

Impact of acute nicotine exposure on monoaminergic systems in adolescent and adult male and female rats

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ABSTRACT

Adolescence is a period of risk for beginning tobacco addiction. Differential neural response to nicotine in adolescents vs. adults may help explain the increased vulnerability to nicotine self-administration seen with adolescent onset. We indexed the effects of acute nicotine ditartrate (0.4 mg/kg, salt weight) administration on dopamine (DA) and serotonin (5HT) as well as the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in several brain regions (nucleus accumbens, striatum and frontal cortex) of 6-week old (adolescent) and 10-week old (young adult) Sprague-Dawley rats. When nicotine was administered DA concentrations in the accumbens were significantly higher in adults than in adolescents, whereas there was no age-related difference without nicotine. However neither age group showed a significant effect of nicotine vs. age-matched controls. DA turnover in the accumbens was significantly greater in adolescent females in response to nicotine, but adult females did not show this effect and neither did males of either age group. DA turnover in the striatum was significantly higher in adolescents than adults regardless of nicotine administration. In the frontal cortex, there was a more complex effect. Without nicotine, adult male rats had higher DA concentrations than adolescent males, whereas female rats did not differ from adolescent to adult ages. When given nicotine, the age effect was no longer seen in males. However, there was not a significant effect of nicotine vs. age-matched controls in either age group. No age or nicotine effects were seen in females. 5HT in the accumbens was significantly increased by nicotine administration in adults but not in adolescents. Altered neural responsivity of adolescents to nicotine-induced neural effects particularly in accumbens DA and 5HT may be related to the increased nicotine dose concentrations they self-administer.

1. Introduction

Adolescence is the life stage when most people who go on to become addicted start tobacco use (Centers for Disease Control and Prevention, 1991; Nelson, 1995; Pierce and Gilpin, 1996; Rigotti, 1990; US Public Health Service, 1994). Many adolescents who use tobacco then quickly develop dependence (Eissenberg and Balster, 2000). The persistence of tobacco addiction and the great difficulty in quitting is significantly worse with people who had started using tobacco use during adolescence (Everett et al., 1999). However, with humans it is difficult to differentiate the relative effect of adolescent nicotine exposure from contributions of genetic and environmental conditions, which can also cause tenacious tobacco addiction and cause early onset of tobacco use. Animal models of nicotine pharmacology can be of great use in determining the causative sequence and the mechanisms underlying greater effects of adolescent nicotine use.

Adolescence includes important finishing phases of neurodevelopment and is a life phase of risk for developing drug abuse (Barron et al., 2005). Behaviorally, adolescence is characterized by increases in sensation seeking and risk taking behavior (Wilson and Daly, 1985). This is a crucial period for central nervous system (CNS) developmental. Metabolic activation is higher in both limbic and frontal regions (Walker, 1994) during adolescence. Many adolescent-specific neuro-behavioral alterations evident in humans are also seen in rats (Spear, 2000; Spear and Brake, 1983). Adolescent rats respond differently from adults to a number of drugs, particularly those that act on DA systems (Bauer and Duncan, 1975; Rezvani and Levin, 2004; Spear, 2000) implicated in nicotine addiction and other drug seeking behaviors (DiChiara and Imperato, 1985; Koob et al., 1998; Li and McBride, 1995; McBride et al., 1992; Weiss et al., 1993). Increased dopamine D₁ receptor actions in adolescence may undelay their increased drive for drug taking (Sonntag et al., 2014). We have found that D₁ receptor

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antagonism in the nucleus accumbens and insular cortex significantly decreases nicotine self-administration (Hall et al., 2015; Kutlu et al., 2013).

There is evidence that not only DA systems but, other monoaminergic transmitter systems undergo significant alterations during adolescence in rats (Spear, 2000). During adolescence 5HT systems are also differentially affected. Subchronic nicotine treatment increased the density of striatal and accumbens dopamine and decreased serotonin reuptake transporters in adolescent rats whereas no effect was seen in adults. (Collins et al., 2004b; Xu et al., 2001).

Animal models are of great utility for addressing the question of whether adolescents are more vulnerable to drug addiction than adults. With randomized assortment of subjects to begin drug access at younger vs. older ages, experimental animals can inform us about the role of initial age of access without the confound of genetic and environmental factors that may predispose individuals to start drug use at younger or older ages as is the case with human observational studies. As might be anticipated, the effects of age are complex and depend greatly on the drug under study (Schramm-Sapayta et al., 2009).

A variety of studies have found that rats that begin nicotine self-administration during adolescence self-administer more nicotine per body weight than those starting in adulthood (Levin et al., 2007; Levin et al., 2006; Levin et al., 2003) (Adriani et al., 2003; Belluzzi et al., 2005; Chen et al., 2007; Klein et al., 2004). There are important sex differences in adolescent-onset nicotine self-administration. There are critical sex differences in adolescent-onset drug use which may be related to males showing generally higher rates of self-administration and greater impulsivity (Kuhn, 2015). Adolescent male rats show a larger increase in nicotine self-administration vs. adolescent females during the initial phases of access (Levin et al., 2007; Levin et al., 2003; Levin et al., 2011). However, adolescent-onset nicotine self-administering females have more persistent increases in self-administration continuing into adulthood, compared with adolescent-onset nicotine self-administering male rats who decrease nicotine intake to adult-onset concentrations with maturation (Levin et al., 2007; Levin et al., 2011). Females initially exposed to nicotine in adolescence exhibited greater sensitivity to nicotine's activity-increasing effects than did females initially exposed to nicotine in adulthood (Elliott et al., 2005). Chen et al. (Chen et al., 2007) found that adolescent female rats acquire nicotine self-administration quite rapidly. Adolescent female rats are more sensitive than male rats, to anxiolytic effects of nicotine (Cheeta et al., 2001), as well as to the persisting effects of nicotine on decreased cell number in the brain (Abreu-Villaca et al., 2003c). Adolescent male rats are more sensitive than adults or adolescent females to the stimulant effects of amphetamine after exposure to nicotine, and this effect is long-lasting, persisting well into adulthood (Collins et al., 2004a). The age of maximal gene expression response to nicotine exposure in female rats corresponds to puberty and corresponds to the age of the greatest behavioral response (Poleskaya et al., 2007). These data suggest that adolescent nicotine use could carry a greater risk than adult nicotine and that there are important sex differences in response (Collins et al., 2004a).

