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Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats

Stephanie L. Collins, Dean Wade, Jennifer Ledon, Sari Izenwasser*

Department of Psychiatry and Behavioral Sciences, University of Miami School of Medicine, 1400 NW 10th Avenue, Suite 704A (D-80), Miami, FL 33136, USA

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Abstract

Chronic treatment with nicotine differentially alters behavior in adolescent rats compared to adult rats. It is not known, however, whether the effects of nicotine on the neurochemical pathways with which it interacts differ in adolescents vs. adults. In the current study, the effects of a 7-day treatment with nicotine on nicotinic, dopaminergic, and serotonergic neurochemistry were examined in the caudate putamen and nucleus accumbens in periadolescent vs. adult male rats. Nicotine treatment increased dopamine transporter densities and decreased serotonin transporter densities in periadolescent rats. There was no change in nicotinic acetylcholine receptor densities or dopamine D1 or D2 receptor densities in nicotine-pretreated periadolescent rats. In adult rats pretreated with nicotine, there was an increase in nicotinic acetylcholine densities, but no change in dopamine transporter, dopamine D1 or D2 receptor, or serotonin transporter densities. Overall, these findings show that periadolescent rats have neurochemical adaptations to nicotine different from adult rats. These alterations may explain, at least in part, the differential behavioral effects of chronic nicotine in adult and adolescent male rats.

Keywords: Adolescence; Autoradiography; Dopamine; Nicotine; Serotonin

1. Introduction

Nicotine use in adolescents is prevalent and problematic. More than 4% of youths reported smoking daily in the year 2000 (NHSDA, 2001), and approximately 80% of smokers start before the age of 18 (CDC, 2002). Adolescents experience higher rates of dependence on nicotine compared to adults (Kandel and Chen, 2000), and smoking in adolescence increases daily consumption and decreases the chance of successfully quitting in adulthood (Chen and Millar, 1998). It is not fully understood, however, whether the effects of nicotine differ on specific neurochemical pathways with which it interacts in adolescents compared with adults.

Nicotine alters various neurotransmitter systems such as the nicotinic, dopaminergic, and serotonergic systems in adult rodent animal models. For example, nicotinic receptor binding was increased after continuous infusion of nicotine (Marks et al., 1985; Nguyen et al., 2003; Pauly et al., 1991, 1996; Trauth et al., 1999), and continuous infusion of nicotine produced tolerance to the inhibition of dopamine uptake by nicotine (Izenwasser and Cox, 1992; Izenwasser et al., 1991). Further, nicotine increased dopamine release in an exocytotic manner (Dwoskin et al., 1995; Goodman, 1974; Grady et al., 1997; Schulz and Zigmond, 1989) and via the dopamine transporter (Drew et al., 2000; Drew and Werling, 2003). There are conflicting results on the effects of chronic nicotine on nicotinic acetylcholine receptor-evoked dopamine release, with both a decrease (Jacobs et al., 2002) and increase (Yu and Wecker, 1994) reported. In addition, daily nicotine injections increased serotonin transporter binding, [^{3}H]serotonin uptake (Awtry and Werling, 2003) and [^{3}H]serotonin release (Kenny et al., 2000;

* Corresponding author. Tel.: +1 305 243 2032; fax: +1 305 243 5475.
E-mail address: s.izenwasser@miami.edu (S. Izenwasser).

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Reuben and Clarke, 2000; Yu and Wecker, 1994). Nicotine has also been shown to produce a biphasic effect on the firing rate of serotonergic neurons (Milhailescu et al., 2002).

Neurochemical changes in response to nicotine administered during periadolescence have been examined as well. Periadolescence is a period of early adolescence that begins just prior to puberty and ends at approximately 42 days of age in male rats (Spear and Brake, 1983). Nicotine injections for 10 days beginning on postnatal day 34 produced an increase in nicotinic acetylcholine receptor gene expression 5 weeks after treatment ended, when the rats were adults (Adriani et al., 2003). Further, nicotinic acetylcholine receptor binding was upregulated after continuous infusion of nicotine for 17 days beginning in adolescence (Trauth et al., 1999), and dopamine turnover was increased at postnatal day 45 after a 17-day nicotine infusion period that began in periadolescence (Trauth et al., 2001). Additionally, continuously infused nicotine for 17 days in adolescent male rats produced a reduction in serotonin transporter densities (Xu et al., 2001) and a decrease in serotonin$_2$ (5-HT$_2$) receptor binding (Xu et al., 2002). The previous studies, however, examined neurochemical changes that occurred at time points past the periadolescent period. In studies examining the effects of nicotine exposure on neurochemical alterations within the adolescent period, daily nicotine injections for 3 days beginning on postnatal day 30, decreased serotonin synthesis and tryptophan expression 3 days later (Jang et al., 2002). Further, continuous infusion or twice daily injections of nicotine for 7 days beginning on postnatal day 30 increased nicotinic acetylcholine receptor binding in the midbrain immediately after treatment ended and persisted at least a month (Abreu-Villaca et al., 2003). Together these data suggest that there are neurochemical alterations occurring in response to adolescent nicotine exposure and that these changes can last into adulthood.

To better determine the effects of nicotine in the periadolescent period compared to adulthood, the effects of a 7-day daily nicotine treatment on nicotinic, dopaminergic, and serotonergic neurochemical markers in periadolescent and adult rats were examined. Since neurochemical systems associated with the effects of nicotine, such as dopaminergic, nicotinic, and serotonergic systems are maturing in the striatum during adolescence, the effects of nicotine on these neurochemical systems were examined in this brain region. In addition, dopamine transporter binding in the cell-body regions of the dopaminergic pathways (the substantia nigra and ventral tegmental area) was also examined.

