preliminary training. Subjects completed four series (two ascending and two descending) during each daily test session that lasted approximately 30 min.

The current threshold was defined as the midpoint between the current intensity level at which the animal made two or more possible responses out of three stimulus presentations and the level where the animal made less that two positive responses. The animals' estimated current threshold for the session was defined as the mean of the four individual series thresholds. In addition to the threshold measure, a measure of performance, response latency, was obtained from the discrete-trial current-threshold paradigm. Response latency was defined as the time between the delivery of the noncontingent stimulus (i.e., initiation of the stimulus) and the animal's response on the wheel manipulandum. Mean response latency was defined as the mean latency of responding during all trials when the animal responded within the 7.5-s interval after the noncontingent stimulus.

Table 1 Postnatal day of adolescent rats for each experiment

Experimental manipulation	Postnatal days
Somatic signs experiment	
Pump implantation	29–33
Withdrawal test—day 7	36–40
ICSS experiment	
Pump implantation	36–37
Withdrawal test—day 8	44–45

After 7 days of ICSS training, animals were anesthesized with an isofluorane/oxygen mixture (1–3% isoflurane) and were prepared with osmotic mini-pumps (model 2ML2 14-day; Alza; Palo Alto, CA) that were placed subcutaneously on the back of the animal parallel to the spine. Both age groups were prepared with mini-pumps that delivered saline or nicotine tartrate (3.2 mg/kg/day expressed as nicotine base). The dose of nicotine was chosen based on previous research from our laboratory demonstrating that this concentration produces reliable nicotine dependence in adult rats within a 7-day period of exposure (Watkins et al. 2000). Before surgical implantation of the pump, the concentration of nicotine tartrate salt was adjusted according to the rats' weight. The surgical wound was closed with 9-mm stainless steel wound clips (Becton Dickinson Primary Care Diagnostics, Sparks, MD) and treated topically with bacitracin ointment. After surgery, all rats received the analgesic, flunixin (2.5 mg/kg, s.c.). Nicotine was delivered for 7 days to induce nicotine dependence in these rats, and this is based on reports demonstrating that nicotine dependence is evident in rats receiving nicotine for at least 3 days (Vann et al. 2005). In addition, several laboratories have observed behavioral (conditioned nicotine withdrawal—Kenny and Markou 2005; somatic signs of withdrawal—Watkins et al. 2000) and neurochemical (decreased DA levels—Rada et al. 2001; protein expression differences—Yeom et al. 2005) changes in rats treated with repeated nicotine via mini-pump administration. Baseline ICSS thresholds were assessed for an additional 7 days to ensure low variability in the baseline values. Eight days after mini-pump implantation, rats received mecamylamine (1.5 mg/kg, i.p.) and 30 min later ICSS thresholds were examined in the same procedures that were used during training.

Experiment 2: Somatic signs of nicotine withdrawal using various doses of nicotine to produce dependence in these age groups Previous results from our laboratory examining somatic signs of withdrawal revealed that there were different levels of blood cotinine in adult (368.3±18.7 ng/ml) and adolescent (264.0±22.9 ng/ml) rats that received the 3.2 mg/kg dose of nicotine in their mini-pumps (O'Dell et al. 2004). Thus, the goal of this experiment was to examine precipitated somatic signs of nicotine withdrawal and plasma nicotine and cotinine levels (ng/ml) in adult and adolescent rats that displayed equivalent blood levels of nicotine. Approximately equal blood levels of nicotine were achieved by adjusting the nicotine dose by 1.5 in the adolescent group. The correction factor that we used to

adjust the adolescent doses is consistent with other laboratories examining the behavioral effects of nicotine in adult and adolescent rats implanted with mini-pumps (Trauth et al. 2000).