Adolescent nicotine exposure slows the development of reward systems, thus, extending an adolescent state indefinitely, which could result in increased vulnerability to substance abuse problems in adulthood. (Nolley and Kelley, 2007). Adolescent rats respond behaviorally to nicotine as being more reinforcing and less aversive, compared to adult response (Wilmouth and Spear, 2004). This shift in the balance between the reinforcing and aversive effects of nicotine may make adolescents more susceptible to continued nicotine use (Shram et al., 2006). Conditioned place preference is more evident in adolescent than in adult rats (Belluzzi et al., 2004; Vastola et al., 2002). Adolescent mice show nicotine conditioned place preference at lower doses than adult mice and showed greater physical withdrawal signs in terms of changes in locomotion, anxiety response, nociception and hypothermia (Kota et al., 2008). Early adolescent rats are more sensitive to nicotine's reinforcing effects and are in accord with studies showing a unique profile of

neurobehavioral alterations following nicotine exposure when compared with adults. Adolescents show diminished nicotine somatic (O'Dell et al., 2004) and other withdrawal symptoms, which raises implications for their addiction vulnerability (O'Dell et al., 2006). This diminished negative consequence of intermittent nicotine use may facilitate continued use. Adolescent rats in nicotine withdrawal show diminished conditioned place aversion vs. adult rats even though there was no age difference in lithium chloride-induced place aversion. (O'Dell et al., 2007).

During adolescence nicotine has greater locomotor stimulatory and PPI disrupting effects than in adulthood (Li et al., 2009). Natividad et al. (Natividad et al., 2013) found that nicotine self-administration was elevated in adolescents vs. adults. Passive administration of nicotine during adolescence increased nicotine SA in adults compared to naïve adults. In contrast to nicotine intake, nicotine naïve adults showed appetite and weight suppressant effects of nicotine, an effect that was absent in adolescents and adults that were pre-exposed to nicotine during adolescence.

Drug actions on the adolescent brain can be long-lasting (Kuhn, 2015). Neural response to nicotine exposure also differs in adolescence vs. adulthood. There is greater and more persistent nicotinic acetylcholine receptor upregulation in response to nicotine in adolescents than in adults; there are neuronal losses in brain areas responsible for learning, memory and affect; and there are relatively larger changes in synaptic activity after nicotine withdrawal in adolescents compared with adults (Abreu-Villaca et al., 2003a; Abreu-Villaca et al., 2003b; Abreu-Villaca et al., 2003c; Slotkin, 2002; Slotkin et al., 2004; Slotkin and Seidler, 2007; Trauth et al., 2001; Trauth et al., 1999; Trauth et al., 2000). With the maturation from adolescence to adulthood nicotinic acetylcholine receptors in the ventral, but not dorsal striatum, show a complex development (O'Dell, 2009). They found a sex-selective effect with significant changes in nicotine potency and efficacy in male, but not female rats. Nicotinic receptors seem to play important roles in DA neuronal maturation (Azam et al., 2007). Importantly, adolescent rats regardless of sex have been found to be less responsive than adults to nicotine effects of increasing nucleus accumbens DA release (Badanich and Kirsteina, 2004).

This study was conducted to see if there are differential effects of acute nicotine on monoamine transmitters (dopamine (DA) and serotonin (5HT)) in the nucleus accumbens, striatum and frontal cortex. Previously, Shearman et al. (Shearman et al., 2008) in a microdialysis study of adolescent rats compared with adults showed that, relative to vehicle-treated controls, acute nicotine, caused lower response of DA and 5HT in the frontal cortex and higher response in the nucleus accumbens of adolescent rats compared with adults. Differential pharmacodynamics of nicotine in adolescence may be important for differential rates of nicotine self-administration between adolescents and adults and differences in their vulnerability to tobacco addiction.

2. Methods

2.1. Subjects

Male and female Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY, USA) during the postweaning period. At 6 and 10 weeks of age were used for the current study ($N = 8/\text{sex}/\text{age}/\text{nicotine treatment}$). At six weeks of age rats are in the midst of adolescent development vs. 10 weeks of age when the rats are sexually mature young adults. These ages have proven useful in previous studies in our laboratory and others to assess adolescent nicotine effects and adolescent-onset nicotine self-administration. We have found that starting nicotine self-administration at six weeks results in higher levels of responding in males and females than if rats are started at ten weeks of age (Levin et al., 2007; Levin et al., 2003; Levin et al., 2011). Indices for sexual maturation were not taken in the current study, but the rats were randomly assigned to treatment groups. This study was conducted

within protocols approved by the Duke University Animal Care and Use Committee. The rats were housed in groups of 2–3 in approved standard lab conditions in a Duke University vivarium facility next to the testing rooms. Animals were maintained on a reversed 12:12 light-dark cycle with lights coming on at 7:00 PM. The rats were fed 5001 Rodent Chow (Lab Diet, Brentwood, MO, USA) and given access to water ad lib. Estrus cyclicity was not gauged in this study.

2.2. Nicotine administration

The rats were injected (SC) with 0.4 mg/kg of nicotine ditartrate (salt weight) 20 min prior to sacrifice for neurochemical analysis. This dose was chosen because it causes moderate neurobehavioral effects in the rat (Kholdebarin et al., 2007; Levin, 1997; Levin et al., 2005; Levin et al., 1997). The volume of injection was 1 ml/kg. The vehicle was sterile saline, which was used as the control injection. The tissue harvest was conducted during the dark phase of the diurnal cycle. The rats were euthanized by decapitation and the brains rapidly removed and dissected into the regions of interest: the nucleus accumbens (shell and core), striatum (dorsal striatum) and frontal cortex as defined by coordinates in the Pellegrino rat brain atlas (Pellegrino et al., 1979).

2.3. Neurochemical analysis

The monoaminergic neurotransmitters dopamine (DA) and serotonin (5HT) as well as metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were analyzed by HPLC methods as previously described (Eddins et al., 2010; Eddins et al., 2009). Tissue samples were weighed and placed in 25× weight/vol homogenization solution (one-part perchloric acid (0.1 N) with 100 micromolar EDTA to ten parts mobile phase). After column purification samples were diluted in mobile phase (1:10) and 20 µL were analyzed for monoamine transmitter and metabolite concentrations.