2. Materials and methods

2.1. Chemicals

Drugs and isotopes were obtained from the following sources: (−)-Nicotine hydrogen tartrate salt from Sigma (St. Louis, MO). [^{125}I]RTI-121 (3beta-(4-iodophenyl) tropane-2beta-carboxylic acid isopropyl ester; approximately 2200 Ci/mmol), [^{125}I]RTI-55 (3beta-(4-iodophenyl)-tropane-2beta-carboxylic acid methylester tartrate; approximately 2200 Ci/mmol), [^{3}H]SCH 23390 (*R*-(−)-8-chloro-2,3,4, 5-tetrahydro-3,1-methyl-5-phenyl-11-3-benzyoepine-7-ol; approximately 75.5 Ci/mmol) and [^{125}I]epibatidine (approximately 2200 Ci/mmol) from Perkin-Elmer (Boston, MA). [^{125}I]iodosulpiride (approximately 2200 Ci/mmol) from Amersham (Arlington Heights, IL).

2.2. Treatments

Sprague–Dawley rats (Charles River, Wilmington, MA) were used. Periadolescent male rats at postnatal day 30 (weighing an average of 90±4 g at the start of the experiment; *n*=6/nicotine group, *n*=4/vehicle group) and adult male rats at postnatal day 60 (weighing 293±3 g at the start of the experiment; *n*=6/nicotine group, *n*=4/vehicle group) were housed two per cage in a temperature and humidity-controlled environment under a 12 h light/dark cycle. Food and water were available ad libitum.

Periadolescent rats and adult rats were injected for 7 days with 0.4 mg/kg nicotine/day (dose was based on weight of the base; i.p.) or vehicle (saline) once daily for 7 days for each assay and killed by decapitation on day 8 for neurochemical analysis. Their brains were quickly removed and frozen in isopentane at −35 °C, then stored at −70 °C prior to slicing. Slices (20 μm) from the caudate putamen, nucleus accumbens, substantia nigra, and ventral tegmental area were thaw-mounted on gelatin/chromate-coated slides and stored at −70 °C prior to assay. Slices were taken that included the rostral caudate putamen and nucleus accumbens (coordinates:+1.7 mm from bregma), the caudal caudate putamen (+0.7 mm from bregma), and substantia nigra and ventral tegmental area (−4.8 mm from bregma) (Paxinos and Watson, 1982).

2.3. Quantitative autoradiography

For the nicotinic acetylcholine receptor autoradiography assay, sections were thawed to room temperature and incubated for 40 min with 0.4 nM [^{125}I]epibatidine in binding buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$), as described previously (Tizabi and Perry, 2000). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive film for 24 h at room temperature. Nonspecific binding was defined as binding in the presence of 300 μM (−)nicotine hydrogen tartrate salt.

For the dopamine transporter autoradiography assay, sections were thawed to room temperature and incubated for 60 min with 0.07 nM [^{125}I]RTI-121 in binding buffer (137

mM NaCl, 2.7 mM KCl, 10.14 mM Na2HPO4 and 10 mM NaI), as described previously (Collins et al., 2001; Izenwasser et al., 1999). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive film for 2 days at room temperature. Nonspecific binding was defined as binding in the presence of 100 μM cocaine HCl.

For the dopamine D1 receptor autoradiography assay, sections were thawed to room temperature and incubated for 30 min with 1 nM [^{3}H]SCH 23390 in binding buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) and 1 μM mianserin (to prevent binding of the ligand to 5-HT_2 receptors), as described previously (Collins et al., 2001; Tella et al., 1996). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{3}H-labeled microscales, Amersham) were apposed to radiosensitive film for 12 days at room temperature. Nonspecific binding was defined as binding in the presence of 10 μM R(+)SCH 23390.

For the dopamine D2 receptor autoradiography assay, sections were thawed to room temperature and incubated for 120 min with 0.1 nM [^{125}I]iodosulpiride in binding buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$), as described previously (Collins et al., 2001; Tella et al., 1996). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive film for 2 days at room temperature. Nonspecific binding was defined as binding in the presence of 1 μM domperidone.

For the serotonin transporter autoradiography assay, sections were thawed to room temperature, preincubated for 30 min with 1 μM benztropine to block dopamine transporters, in binding buffer (137 mM NaCl, 2.7 mM KCl, 10.14 mM Na2HPO4 and 10 mM NaI), and incubated for 60 min with 0.01 nM [^{125}I]RTI-55 in binding buffer, as described previously (Boja et al., 1992; Sellings and Clarke, 2003). Sections were then washed twice in ice-cold buffer, dipped in ice-cold deionized water, and dried with a stream of cool dry air. Slides and standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive film for 5 days at room temperature. Nonspecific binding was defined as binding in the presence of 10 μM fluoxetine.

Films were developed in Kodak GBX developer and fixative, and autoradiograms were analyzed using a Macintosh-based image analysis system (NIH, Image 1.60 software). Brain images were quantified using curves generated from the labeled standards. Data were analyzed by Analysis of Variance and Fisher's Protected Least Significant Difference.