Precipitated somatic signs of nicotine withdrawal were compared in adult and adolescent rats (n=4–8 per group) prepared with osmotic mini-pumps that delivered various doses of nicotine (adult doses= 0, 1.0, 2.1, and 3.2 mg/kg/day salt and adolescent doses=0, 1.6, 3.2, and 4.7 mg/kg/day salt). Seven days after mini-pump implantation, all rats received the same dose of mecamylamine (1.5 mg/kg/salt, i.p.) to precipitate withdrawal. The animals were then immediately placed into a plastic opaque cylindrical container (30×29 cm) in which the rat could move around freely. Twenty minutes later, the frequency and time of occurrence of the following signs were recorded for 10 min: eye blinks, body shakes, chews, cheek tremors, escape attempts, foot licks, gasps, writhes, headshakes, ptosis, teeth chattering, and yawns. Animals were continuously observed for 10 min, and during this time, the number of times the animals exhibited any of the above signs were recorded. Multiple successive counts of any sign required a distinct pause between episodes. If present continuously, ptosis was counted only once. The total number of somatic signs per 10-min observation period was defined as the sum of individual occurrences of the aforementioned withdrawal signs. Subjects were habituated to the observation room and containers for 2 days before the test day by placing them into the containers for 10 min on each day. Control rats prepared with saline mini-pumps also received mecamylamine on the test day. Approximately 8 h after the test, tail blood was drawn and both nicotine and cotinine were extracted from plasma and analyzed using gas or liquid chromatography coupled with mass spectrometry.

Blood nicotine and cotinine analysis For extraction of nicotine and cotinine, a 100-μl aliquot of plasma was added to a 1.5-ml Eppendorf micro-centrifuge tube and spiked with 10 μl of a 1-μM solution of 2-phenylimidazole as an internal standard to account for extraction efficiency. To this, 20 μl of 20% NaOH was added, vortexed well for 10 s, followed by the addition of 400 μl of dichloromethane (DCM) and an additional 10 s of vortexing. After centrifugation at 1,500×g for 10 min, the upper aqueous layer was discarded, and 10 μl of 6 M HCL was added to the DCM layer. Samples were vortexed as above, centrifuged for 10 min and the acidified aqueous layer was removed along with any protein precipitate and discarded, while the bottom DCM layer was transferred to a new micro-centrifuge tube and evaporated to dryness under a gentle stream of nitrogen. Once fully lyophilized, the samples were stored at −70°C. Subsequently, plasma levels of nicotine and cotinine were determined using a 1100 series liquid chromatography coupled mass spectroscopy (LC-MS) from Agilent Technologies. The lyophilized samples were reconstituted with 25 μl of the LC mobile phase containing 0.25 pmol ethyl-nor-cotinine as the MS internal standard. After mixing, a 1−μl aliquot was injected into the LC-MS using a refrigerated capillary autosampler (tray temperature maintained at 5°C).

Nicotine, cotinine, and the two internal standards were separated by hydrophilic interaction chromatography using a 1×150 mm polyhydroxyethyl-A column (5-μm spheres, 100-Å pore size; Poly LC, Columbia MD, USA) and an isocratic mobile phase composed of 20-mM ammonium formate in 72% (v/v) acetonitrile with 0.1% (v/v) formic acid delivered at 20 μl/min using a capillary LC pump. The column eluent was delivered to the mass spectrometer via a microelectrospray interface using 13 psig nebulization pressure and a 300°C nitrogen drying gas delivered at 7 l/min. The analytes and internal standards were detected in their protonated form (M+H) and were quantified using selected ion monitoring at the following mass to charge (m/z) ratios: nicotine (m/z= 163.1); cotinine (m/z=177.1); ethyl-nor-cotinine (m/z= 191.2); 2-phenylimidazole (m/z=145.2). External calibration curves were generated daily and were constructed from three standard concentrations run in triplicate. All standards and reagents were the highest grade available and were obtained from Sigma-Aldrich (St. Louis, MO, USA). Our nicotine assay is able to detect the absence of nicotine. This was achieved via an external standard curve that consisted of samples containing zero nicotine levels. Our system did not detect significant levels of nicotine in these zero blank samples, even when the control sample was run between nicotine-containing samples. Furthermore, there was no carryover from nicotine samples because our daily calibration of the instrument indicated that our control samples were always blank regardless of what preceded it.

Experiment 3: Somatic signs of nicotine withdrawal after administration of various doses of mecamylamine to precipitate withdrawal in these age groups This study compared the dose-dependent effects of mecamylamine across adolescent and adult rats. Precipitated somatic signs of nicotine withdrawal were compared in adult and adolescent rats (n=7–14 per group) prepared with osmotic mini-pumps containing a dose of nicotine that was demonstrated in the previous study to produce equivalent blood levels of nicotine (see Table 2; adult dose=3.2 mg/kg/day and adolescent dose=4.7 mg/kg/day, expressed as nicotine base). Seven days after mini-pump implantation, separate groups of adult and adolescent rats received various doses of mecamylamine (0, 0.75, 1.5, 3.0 mg/kg/salt, i.p.) to precipitate withdrawal, and 20 min later, somatic signs of withdrawal were recorded for 10 min, as described above.