The HPLC system consisted of an isocratic pump (model LC1120, GBC Separations), a Rheodyne injector (model 7725i) with a 20 µL PEEK loop, and an INTRO amperometric detector (Antec Leyden, Zoeterwoude, The Netherlands). The electrochemical flow cell (model VT 03, Antec Leyden) had a 3 mm glassy carbon working electrode with a 25 µm spacer, and a Ag/AgCl reference electrode. The cell potential was set at 700 mV. The signal is filtered with a low-pass in-line noise killer (LINK, Antec Leyden, Zoeterwoude, The Netherlands) set at a 14 s peak width and a cut off frequency of 0.086 Hz. The signal was integrated using the EZChrom elite chromatography software (Scientific Software Inc., Lincolnwood, IL, USA). The injector, flow cell, and analytical column were placed in the Faraday-shielded compartment of the detector where the temperature was maintained at 30 °C. The C-18 column, product number MD-150, (15 cm × 3.2 mm, pore size 120 Å, particle size 3 µm) was purchased from ESA (Chelmsford, MA, USA). The stationary phase was a reverse phase BDS Hypersil C18 column 100mm × 2.1 mm, with 5 µm particle size and 120 pore size (Keystone Scientific). The mobile phase was 50 mM H₃PO₄, 50 mM citric acid, 100 mg/l 1-octanesulfonic acid (sodium salt), 40 mg/l EDTA, 2 mM KCl and 3% methanol. The mobile phase was purchased (ESA, Chelmsford, MA, USA) and consisted of 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octanesulfonic acid, 100 µL/L triethylamine, 25 µM EDTA, 10% acetonitrile adjusted to pH 3.0 with NaOH. The mobile phase was continually degassed with a Degasys Populaire on-line degasser (Sanwa Tsusho Co. Tokyo, Japan) and delivered at a flow rate of 0.50 ml/min. The limit of quantitation was approximately 1.56 pg/mg tissue. The limit of detection was approximately 1.07 pg/mg tissue. There were external standards run with the analyses. The standard curve was run across the relevant concentration curve. Concentrations of the monoaminergic neurotransmitters DA and 5HT and the DA metabolite DOPAC were analyzed. The ratio of DOPAC/DA was analyzed as indices of neurotransmitter turnover.

2.4. Statistical analysis

The data were assessed for significance by the analysis of variance. Between subjects factors were age, sex and nicotine administration. There were two concentrations of each factor (Nicotine: 0 and 0.4 mg/kg; Age: adolescent and young adult; Sex: male and female). The degrees of freedom varied slightly between assays due to occasional unusable sample. Interactions with $p < 0.10$ were followed-up with tests of the simple main effects as recommended by the classic statistics text Snedecor and Cochran (Snedecor and Cochran, 1967). Tests of the simple main effects refers to comparisons of one factor at each level of the interacting factor such as the effect of nicotine at each age. For example, if there was such an interaction of age x nicotine treatment or sex x nicotine treatment simple main effects tests were conducted to determine the source of the interaction. Alpha was set at $p < 0.05$ (two-tailed) as the cut-off for significance in the final analyses.

3. Results

3.1. Dopamine

In the nucleus accumbens, there was an age x nicotine interaction ($F(1,46) = 3.07, p < 0.09$) DA, which triggered tests of the simple main effects. No age effect was seen in saline injected controls, but when nicotine was given, the adults had a significantly ($F(1,46) = 4.76, p < 0.05$) higher concentration of DA than the adolescents (Fig. 1A). Neither age group showed significant effects of nicotine vs. age-matched controls on DA concentrations in the nucleus accumbens. Male and females did not have significantly differential effects (Fig. 1B). Concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) the DA metabolite in the nucleus accumbens were not significantly changed. With DA turnover (DOPAC/DA) in the nucleus accumbens there was an age x sex x nicotine interaction ($F(1,46) = 2.98, p < 0.10$) that prompted tests of the simple main effects. DA turnover in the accumbens was significantly ($F(1,46) = 7.93, p < 0.01$) raised by nicotine in adolescent females but not adult females (Fig. 1C). With nicotine challenge, accumbens DA turnover in adolescent females rose to concentrations significantly ($F(1,46) = 4.77, p < 0.05$) higher than nicotine-treated adult females. In contrast, no significant effects of nicotine on DA turnover in the accumbens were seen in males.

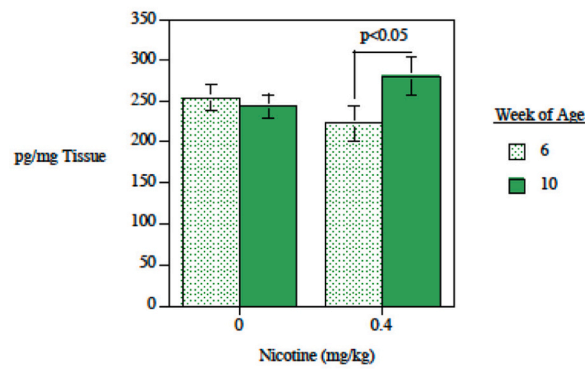
In the striatum, no significant effects of nicotine, age or sex were seen in terms of concentrations of DA (Figs. 2A-2B). DOPAC concentrations in the striatum were not significantly altered by nicotine, age or sex. While DA turnover in the striatum was significantly ($F(1,46) = 4.51, p < 0.05$) lower in adult (0.151 ± 0.008) vs. adolescent rats (0.166 ± 0.003), but striatal DA turnover was not significantly affected by nicotine or sex.

In the frontal cortex DA concentrations showed a significant three-way interaction of age x sex x nicotine ($F(1,46) = 6.26, p < 0.025$). Although there was not a significant main effect of nicotine (Fig. 3A), there were differential dopamine concentrations in adolescent vs. adult males (Fig. 3B). In males, there was a significantly ($F(1,46) = 5.88, p < 0.025$) higher DA concentration in the frontal cortex of adults vs. adolescents. When nicotine was given, the age effect was no longer seen in males. However, there was not a significant effect of nicotine vs. age-matched controls in either age group. No age or nicotine effects were seen in females. Frontal cortical DOPAC concentrations showed a significant effect of age ($F(1,46) = 4.95, p < 0.05$, adolescent = 4.64 ± 0.29 , adult = 5.72 ± 0.45), but not nicotine or sex. DA turnover in the frontal cortex was not significantly affected by nicotine, age or sex.