3. Results

3.1. Nicotinic acetylcholine receptor densities

There were no significant differences in nicotinic acetylcholine receptor densities in the caudate putamen or nucleus accumbens in periadolescent vs. adult rats pretreated with vehicle for 7 days ($P \geq 0.05$; Fig. 1A,B). Thus, it appears that

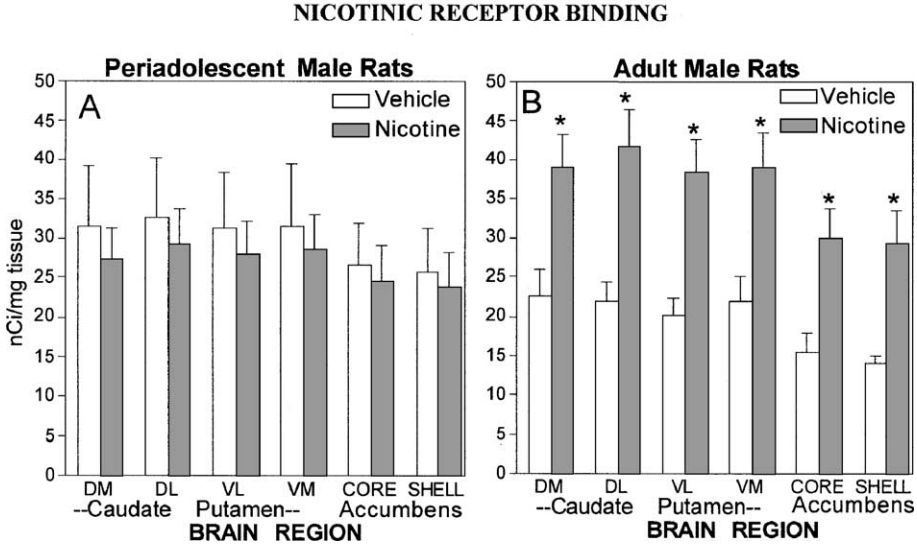

Fig. 1. Periadolescent and adult rats were pretreated with nicotine or vehicle for 7 days and nicotinic acetylcholine receptor densities were measured 1 day later on Day 8. (A) There were no changes in nicotinic acetylcholine receptor densities in the caudate putamen or the nucleus accumbens in the periadolescent rats pretreated with nicotine for 7 days compared to periadolescent rats pretreated with vehicle. (B) Adult rats pretreated with nicotine had significantly higher nicotinic acetylcholine receptor densities in the caudate putamen and nucleus accumbens core and shell than adult rats pretreated with vehicle. Nicotinic acetylcholine receptor densities were measured in four quadrants of the rostral caudate putamen (DM: dorsomedial, DL: dorsolateral, VL: ventrolateral, VM: ventromedial) and the nucleus accumbens core and shell. * indicates a significant difference from rats pretreated with vehicle, $P \leq 0.05$.

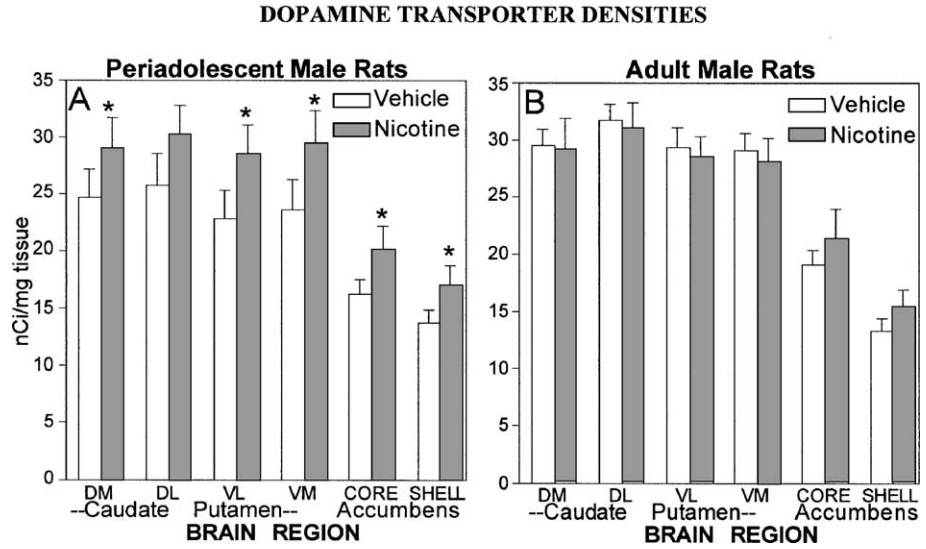

Fig. 2. Periadolescent and adult rats were pretreated with nicotine or vehicle for 7 days and dopamine transporter densities were measured 1 day later on Day 8. (A) Periadolescent rats pretreated with nicotine had significantly higher dopamine transporter densities in the caudate putamen and nucleus accumbens core and shell than periadolescent rats pretreated with vehicle. (B) There were no changes in dopamine transporter densities in the caudate putamen or the nucleus accumbens in the adult rats pretreated with nicotine for 7 days compared to adult rats pretreated with vehicle. Dopamine transporter densities were measured in four quadrants of the rostral caudate putamen (DM: dorsomedial, DL: dorsolateral, VL: ventrolateral, VM: ventromedial) and the nucleus accumbens core and shell. * indicates a significant difference from rats pretreated with vehicle, $P \leq 0.05$.