Statistical analyses An overall analysis of variance (ANOVA) was performed on the total number of withdrawal signs with group (adolescent vs adult) and treatment (saline vs nicotine) as between subject variables. The total number of withdrawal signs reflects the overall sum frequency of all of the recorded signs in the 10-min observation period. Significant interaction effects were further analyzed using post hoc tests to compare group differences and individual dose effects (Fisher's test,

Table 2 Nicotine and cotinine levels (ng/ml) and withdrawal signs following mecamylamine administration (1.5 mg/kg) 7 days after implantation with pumps delivering various nicotine doses

Dose of nicotine (mg/kg/day)	Plasma levels (ng/ml)		Withdrawal signs
	Nicotine	Cotinine	
Adolescent			
0 (*n*=4)	–	–	13.5±3.3
1.6 (*n*=8)	26.6±5.2	150.4±18.8	11.5±1.9
3.2 (*n*=6)	40.5±2.0	265.6±50.1	14.3±2.1
4.7 (*n*=8)	76.2±7.6	460.5±74.2	14.8±2.6
Adult:			
0 (*n*=4)	–	–	9.2±1.5
1.0 (*n*=8)	22.9±3.2	143.0±42.2	15.2±2.7
2.1 (*n*=8)	33.8±6.4	179.3±38.1	21.7±2.6*
3.2 (*n*=7)	65.4±9.5	297.8±53.6	27.1±3.3*

*Denote a significant difference from saline controls $P<0.05$

$P<0.05$). Changes in ICSS thresholds (expressed as a percentage of the mean±SEM baseline values) were treated as the dependent measure and subjected to the same ANOVA and post hoc analyses.

Results

Overall, the results revealed that adolescent rats display less motivational and somatic signs of nicotine withdrawal compared to their adult counterparts. Our analyses of experiment 1 were performed on ICSS thresholds, which were expressed as percentage change from the animal's baseline threshold value. There were no overall group differences in the average of the four baseline ICSS threshold values [age × treatment interaction effect: [$F(1,19)=0.10$, $P=0.15$]; adult saline=82.1±10.0; adult nicotine=72.2±7.2; adolescent saline=103±10.5; adolescent nicotine=87.7±6.3]. The results from the ICSS study revealed that adult rats receiving nicotine for 8 days displayed elevated ICSS reward thresholds after mecamylamine administration relative to all other groups (age × treatment interaction effect [$F(1,19)=12.3$, $P<0.01$]; Fig. 1). Subsequent analyses revealed that nicotine-treated adult rats exhibited a significant increase in ICSS thresholds relative to saline controls ($P<0.05$). In contrast, there was no difference in thresholds of adolescent rats receiving nicotine relative to saline controls after mecamylamine administration.

Our analyses of experiment 2 were conducted on the total observed signs of nicotine withdrawal throughout the 10-min test period in adult and adolescent rats that received various doses of nicotine to produce dependence. These analyses revealed that adult, but not adolescent rats, displayed a dose-dependent increase in somatic signs of withdrawal after mecamylamine administration relative to saline controls ([$F(3,45)=2.7$, $P<0.05$]; Table 2). Subsequent analyses revealed that adult rats treated with the two highest doses of nicotine (2.1 and 3.2 mg/kg) exhibited