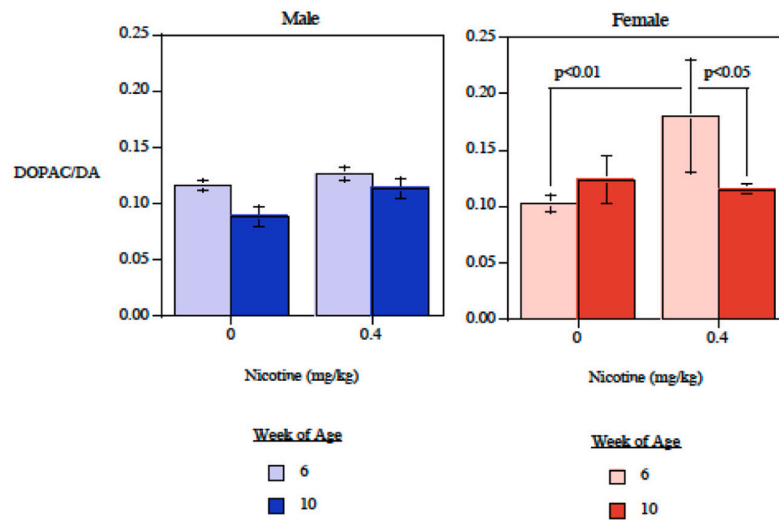
3.2. Serotonin

In the nucleus accumbens, there was a significant ($F(1,53) = 4.16, p < 0.05$) nicotine-induced increase in 5HT concentrations. This effect of nicotine appeared to be more evident in adults than adolescents, but the nicotine x age interaction did not trigger tests of the simple main effects

(A) Acute Nicotine Effects on Dopamine Levels in the Nucleus Accumbens of Adolescent and Young Adult Rats



(B) Acute Nicotine Effects on Dopamine Turnover in the Nucleus Accumbens of Adolescent and Young Adult Male and Female Rats



(C) Acute Nicotine Effects on Dopamine Levels in the Nucleus Accumbens of Adolescent and Young Adult Male and Female Rats

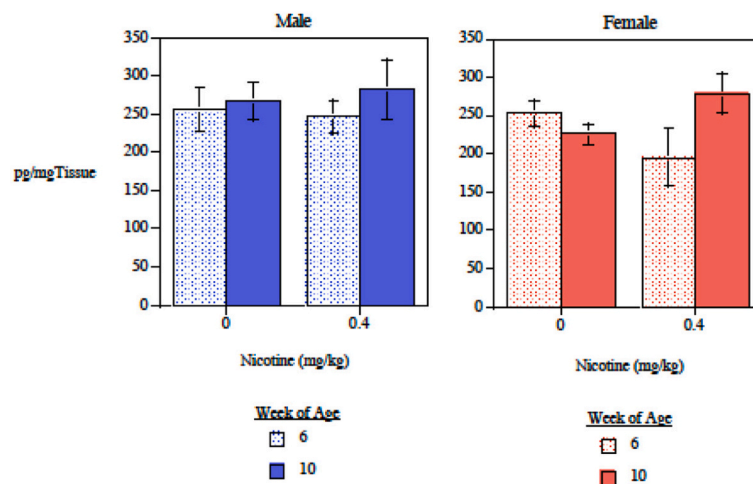


Fig. 1. Nicotine (0.4 mg/kg, sc) effects on dopamine in the nucleus accumbens in adolescent (6 weeks of age) and young adult (10 weeks of age) rats A) Averaged across sexes, B) Separately shown for male and female rats, C) Effects on dopamine turnover (DOPAC/DA) in the nucleus accumbens of male and female rats (mean \pm sem).