basal levels of nicotine receptors in these brain regions are not different at these two ages. In periadolescent rats, there were no significant differences in nicotinic acetylcholine receptor densities in the rostral caudate putamen or nucleus accumbens core or shell 1 day after pretreatment with nicotine for 7 days compared to vehicle controls ($P \geq 0.05$; Fig. 1A). Adult rats pretreated with nicotine for 7 days, however, had significantly greater nicotinic acetylcholine receptor densities in the rostral caudate putamen and the nucleus accumbens compared to vehicle controls (Fig. 1B). There were significant increases in nicotinic acetylcholine receptor densities in the dorsomedial [$F(1,6)=12.30$, $P \leq 0.01$], dorsolateral [$F(1,6)=13.83$, $P \leq 0.01$], ventromedial [$F(1,6)=14.25$, $P \leq 0.007$], and ventrolateral [$F(1,6)=17.56$, $P \leq 0.004$] quadrants of the rostral caudate putamen and in the nucleus accumbens core [$F(1,6)=13.19$, $P \leq 0.008$] and shell [$F(1,6)=12.41$, $P \leq 0.01$] in nicotine-pretreated adults compared to vehicle-pretreated adults. Nicotine did not produce any significant differences, however, in nicotinic acetylcholine receptor densities in the more caudal regions of the

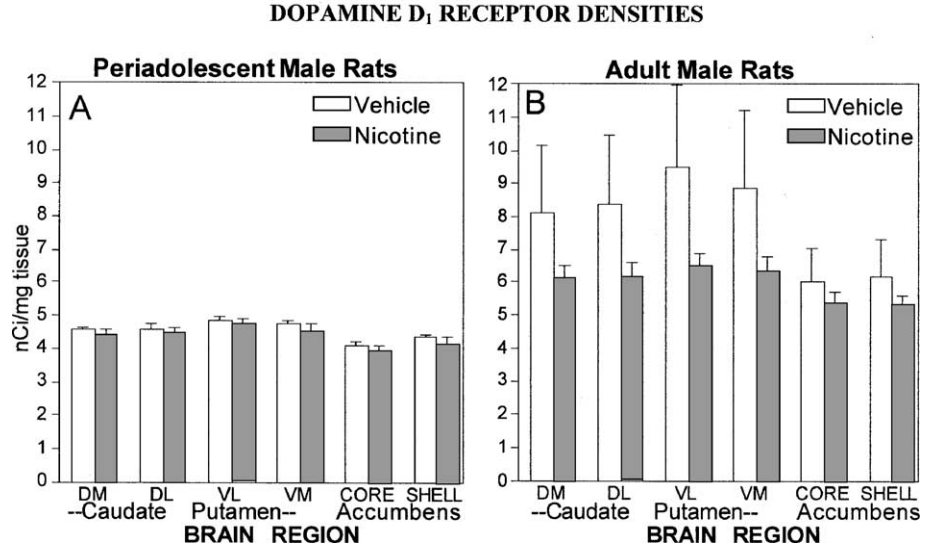

Fig. 3. Periadolescent and adult rats were pretreated with nicotine or vehicle for 7 days and dopamine D1 receptor densities were measured 1 day later on Day 8. There were no changes in dopamine D1 receptor densities in the caudate putamen or the nucleus accumbens in the (A) periadolescent or (B) adult rats pretreated with nicotine compared with vehicle. Dopamine D1 receptor densities were measured in four quadrants of the rostral caudate putamen (DM: dorsomedial, DL: dorsolateral, VL: ventrolateral, VM: ventromedial) and the nucleus accumbens core and shell.

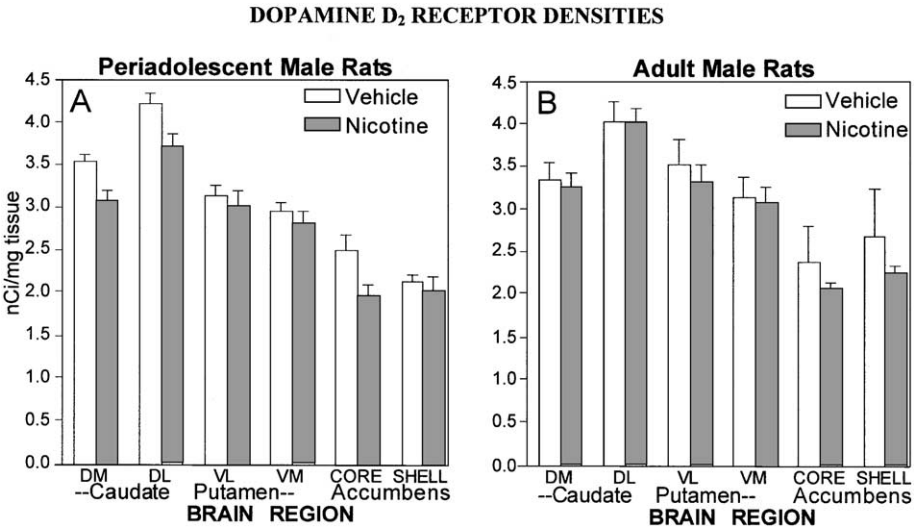

Fig. 4. Periadolescent and adult rats were pretreated with nicotine or vehicle for 7 days and dopamine D2 receptor densities were measured 1 day later on Day 8. There were no changes in dopamine D2 receptor densities in the caudate putamen or the nucleus accumbens in the (A) periadolescent or (B) adult rats pretreated with nicotine for 7 days compared with vehicle. Dopamine D2 receptor densities were measured in four quadrants of the rostral caudate putamen (DM: dorsomedial, DL: dorsolateral, VL: ventrolateral, VM: ventromedial) and the nucleus accumbens core and shell.

caudate putamen in either the periadolescent or adult rats ($P \geq 0.05$; data not shown).