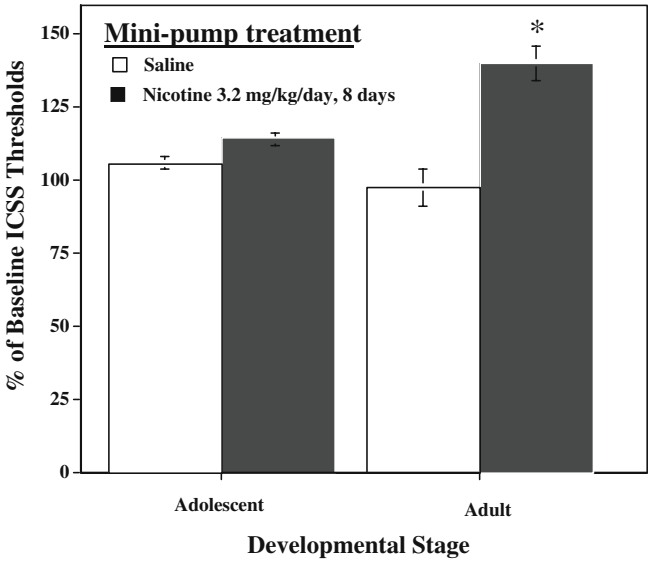

Fig. 1 *ICSS* thresholds (expressed as a percentage of the mean±SEM baseline threshold) 30 min after mecamylamine administration (1.5 mg/kg, s.c.) in adult and adolescent rats that received nicotine (3.2 mg/kg/day; *solid bars*) or saline (*open bars*) via osmotic minipumps for 8 days. The group animal numbers were as follows: adolescent/saline, *n*=4; adolescent nicotine, *n*=7; adult/saline, *n*=6; adult/nicotine, *n*=6. There were no significant differences in baseline values across the treatment groups (adult/nicotine=72.2±7.1; adolescent/nicotine=87.7±6.3; adult/saline=82.1±10.0; adolescent/saline=103.1±10.5). *Asterisks* reflect a significant threshold elevation in adult rats relative to all other treatment groups following a significant age × treatment interaction effect ($P<0.05$)

significantly more precipitated signs of withdrawal relative to saline controls ($P<0.05$). Both nicotine and cotinine plasma levels in both age groups dose-dependently increased across the dose range that was administered (main effect of dose for nicotine [$F(2, 39)=33.5$, $P<0.001$] and cotinine [$F(2, 39)=13.7$, $P<0.001$]; Table 2) with a factor of 1.5 in the dose required to produce equivalent plasma levels.

Our analyses of experiment 3 examining somatic signs of nicotine withdrawal after administration of various doses of mecamylamine revealed a dose-dependent increase in somatic signs of withdrawal in adult and adolescent rats receiving various doses of mecamylamine (age × treatment interaction effect [$F(3,69)=7.3$, $P<0.001$]; Fig. 2). Subsequent analyses revealed that adult rats treated with mecamylamine (1.5 and 3.0 mg/kg) exhibited significantly more precipitated signs of withdrawal relative to adolescent rats receiving the same respective dose of the antagonist ($P<0.05$).

Discussion

The present study demonstrated that adolescent rats treated with nicotine display less motivational and somatic signs of nicotine precipitated withdrawal relative to their adult counterparts. Based on these findings, it is hypothesized that the negative effects of nicotine withdrawal are lower during adolescence relative to later periods of develop-

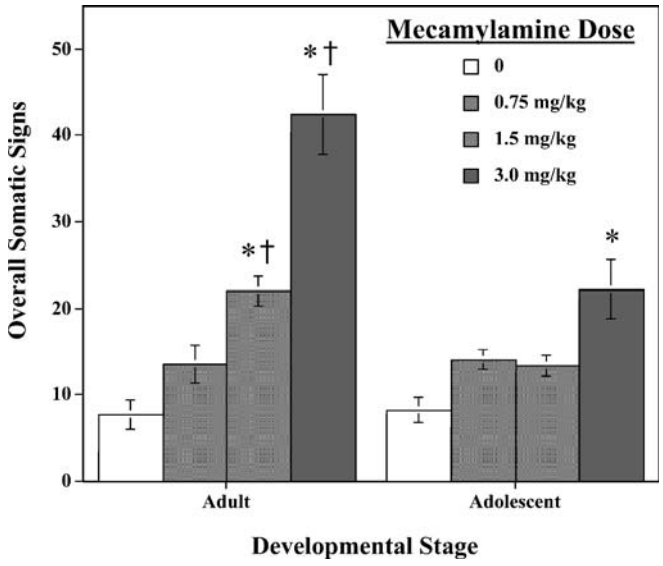

Fig. 2 Precipitated withdrawal 20 min after administration of various doses of mecamylamine (0, 0.75, 1.5, and 3.0 mg/kg) in separate groups of adult and adolescent rats that received a dose of nicotine (adult dose=3.2 mg/kg/day, salt and adolescent dose=4.7 mg/kg/day, salt) that produces equivalent blood nicotine levels across these age groups. The group numbers were the same for adolescents and adults receiving various doses of mecamylamine as follows: 0 mg/kg, n=7; 0.75 mg/kg, n=9; 1.5 mg/kg, n=10; and 3.0 mg/kg, n=9. *Asterisks* reflect a significant increase in somatic signs in mecamylamine-treated rats relative to saline controls following a significant age × treatment interaction effect (P<0.001). *Daggers* (†) reflect a significant increase in somatic signs in adult rats receiving mecamylamine relative to adolescent rats receiving the same dose of the antagonist (P<0.05)