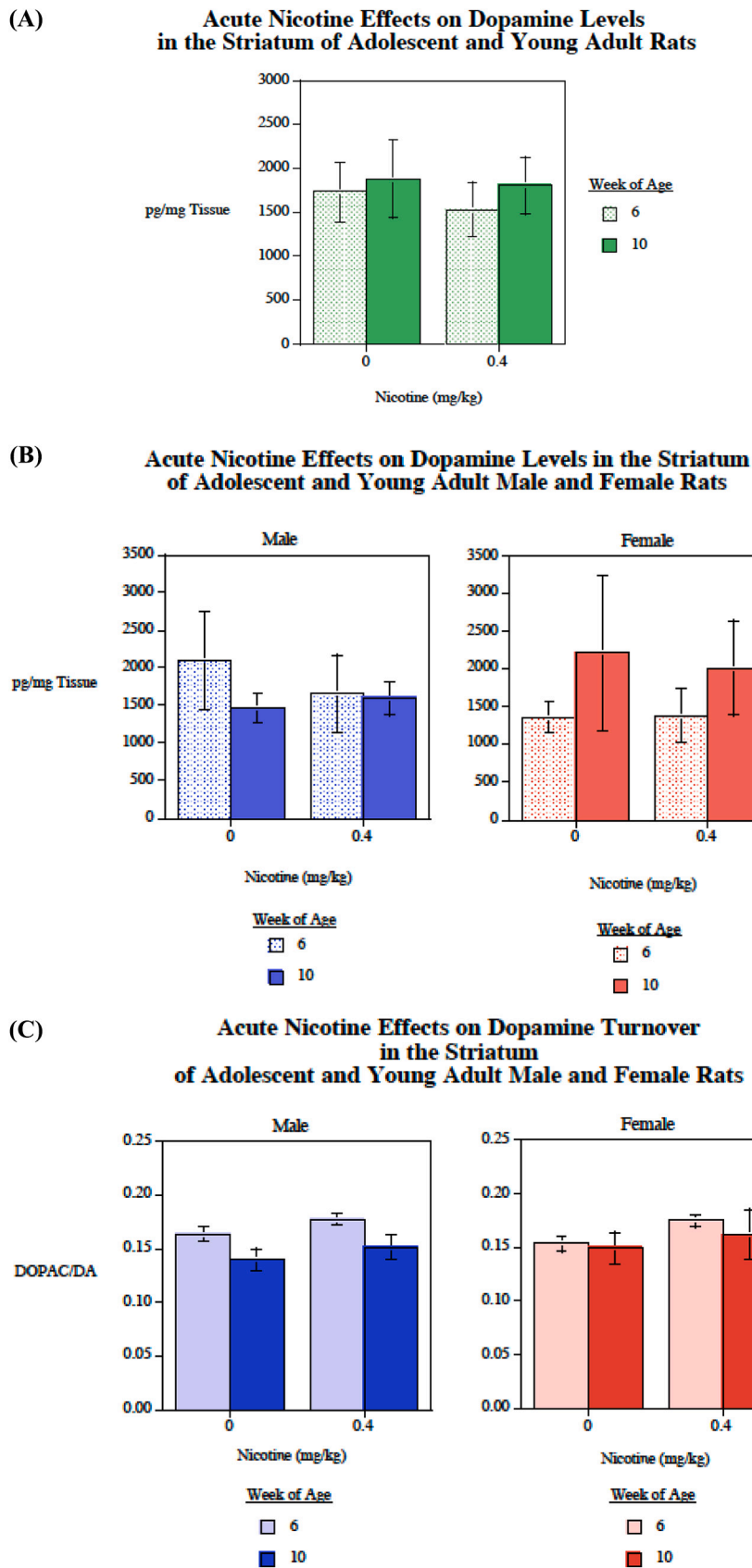


Fig. 2. Nicotine (0.4 mg/kg, sc) effects on dopamine in the striatum in adolescent (6 weeks of age) and young adult (10 weeks of age) rats A) Averaged across sexes, B) Separately shown for male and female rats, C) Effects on dopamine turnover (DOPAC/DA) in the striatum of male and female rats (mean \pm sem), This was significantly ($p < 0.05$) lower in adult vs. adolescent rats.

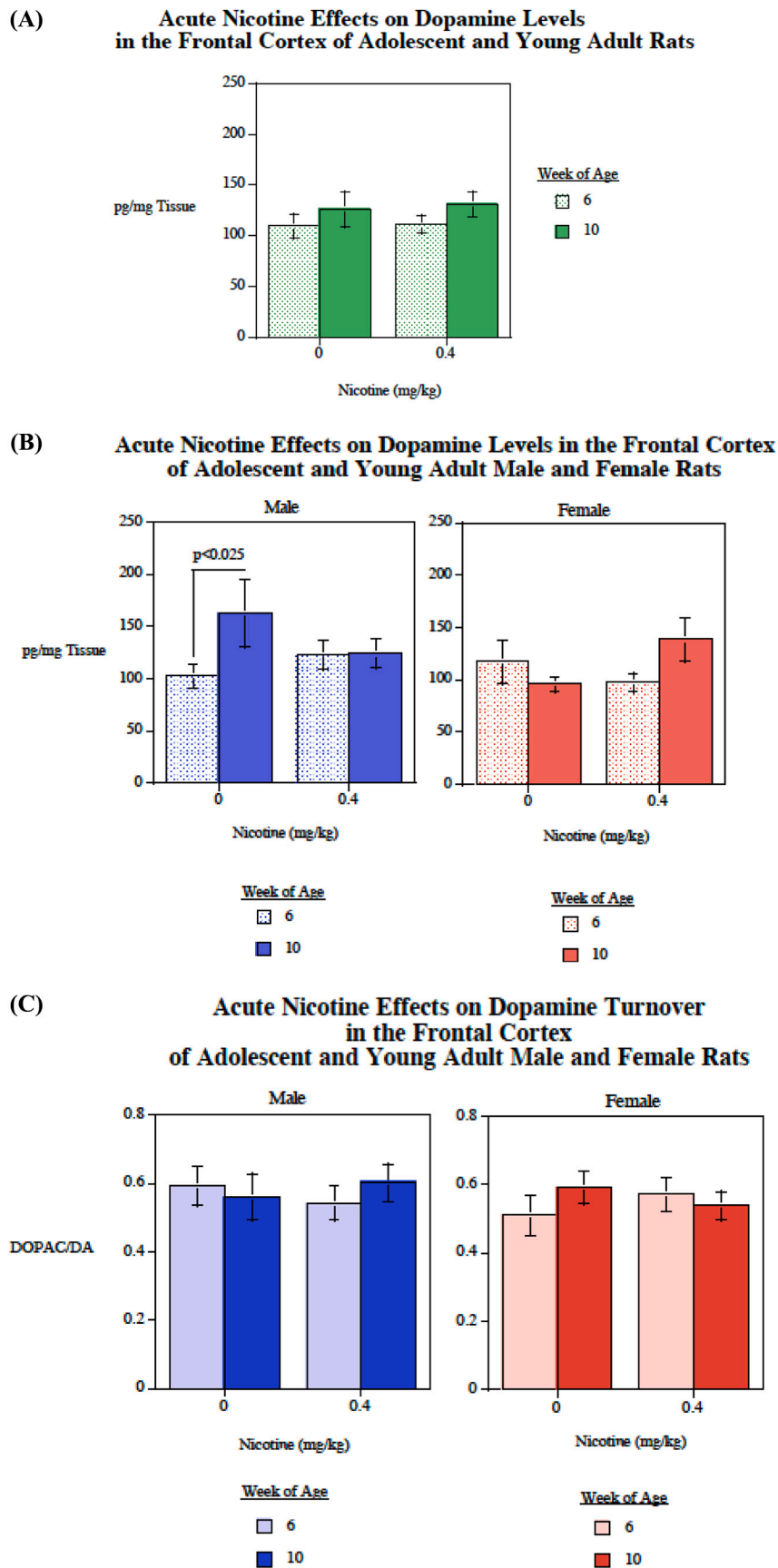


Fig. 3. Nicotine (0.4 mg/kg, sc) effects on dopamine in the frontal cortex in adolescent (6 weeks of age) and young adult (10 weeks of age) rats A) Averaged across sexes, B) Separately shown for male and female rats \, C) Effects on dopamine turnover (DOPAC/DA) in the frontal cortex of male and female rats (mean \pm sem).

at each age (Fig. 4A). No significant differential effects in male and female rats were seen (Fig. 4B). In the striatum there were no significant effects of age, nicotine treatment or sex on 5HT concentrations (Figs. 5A-5B). In the frontal cortex there were also no significant effects of age, nicotine treatment or sex on 5HT concentrations (Figs. 6A-6B).