3.2. Dopamine transporter densities

There were no significant differences in dopamine transporter densities in periadolescent vs. adult rats pretreated with vehicle for 7 days ($P \geq 0.05$; Fig. 2A,B), suggesting that basal dopamine transporter densities do not differ in these two groups. Periadolescent rats pretreated with nicotine for 7 days had significantly greater dopamine transporter densities in the rostral caudate putamen and the nucleus accumbens compared to vehicle controls 1 day after treatment ended (Fig. 2A). There were significant increases in dopamine transporter densities in the dorsomedial [$F(1,6)=6.15$, $P \leq 0.05$], ventromedial [$F(1,6)=9.94$, $P \leq 0.02$], and ventrolateral [$F(1,6)=11.85$, $P \leq 0.01$] regions of the rostral caudate putamen and in the nucleus accumbens core [$F(1,6)=14.20$, $P \leq 0.009$] and shell [$F(1,6)=16.14$, $P \leq 0.007$] in nicotine-pretreated periadolescents compared to vehicle-pretreated periadolescents. There were no significant changes in dopamine transporter densities, however, in the rostral

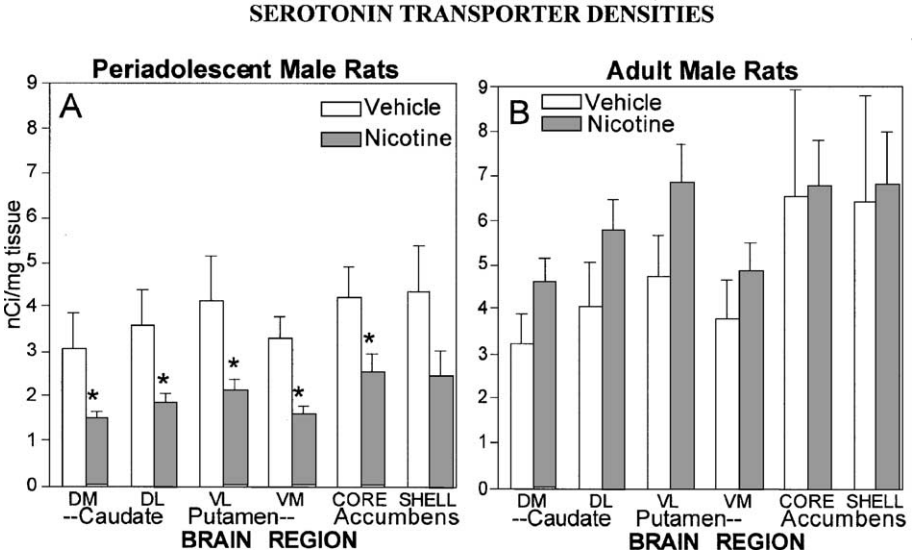

Fig. 5. Periadolescent and adult rats were pretreated with nicotine or vehicle for 7 days and serotonin transporter densities were measured 1 day later on Day 8. (A) Periadolescent rats pretreated with nicotine had significantly lower serotonin transporter densities in the caudate putamen and nucleus accumbens core than periadolescent rats pretreated with vehicle. (B) There were no changes in serotonin transporter densities in the caudate putamen or the nucleus accumbens in the adult rats pretreated with nicotine for 7 days compared to adult rats pretreated with vehicle. Serotonin transporter densities were measured in four quadrants of the rostral caudate putamen (DM: dorsomedial, DL: dorsolateral, VL: ventrolateral, VM: ventromedial) and the nucleus accumbens core and shell. * indicates a significant difference from rats pretreated with vehicle, $P \leq 0.05$.

caudate putamen or the nucleus accumbens core or shell in adult rats pretreated with nicotine for 7 days compared with vehicle controls ($P \geq 0.05$; Fig. 2B). Further, there were no significant differences in dopamine transporter densities in the caudal caudate putamen, substantia nigra, or ventral tegmental area of periadolescent or adult rats compared to their respective controls ($P \geq 0.05$; data not shown).

3.3. Dopamine receptor densities

There were no significant differences in dopamine D1 or D2 receptor densities in the rostral caudate putamen or the nucleus accumbens core or shell in periadolescent rats pretreated with nicotine for 7 days compared to periadolescent rats pretreated with vehicle ($P \geq 0.05$). Further, neither dopamine D1 nor D2 receptor densities were significantly altered in the rostral caudate putamen or nucleus accumbens core or shell in adult rats pretreated with nicotine compared to adult rats pretreated with vehicle ($P \geq 0.05$) (Figs. 3 and 4).

3.4. Serotonin transporter densities

There were no significant differences in serotonin transporter densities in periadolescent vs. adult rats pretreated with vehicle for 7 days ($P \geq 0.05$; Fig. 5A,B), suggesting that basal levels of the serotonin transporter are not different in these two groups. Nicotine treatment significantly decreased serotonin transporter densities in the rostral caudate putamen and the nucleus accumbens compared to vehicle controls in the periadolescent rats (Fig. 5A). There were significant decreases in serotonin transporter densities in the dorsomedial [$F(1,6)=10.50$, $P \leq 0.02$], dorsolateral [$F(1,6)=10.56$, $P \leq 0.02$], ventromedial [$F(1,6)=25.08$, $P \leq 0.002$], and ventrolateral [$F(1,6)=8.31$, $P \leq 0.03$] regions of the rostral caudate putamen and in the nucleus accumbens core [$F(1,6)=6.54$, $P \leq 0.04$] in nicotine-pretreated periadolescents compared to vehicle-pretreated periadolescents. The number of serotonin transporters in the nucleus accumbens shell, however, was not altered significantly by treatment with nicotine. In contrast to the periadolescent rats, although there appeared to be a trend toward an increase, serotonin transporter densities were not significantly altered in the rostral caudate putamen or nucleus accumbens of adult rats after treatment with nicotine compared to vehicle ($P \geq 0.05$; Fig. 5B).