ment. This hypothesis is based on our finding that adult rats receiving nicotine for 8 days displayed elevated ICSS reward thresholds after mecamylamine administration. However, adolescent rats did not display decreases in brain reward function during nicotine withdrawal. Our hypothesis is further supported by dose–response studies illustrating that adult rats receiving various doses of nicotine displayed a dose-dependent increase in somatic signs of withdrawal relative to adolescent rats that did not display withdrawal signs at any dose of nicotine using a low dose of mecamylamine. Additionally, adolescent rats receiving the highest dose of mecamylamine displayed fewer somatic signs of nicotine withdrawal relative to adult rats receiving the same high dose of mecamylamine. Collectively, these findings suggest that both the motivational and somatic effects of nicotine withdrawal are lower during the adolescent period relative to the later stages of development.

The finding that adolescents are less sensitive to nicotine withdrawal does not appear to be due to age-dependent differences in drug metabolism. First, adolescent rats displaying equivalent blood levels of nicotine exhibited fewer somatic signs of nicotine withdrawal relative to their adult counterparts. In fact, the adjustment factor that was used to produce equivalent nicotine levels in adolescent rats (1.5-fold higher) is consistent with other laboratories examining the behavioral effects of repeated nicotine administration via mini-pumps in these age groups (Trauth et al. 2000). Second, adolescent rats displayed lower levels of withdrawal signs relative to adults across a range of nicotine/mecamylamine doses. We recognize that adolescents may not have had enough days of nicotine exposure to produce dependence. Indeed, we have observed somatic signs of nicotine withdrawal in adolescent rats exposed for longer nicotine exposure times (i.e., 14 days). It should be emphasized that the goal of this report is to present a time point where a lack of motivational and somatic signs of withdrawal are observed in two different age groups exhibiting equivalent nicotine blood levels.

Motivational measures of nicotine withdrawal have been widely assessed using ICSS procedures. An increase in current intensity threshold is interpreted as a decrease in brain reward function because the animal requires higher current levels to maintain responding for electrical brain stimulation. Previous work has demonstrated that withdrawal from nicotine produces an increase in current intensity thresholds (Watkins et al. 2000). This study replicates this finding and extends this work by demonstrating that adolescent rats do not display decreased brain reward function during nicotine withdrawal. It seems unlikely that pharmacokinetic factors can explain the lack of nicotine withdrawal in the adolescent rats in the ICSS study in light of the findings from the somatic signs of withdrawal study. Specifically, when a higher nicotine dose was administered to adolescent rats (4.7 mg/kg) that produced nicotine plasma levels that were similar to that produced by the highest dose in the adults, the adolescent rats showed little or no nicotine withdrawal.

Several different neural mechanisms may mediate developmental sensitivity to the negative effects of nicotine withdrawal. Previous research demonstrates that the motivational and somatic signs of nicotine withdrawal are mediated by cholinergic transmission in the ventral tegmental area (VTA; Bruijnzeel and Markou 2004; Watkins et al. 2000). Therefore, it is possible that cholinergic systems mediating nicotine withdrawal in this region are not fully developed in adolescent rats. Adolescent rats may be less sensitive to nicotine withdrawal due to decreased nicotinic receptor function. However, this suggestion is not consistent with the literature demonstrating that nicotine exposure during adolescence is associated with an upregulation of nicotinic receptor number and function during adolescence and into adulthood (see Abreu-Villaca et al. 2003; Slotkin 2002).