4. Discussion

This study found that in specific brain areas, adolescent vs. adult rats had differential response when given nicotine. Differential response in adolescents compared with adults was seen with dopamine in the nucleus accumbens and frontal cortex (Table 1). In contrast, no differential effects of nicotine in adolescents vs. adults was seen regarding dopamine in the striatum nor in any of the three regions examined (nucleus accumbens, striatum and frontal cortex) for serotonin. The altered dopamine concentrations in response to nicotine in adolescents vs. adults in the nucleus accumbens and frontal cortex may be related to altered behavioral effects of nicotine during adolescence.

With nicotine, adult rats had significantly greater concentrations of DA in the nucleus accumbens, whereas no age-related differences were seen without nicotine. This is similar to the finding of Badanich and coworkers who found that adult rats had an enhanced DA response to acute nicotine challenge compared with adolescents (Badanich and

Kirsteina, 2004). Furthermore, we found both age and sex-specific effects with nicotine effects on DA turnover in the nucleus accumbens. Acute nicotine significantly increased DA turnover in the nucleus accumbens of female adolescent rats to concentrations greater than adult female rats given the same dose of nicotine, whereas no significant effects of this dose of nicotine were seen in male rats of either age. In contrast to the nucleus accumbens, in the dorsal striatum, no significant effects of nicotine, age or sex were seen in terms of concentrations of DA. Striatal DA turnover was lower in adult vs. adolescent rats, but this was not significantly affected by either nicotine or sex. In the frontal cortex, DA concentrations were differentially affected by age in each sex. In males, there was higher DA concentration in the frontal cortex of adults vs. adolescents when no nicotine was given, but when nicotine was given the age effect was no longer seen. However, there was not a significant effect of nicotine vs. age-matched controls in either age group. No age or nicotine effects were seen in females. DA turnover in the frontal cortex was not significantly affected by nicotine, age or sex. The higher frontal cortical DA concentrations seen in adult vs. adolescent males are not seen when nicotine is administered, rather with nicotine in adult makes frontal cortical DA did not differ that seen in adolescent males.

In contrast to dopamine, serotonin concentrations were not seen to be differentially altered in adolescent vs. adult rats by this dose of

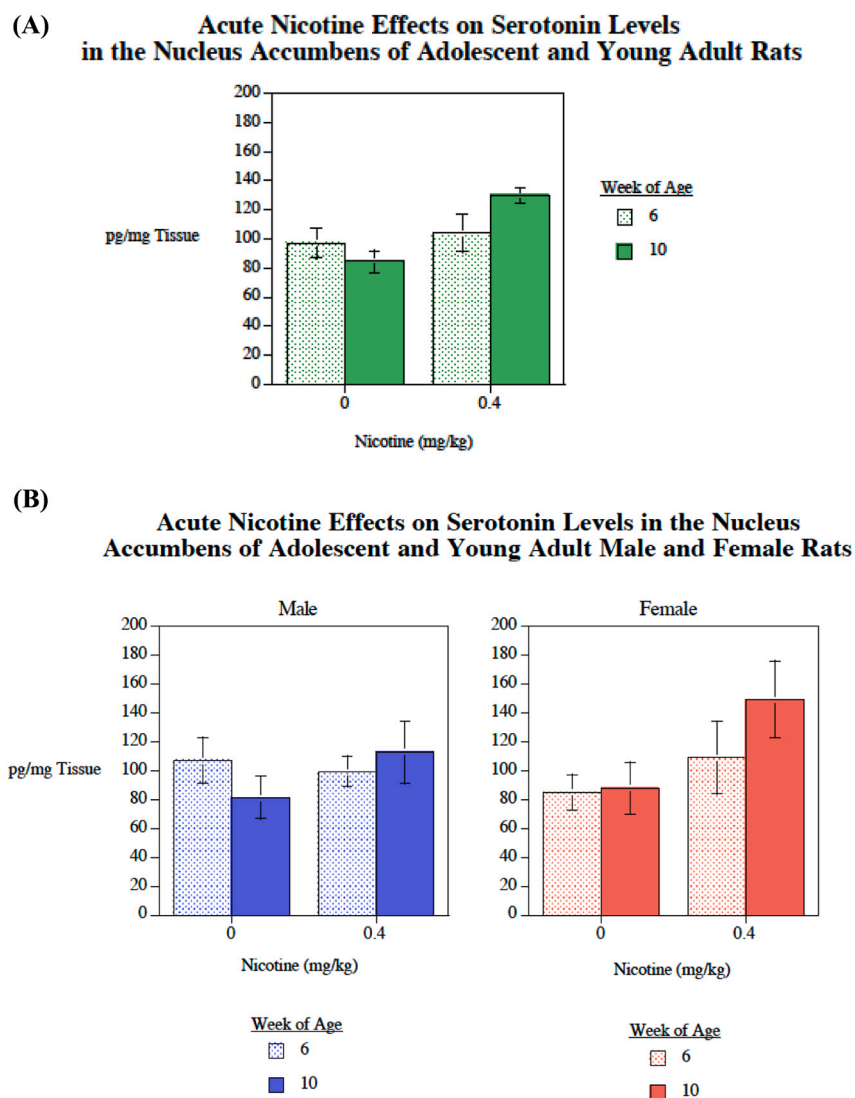


Fig. 4. Nicotine (0.4 mg/kg, sc) effects on serotonin in the nucleus accumbens in adolescent (6 weeks of age) and young adult (10 weeks of age) rats A) Averaged across sexes. There was a significant ($p < 0.05$) nicotine-induced increase in 5HT concentrations. B) Separately shown for male and female rats (mean \pm sem).