4. Discussion

4.1. Nicotinic acetylcholine receptor densities

In the current study, nicotinic acetylcholine receptor density was increased in the rostral caudate putamen and nucleus accumbens in the adult rats after treatment with nicotine for 7 days. This is similar to previous studies showing that continuously infused nicotine produced an upregulation of nicotinic receptor binding in adult rats (Nguyen et al., 2003; Trauth et al., 1999) and mice (Marks et al., 1985; Pauly et al., 1991; Pauly et al., 1996). Further, this is consistent with increases seen in brains from smokers compared to non-smokers (Court et al., 1998; Perry et al., 1999).

In contrast to the adult rats, there were no changes in nicotinic acetylcholine receptor densities in the caudate putamen or the nucleus accumbens of periadolescent rats after treatment with nicotine. Other studies have shown that nicotinic acetylcholine receptor binding is upregulated in the cerebral cortex, midbrain, and hippocampus in adolescent rats after continuous infusion or twice-daily injections of 0.6, 2, or 6 mg/kg nicotine for 1 week (Abreu-Villaca et al., 2003) or after continuous infusion of 6 mg/kg nicotine for 17 days when rats were past the periadolescent period (Trauth et al., 1999). Thus, it is possible that changes in nicotinic receptor binding after nicotine treatment in periadolescent rats occur, if at all, in different brain regions than in rats in later stages of adolescence or adult rats.

By postnatal day 27, the distribution of nicotinic cholinergic binding sites in the male rat brain is similar to that of adults in almost all brain regions (Naeff et al., 1992). Similarly, [^{3}H]nicotine binding sites in male mice reached adult levels by postnatal day 28 (Zhang et al., 1990). This is consistent with the lack of difference in nicotinic receptor densities in the caudate putamen and nucleus accumbens in periadolescent and adult rats pretreated with vehicle in the current study. This suggests that any differences in the effects of nicotine on nicotinic acetylcholine receptors in the adults vs. the adolescents were not due to differences in basal levels of nicotinic acetylcholine receptors.

It is interesting to note that the changes in nicotine receptor binding are consistent with our previous behavioral data showing that adult male rats became sensitized to the locomotor-activating effects of nicotine during this treatment regimen, while periadolescent rats did not develop sensitization (Collins and Izenwasser, 2004). Thus, the possibility exists that nicotine receptors in the rostral caudate putamen and nucleus accumbens core are involved in mediating the locomotor-stimulant effects of chronic nicotine, since these increased in the adults and so did the adult behavior, while there was no change in either receptor number or behavior in the periadolescents.

4.2. Dopamine transporter densities

In the current study, dopamine transporter densities were significantly increased in the rostral caudate putamen and nucleus accumbens on the day after nicotine treatment for 7 days in the periadolescent rats. In addition, it has been shown that there was an increase in dopamine turnover in the striatum and midbrain of rats at postnatal day 45 during a 17-day nicotine infusion period that began at postnatal day 30 (Trauth et al., 2001). An increase in dopamine transporter sites observed in the current study and an increase in

dopamine metabolism (Trauth et al., 2001) in the striatum after nicotine treatment suggests that there may be a decrease in extracellular dopamine in terminal regions in response to nicotine in periadolescent rats that may last into late adolescence. This is interesting in light of the fact that we have shown previously that under the same conditions as in the current study (daily injections of nicotine for 7 days), sensitization to cocaine and amphetamine was evident on the day after pretreatment ended and was still present 30 days later (Collins and Izenwasser, 2004; Collins et al., in press). Although it has been suggested that an increase in extracellular dopamine is associated with behavioral sensitization to stimulants (Benwell and Balfour, 1992; Kalivas et al., 1993), it has also been shown that increases in the behavioral response to stimulants can occur in the absence of a change (Hurd et al., 1990; Imperato et al., 1996; Kalivas et al., 1993; Unterwald et al., 1994) or in the presence of a decrease in extracellular dopamine (Kalivas et al., 1993). It has been suggested further that the relationship between dopamine levels and behavioral sensitization may be dependent upon the treatment regimen (Kuczenski et al., 1997). Under the current conditions, behavioral sensitization to cocaine and amphetamine occurred simultaneously with an increase in dopamine transporter density and a possible decrease in dopamine levels in adolescent rats pretreated with nicotine. Thus, the change in dopamine transporter density may play a role in the behavioral response to stimulants in this age group.