The neurochemical mechanisms that mediate nicotine withdrawal involve changes in dopamine and acetylcholine transmission in the nucleus accumbens (NAcc). Microdialysis studies have demonstrated that NAcc dopamine is decreased and acetylcholine levels are increased during precipitated withdrawal in nicotine-dependent rats (DiChiara 2000; Hildebrand et al. 1998; Rada et al. 2001) and mice (Gåddnås et al. 2002). The decreases in NAcc dopamine are observed with concomitant decreases in the dopamine metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid. Basal dopamine levels are also reduced during abstinence from nicotine self-administra-

tion (Rahman et al. 2004). The ability of mecamylamine to reduce NAcc dopamine levels is believed to be due to a blockade of nicotinic acetylcholine receptors residing on dopamine neurons in the VTA. This is based on the finding that intra-VTA infusions of mecamylamine elicit nicotine withdrawal signs (Hildebrand and Svensson 2000). Therefore, the possibility exists that adolescent rats are less sensitive to nicotine withdrawal due to developmental differences in NAcc dopamine and/or acetylcholine transmission. However, to our knowledge, these neural measures of nicotine withdrawal have not been examined in adolescent rats.

Treatment strategies in adult smokers focus on alleviating negative symptoms of nicotine withdrawal with antidepressant, anti-anxiety, or nicotine replacement therapies (see Rose 1996). This treatment approach reflects the intense nature of the negative effects of nicotine withdrawal that are believed to be one of the primary reasons that the relapse rate among smokers is highest among other drugs of abuse (Sofuoglu and Kosten 2004). The finding that nicotine patches are effective in adult but not adolescent smokers suggests that there are fundamental differences in the mechanisms that drive smoking behavior at different stages of development (see Hurt et al. 2000; Smith et al. 1996). Moreover, our finding that adolescent rats do not display motivational/affective signs of nicotine withdrawal suggests that these treatment strategies may not be effective as nicotine withdrawal does not significantly contribute to adolescent smoking behavior.

Smoking behavior appears to be maintained by a balance between the positive reinforcing effects of nicotine and avoiding the negative consequences of nicotine withdrawal. The present results combined with the existing literature showing that adolescent rats appear to be more sensitive to the reinforcing (Adriani et al. 2003b; Levin et al. 2003) and stimulant (Faraday et al. 2003) effects of nicotine suggest that adolescent rats are more sensitive to the positive effects and less sensitive to the negative effects of nicotine exposure. This hypothesis is further supported by a recent report demonstrating that adolescent rats display a reduced aversion for flavors paired with nicotine relative to adult rats (Wilmouth and Spear 2004). The notion that the negative effects of nicotine withdrawal are less evident during adolescence is also consistent with clinical and anecdotal evidence that negative effects of drugs of abuse, such as "hangover," are less prominent during adolescence (Spear 2000). Therefore, it is hypothesized that during adolescence, the positive effects of nicotine are enhanced, whereas, the negative effects associated with withdrawal are reduced. Therefore, in adolescents, the balance that regulates smoking behavior is tipped in favor of experiencing more of the positive effects associated with nicotine use. Taken together, the enhanced sensitivity to the reinforcing effects of nicotine and resistance to the negative effects of nicotine and nicotine withdrawal when combined could constitute a powerful basis for rapid increases in tobacco smoking behavior in adolescents that set the stage for vulnerability to addiction.

Our previous work posits that in addiction, drug taking behavior progresses from impulsivity to compulsivity in two stages (Koob and Le Moal 2005). In the impulsive stage, the motivation for drug-taking behavior is positive reinforcement in which stimuli increase the probability of the response. As individuals move towards the compulsive stage, the drive for drug taking progresses to negative reinforcement, in which removal of the aversive state increases the probability of the response. Over the course of development, we hypothesize that addiction progresses from impulsivity, observed largely in adolescence, to compulsivity that would be largely observed in adults. Young persons that are inherently impulsive are driven by positive reinforcement that is heightened during adolescence, an effect that is observed in both animal (Adriani et al. 2003a) and human (Chambers et al. 2003) subjects. The degree to which positive reinforcement plays a role in drug taking cannot be overestimated during this highly impulsive and reward driven stage of development. As addiction develops, we previously posited that the impulsive and compulsive stages of addiction feed into each other and mediate decreased function of brain reward systems and recruitment of anti-reward systems that drive aversive states. However, our present results suggest that the recruitment of anti-reward systems may not play an important role in drug taking during adolescence. Therefore, the current hypothesis is that positive reinforcement that is heightened during this stage of development plays a larger role in drug taking during this young stage of development that is characterized by impulsive behavior.

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