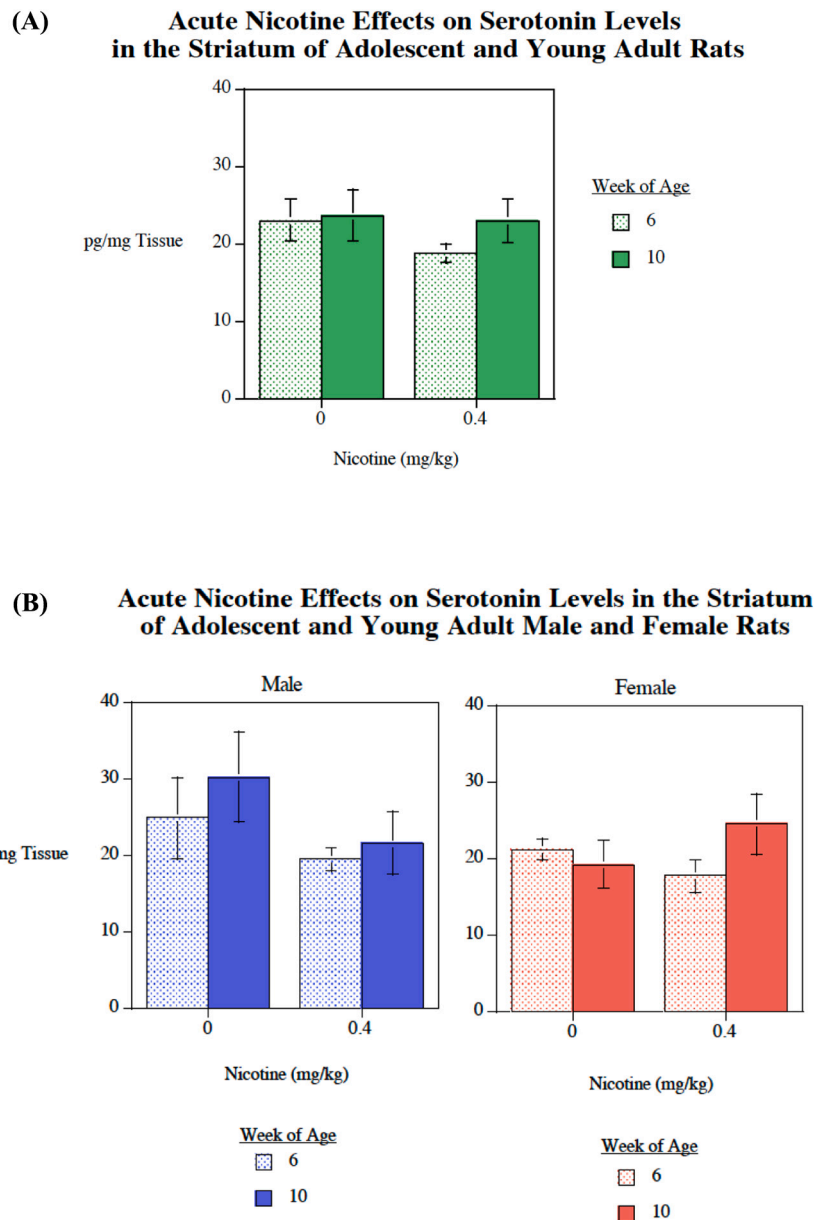


Fig. 5. Nicotine (0.4 mg/kg, sc) effects on serotonin in the striatum in adolescent (6 weeks of age) and young adult (10 weeks of age) rats A) Averaged across sexes, B) Separately shown for male and female rats (mean \pm sem),

nicotine. In the nucleus accumbens, there was a nicotine-induced increase in 5HT concentrations. Although this effect of nicotine appeared to be somewhat greater in adults than adolescents, a nicotine \times age interaction was not evident. No differential sex effects were seen regarding serotonin in the nucleus accumbens. In the striatum or frontal cortex there were no significant effects of age, nicotine treatment or sex on 5HT concentrations.

The kinetics of nicotine administration appear to make a difference in its impacts on DA. Kane et al. (Kane et al., 2004) found that with quicker nicotine distribution with IV doses, acute nicotine significantly increased DA concentration in the nucleus accumbens in both sexes. In the current study adult rats had increased accumbens DA concentrations vs. adolescent rats after nicotine administration. In contrast, there were no significant age differences without nicotine administration. The route of administration may have been the critical difference between the studies. Quicker distribution via the IV route would more closely resemble that of inhalation and the slower distribution via the sc route would have more closely resembled chewing tobacco or snuff. The

current study was limited with its test of a single dose of nicotine at a single interval between administration and measurement.

Interestingly, adolescent females did show a significantly greater increase in DA turnover with nicotine than adult females or males of either age. This may be related to larger increase in the approach to nicotine by adolescent female rats compared with adolescent males and adults of both sexes (Espinoza et al., 2022). The effect in the appeared to be regionally specific. In contrast, in the striatum dorsal to the accumbens, no age-related differential effects of nicotine on DA or 5HT were seen. In the frontal cortex, there were complex effects. Adult males had higher frontal cortical DA than adolescent males. Nicotine ablated this effect. Females did not show an age-related effect without nicotine, but with nicotine showed a more male-like pattern with higher DA in adults. No age or nicotine effects were seen with frontal cortical 5HT. The mechanisms for this sex-selective effect is not currently clear. Further study is needed.

Consistent with an earlier report investigating effects of nicotine in adolescent vs. young adult Sprague-Dawley rats (Badanich and