In contrast to the periadolescent rats, there was no effect of nicotine on dopamine transporter densities in the adult rats in the current study. Although dopamine turnover was decreased and dopamine levels were increased in post-mortem brains of long-term smokers vs. non-smokers, dopamine transporter binding was not different (Court et al., 1998). Further, there was no difference in dopamine transporter availability in the striatum of living smokers vs. non-smokers (Staley et al., 2001). In a previous study in rats, chronic nicotine treatment with 6 mg/kg/day administered to adult rats for 7 days via osmotic pump produced tolerance to the inhibition of dopamine uptake by nicotine in the striatum (Izenwasser and Cox, 1992; Izenwasser et al., 1991). Another study found that there was an increase in amphetamine-stimulated dopamine release, but not uptake, in the prefrontal cortex of adult rats injected twice daily with 2 mg/kg nicotine for 10 days (Dluzen and Anderson, 1998; Drew et al., 2000; Drew and Werling, 2003). Thus, in adult rats, nicotine may have different effects on uptake and release of dopamine than on dopamine transporter binding sites and these effects may be dependent upon treatment regimen (daily single injections vs. continuous infusion or twice daily injections). It is interesting to note once again that the lack of change in dopamine transporter density in the caudate putamen and nucleus accumbens in the adult rats is consistent with the behavioral effects produced by other psychostimulant drugs as a result of chronic treatment with nicotine. We have shown previously that there is a small increase in the behavioral effect of cocaine (Collins and Izenwasser, 2004), but no change in the effect of amphetamine (Collins et al., in press) on locomotor activity in the adult male rats after nicotine treatment. This is in contrast to a large increase in the locomotor-activating effects of cocaine (Collins and Izenwasser, 2004) and amphetamine (Collins et al., in press) in the periadolescent rats pretreated with nicotine. Thus, it may be that the significant increase in dopamine transporter densities contributes to the greater sensitized response to subsequent stimulant administration seen in the periadolescent male rats compared to the adult male rats.

In the current study there were no significant differences in dopamine transporter densities in the caudate putamen and nucleus accumbens in periadolescent and adult rats pretreated with vehicle in the current study. This is consistent with previous studies showing that by postnatal day 21, dopamine transporter levels are not significantly different from that seen in adult rats (Coulter et al., 1997; Coulter et al., 1996). Thus, the differences observed in dopamine transporter binding after nicotine treatment in the periadolescents and adults are not due to differences in basal levels of dopamine transporter densities.

4.3. Dopamine receptor densities

In the current study we observed no significant changes in dopamine D1 or D2 receptor densities in any brain region examined in nicotine-pretreated adult rats or periadolescent rats compared to vehicle controls. Similarly, it has been shown that dopamine D1 and D2 receptor densities were not different in the striatum or hippocampus in smokers compared to non-smokers or ex-smokers (Court et al., 1998). Further, it was found that there were no changes in dopamine D1 or D2 receptor expression after 5 days of daily 0.5 mg/kg nicotine injections in adult rats (Le Foll et al., 2003). In contrast, another study showed that after 14 days of continuously infused nicotine (0.125 mg/kg/day), there was a decrease in dopamine D2 receptor binding in the nucleus accumbens and olfactory tubercle in adult rats (Janson et al., 1992). Thus, it may be that changes in dopamine receptors require a longer period of nicotine exposure. Under the current conditions, however, dopamine receptors were not altered by daily nicotine exposure over a week-long period in either periadolescent or adult rats.

Some studies have suggested that levels of dopamine receptors are undergoing changes in adolescent rats (Bolanos et al., 1998; Teicher and Andersen, 1995), which may interfere with adaptations in these receptors that may occur in response to nicotine. It has been shown that, in rats, dopamine D1 and D2 receptor binding peaked at postnatal day 40 and then decreased after postnatal day 60 in the striatum and nucleus accumbens (Teicher and Andersen, 1995) or that dopamine D1 receptor density increased from postnatal day 7 to 28 and then declined to adult levels between postnatal days 35 and 60 in the caudate putamen and nucleus accumbens (Tarazi et al., 1999), suggesting that

dopamine receptors may be at higher levels during adolescence than in adulthood. Other studies, however, found that dopamine D1 and D2 receptor binding reached adult levels by postnatal day 30 in the caudate putamen (Rao et al., 1991) or increased to adult levels between postnatal day 14 and 21 (Schambra et al., 1994), suggesting that dopamine receptors are reaching maturity by the early adolescent period. Thus, it appears that the exact level of dopamine receptors during adolescence is controversial. Whether the dopaminergic system is reaching maturity or continuing to change during adolescence, this may lead to unique adaptations of these receptors in response to stimulant drugs. The fact that the dopamine receptor densities are not significantly different in the periadolescents or adults treated with nicotine or vehicle in the current study, however, suggests that the dopamine receptors do not play a role in nicotine sensitization or cross-sensitization to psychostimulants in these two age groups under the conditions used in our laboratory.

4.4. Serotonin transporter densities

Periadolescent rats pretreated with nicotine showed a reduction in serotonin transporter densities in the rostral caudate putamen and nucleus accumbens. A recent study showed that daily nicotine injections (0.1–10 mg/kg) for 3 days given to adolescent rats also decreased serotonin synthesis and tryptophan expression in the dorsal and medial raphe nucleus (Jang et al., 2002). Further, 2 or 6 mg/kg nicotine continuously infused for 17 days in adolescent male rats reduced serotonin transporter densities in the striatum (Xu et al., 2001) and continuous infusion of 6 mg/kg nicotine decreased 5-HT_2 receptor binding in the midbrain (Xu et al., 2002), when measured subsequent to the adolescent period. Thus, it appears that nicotine exposure during adolescence produces an overall downregulation of the serotonergic system regardless of the treatment regimen, and further, that these effects are still evident in adulthood.

Adult rats pretreated with nicotine did not have significantly different serotonin transporter densities in any brain region examined compared with their vehicle controls. It has been shown that there was no change in serotonin transporters in the diencephalon of human living smokers compared to non-smokers (Staley et al., 2001). In contrast, in adult rats, nicotine treatment twice a day for 10 days increased serotonin transporter binding and [^{3}H]5-HT uptake in the prefrontal cortex and hippocampus (Awtry and Werling, 2003). Since [^{3}H]5-HT uptake was measured at only a single concentration in that study, it is not clear whether the change represents an alteration in V_{max} or K_m for uptake, although the increased B_{max} for binding suggests that V_{max} is increased. While the striatum was not examined in that study, an earlier study showed that [^{3}H]5-HT release was increased in the striatum after twice daily nicotine injections (1.76 mg/kg nicotine, corresponding to 0.62 mg of nicotine base) for 10 days (Yu and Wecker, 1994). Thus, it is possible that serotonergic uptake and release and serotonin transporter binding sites are differentially altered by nicotine in adult rats, although it is not clear since different brain regions were examined in each study.

Serotonin transporter binding was not significantly different in the periadolescent and adult rats treated with vehicle in the current study, suggesting that the effects of nicotine on serotonin transporter binding most likely were not due to differences in basal levels of serotonin transporter densities. It has been shown, however, that serotonin transporter densities increased from postnatal day 25 until postnatal day 50 and then decreased until postnatal day 240 in the striatum and increased from postnatal day 25 until postnatal day 240 in the frontal cortex, but there were no differences in the ontogeny of serotonin transporter binding in the midbrain or brainstem (Moll et al., 2000). Another study showed that there was a marked increase in serotonin transporter densities between postnatal day 7 and 35 with smaller increases seen up until postnatal day 60 in the caudate putamen and nucleus accumbens (Tarazi et al., 1998). Differences in our findings and the previous studies could stem from the fact that the previous studies did not look at any day between postnatal day 28 and 35, which is the time period when the rats were treated in the current study. Thus, it is possible that there are slight fluctuations during that time and serotonin transporter levels in periadolescent rats in this age range are closer to the levels observed in adult rats. Further, our rats were handled and exposed to vehicle injections daily during the 7-day treatment period, which could alter their neurochemistry.

After nicotine pretreatment, there was a decrease in serotonin transporter density observed in the current study at the same time point as the sensitization to the locomotor-activating effects of cocaine (Collins and Izenwasser, 2004) or amphetamine (Collins et al., in press) previously shown in nicotine-pretreated periadolescent rats. Further, there was little or no change in any of these measures in adult rats pretreated with nicotine (Collins and Izenwasser, 2004; Collins et al., in press). Thus, serotonin transporters may play a role in behavioral sensitization to psychostimulants after treatment with nicotine.

4.5. Summary and conclusion

The underlying mechanisms of the differential changes in receptors and transporters in the adolescents and adults is not known. During adolescence, most neurochemical systems are reaching adult levels or continue to change. Cortical synapses are decreased dramatically (Lidow et al., 1991) and dopamine autoreceptors are just becoming functional (Hedner and Lundborg, 1985). There is an increase in dopamine input to the prefrontal cortex compared to both earlier and later ages (Rosenberg and Lewis, 1994, 1995). Dopamine transporter binding has been shown to reach adult levels in the striatum immediately prior to adolescence, peak prior to adolescence and then decline in the substantia nigra, and increase

continuously until adulthood in the nucleus accumbens (Coulter et al., 1996). Studies examining the ontogeny of the dopamine receptors in the striatum have shown that dopamine D1 and D2 receptors reached adult levels immediately prior to (Schambra et al., 1994) or during adolescence (Rao et al., 1991) or increased until adolescence and then decreased to adult levels thereafter (Creese et al., 1992; Tarazi et al., 1999; Teicher and Andersen, 1995). Nicotinic receptors have been shown to reach adult levels during adolescence in the hippocampus of male rats (Adams et al., 2002) and α3 and β4 nicotinic acetylcholine receptor subunit mRNAs reached adult levels in most brain regions during adolescence (Winzer-Serhan and Leslie, 1997). In mice, [^{3}H]nicotine binding decreased in midbrain and hypothalamus and remained unchanged in hippocampus and cortex from birth to adulthood while binding in the striatum reached adult levels during adolescence (Fiedler et al., 1990). Serotonin transporter binding increases continuously into adulthood in the frontal cortex but remains stable in the striatum and midbrain after weaning (Moll et al., 2000). Another study, however, showed that serotonin transporter binding increased until adolescence and then remained stable in the caudate putamen and nucleus accumbens (Tarazi et al., 1998). Thus, there is considerable change and growth occurring in multiple neurochemical systems during the adolescent period, in comparison to adults. It is likely that some of these neurochemical differences may play a role in the unique effects of stimulant drugs in adolescents compared to adults.

The different neurochemical adaptations in adolescent and adult rats after treatment with nicotine correlate well with the differential effects of nicotine on behavior and on subsequent psychostimulant-induced behavior observed previously in response to 7 daily injections of nicotine (Collins and Izenwasser, 2004; Collins et al., in press). Sensitization to nicotine-stimulated locomotor activity occurred in the adult but not the adolescent male rats (Collins and Izenwasser, 2004). In contrast, 7 days of nicotine injections produced sensitization to the locomotor-activating effects of both cocaine (Collins and Izenwasser, 2004) and amphetamine (Collins et al., in press) 1 day after treatment in the adolescent rats, and this persisted into adulthood. This was not seen in rats that were treated with nicotine during adulthood. Thus, behavioral sensitization to nicotine in the adult rats corresponds with increases in nicotinic acetylcholine receptor binding, while the subsequent sensitization to cocaine and amphetamine in periadolescent rats treated with nicotine is consistent with increases in dopamine transporter densities and decreases in serotonin transporter densities.

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