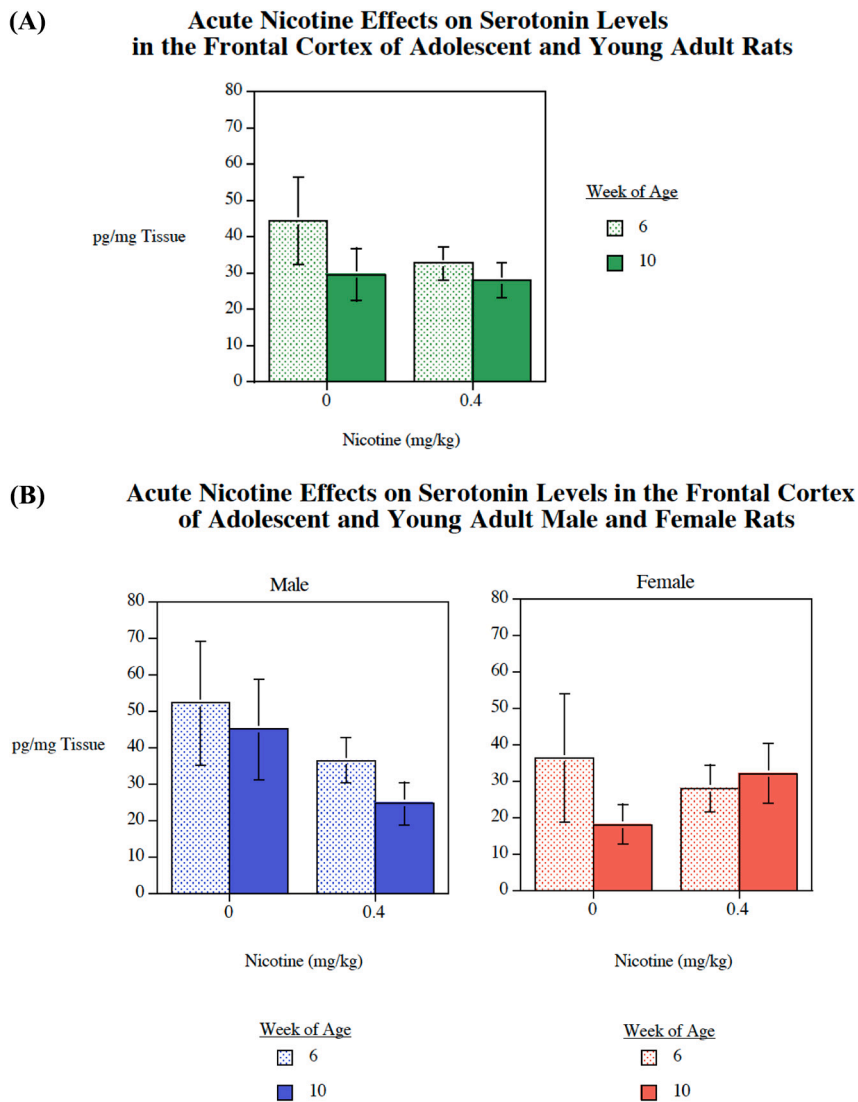


Fig. 6. Nicotine (0.4 mg/kg, sc) effects on serotonin in the frontal cortex in adolescent (6 weeks of age) and young adult (10 weeks of age) rats A) Average across sexes, B) Separately shown for male and female rats (mean ± sem).

Table 1
Summary of nicotine effects on dopamine and serotonin systems in adolescents and adults (mean ± sem).

Nucleus accumbens	
Dopamine	With nicotine higher in 10wk vs. 6wk regardless of sex
DA Turnover	6wk females with nicotine higher than 10wk females with nicotine and higher than 6wk females without nicotine
Serotonin	Overall nicotine induced increase
Striatum	
Dopamine	No significant differences
DA Turnover	Lower in 10wk vs. 6wk
Serotonin	No significant differences
Frontal cortex	
Dopamine	Without nicotine 10wk males higher than 6wk males
DA Turnover	No significant differences
Serotonin	No significant differences

Kirsteina, 2004), the current study found that with acute nicotine challenge adults showed higher DA concentrations in the nucleus accumbens than adolescents. The greater effect of nicotine in the nucleus accumbens in adults vs. adolescents may be helpful in explaining the greater nicotine self-administered by adolescents. Perhaps

adolescent rats require more nicotine to affect the dopamine-based reward mechanisms in the nucleus accumbens compared to adults. However, Shearman et al. ((Shearman et al., 2008) found modestly higher DA concentrations in the nucleus accumbens after nicotine challenge in adolescent compared with adult rats regardless of sex. The critical differences between that study and the current one as well as an earlier one (Badanich and Kirsteina, 2004) may have been due to the exact dose and timing of nicotine administration.

Pharmacokinetic and pharmacodynamic differences between adolescent and adult rats may have contributed to the differences in effects on dopamine response to nicotine challenge. Adolescent rats metabolize nicotine more quickly than adult rats (Craig et al., 2014). Adolescents and adults have different concentrations of nicotinic acetylcholine receptors. Duora et al. (Duora et al., 2008) found significantly greater binding of nicotinic $\alpha 7$ and $\alpha 4\beta 2$ receptors in adolescent rats than in adults, with more substantial increases with $\alpha 4\beta 2$ receptors. Nicotine receptors containing the $\alpha 6$ subunit were not substantially altered between adolescents and adults. They found increased $\alpha 4\beta 2$ receptors on both dopamine cell bodies as well as terminals. After chronic nicotine exposure, the increase in $\alpha 7$ and $\alpha 4\beta 2$ receptors was greater in adults than in adolescents showing differential response of adolescent and adult rats to chronic nicotine exposure. In contrast in response to

chronic nicotine the decrease in $\alpha 6$ containing nicotinic receptors was greater in adolescents than adults.

The finding in the current study that there was little age-related difference in the dorsal striatum may be relevant to higher rates of nicotine self-administration in adolescent rats. When drugs are administered or self-administered systemically, they go to every part of the brain. Perhaps in the drive to deliver more nicotine to the accumbens to provide the desired rewarding effect, adolescents also provide excessive amounts of nicotine to the dorsal striatum systems. This in turn facilitates transition of reward-driven drug taking to compulsive habit driven drug taking, for which the dorsal striatum is an important neural substrate. With regard to the complex interactions in the frontal cortex, the sex differences may be relevant to sex differences in nicotine self-administration (Becker and Hu, 2008). Given the important sex differences in nicotine self-administration and tobacco smoking addiction (Becker and Hu, 2008) sex-selective effects on DA turnover in the nucleus accumbens may be a critical mechanism in the behavioral effects of nicotine.

Interestingly, when DA turnover is measured with the DOPAC/DA ratio, a sex-selective nicotine effect was seen with regard to DA turnover in the nucleus accumbens. Nicotine treatment caused a significantly higher DA turnover in the accumbens in adolescent vs. adult females. No such effect was seen in adolescent males. This increased DA turnover in adolescent females may be related to the induction of long-term increases in nicotine self-administration relative to adolescent males and adult females. Differential effects of nicotine on the shell and core of the nucleus accumbens have been reported (Cadoni et al., 2009). These portions were not parceled out in the current study, so further study will be needed to determine how they may be differentially impacted by nicotine in adolescent and adult male and female rats. Shearman et al. (Shearman et al., 2008) found higher 5HT concentrations in the nucleus accumbens in adolescent compared with adult rats. As with their finding of higher nicotine-elicited DA in the accumbens this difference from this study may have resulted from differences in nicotine dose and timing.

In summary, these results demonstrate critical differences between adolescent and adult DA systems in the nucleus accumbens and frontal cortex and their differential response to acute nicotine challenge. In particular, adolescent females appear uniquely vulnerable to effects of nicotine on DA in NA than adolescent males or adult females. These age and sex-related differences in neural maturation may be important for the documented age and sex-related differences in nicotine self-administration (Becker and Hu, 2008).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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