Gestational Nicotine Exposure Attenuates Nicotine-Stimulated Dopamine Release in the Nucleus Accumbens Shell of Adolescent Lewis Rats

Victoria B Kane, Yitong Fu, Shannon G. Matta, and Burt M. Sharp

Department of Pharmacology, Health Science Center, University of Tennessee, Memphis, TN 38163
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Corresponding author:
Burt M. Sharp, M.D., Department of Pharmacology, University of Tennessee Health Science Center, 874 Union Avenue, Memphis, TN 38163; Phone: 901-448-6000; FAX 901-448-7206; E-mail: bsharp@utmem.edu

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Abbreviations: HPLC, high pressure liquid chromatography;
Abstract

The effects of chronic gestational exposure to nicotine on the nucleus accumbens dopamine response to acute nicotine were determined during adolescence (postnatal day 29-36) in cross-fostered and non-cross-fostered Lewis rats. In both males and females, gestational nicotine exposure diminished the adolescent nucleus accumbens dopamine response to 0.07 mg/kg nicotine i.v. (p<0.05). However, dopamine responses to 0.105 mg/kg nicotine were unaffected by gestational nicotine treatment and were similar in both genders. Furthermore, in both female and male gestational nicotine and control groups, the dopamine response to nicotine (0.105) was the same as that observed to the lower dose of nicotine in gestational controls. Thus, in adolescent male and female Lewis rats, gestational nicotine exposure attenuated nucleus accumbens dopamine release to a maximally stimulative dose of nicotine. Unexpectedly, in female gestational controls cross-fostering *per se* reduced nucleus accumbens dopamine secretion to 0.07 mg/kg nicotine (p<0.05). These investigations suggest that gestational nicotine exposure could modify the acute reinforcing effects of nicotine in adolescent rats, while early postnatal stressors, (e.g. cross-fostering) may affect nicotine-induced reinforcement in female but not male adolescents.
Less than ten percent of smokers initiate tobacco use as adults (Chassin et al., 1996; Dappen et al., 1996; USDHHS, 1989), yet animal models of nicotine treatment and self-administration focus, almost exclusively, on adult males. In the United States, the prevalence of cigarette use increases across genders until the age of 20 (USDHHS, 2001). Thereafter, cigarette use stabilizes and then begins to decline. However, in 2000 compared to 1999, the current use of cigarettes declined in males age 12-17, whereas cigarette use in adolescent females remained stable. Thus, there is a need to understand the gender-related neurochemical and behavioral correlates of nicotine intake during adolescence.

The initiation of smoking during adolescence has been associated with mothers who smoke cigarettes (Kandel et al., 1994). Furthermore, after controlling for concurrent maternal smoking and childhood exposure to cigarette smoke, daughters whose mothers smoked during pregnancy were shown to be at greater risk for both the occurrence and persistence of smoking. Despite the recent emphasis on educating the public about the dangers of tobacco use during pregnancy, 18.6% of pregnant women age 15-44 continue to smoke during pregnancy (USDHHS, 2001). Smoking during pregnancy may modify CNS responses to nicotine in the offspring, thus affecting their vulnerability to initiate and/or become a chronic smoker. In support of this is Kandel’s hypothesis that prenatal exposure to nicotine may modify CNS dopaminergic systems, thereby altering the dopaminergic response to nicotine later in life. Significantly, it is the mesocortical limbic dopamine that is known to be central to drug taking behavior (Di Chiara, 1999).

In a rat model, the administration of nicotine (6 mg/kg/day, via miniosmopump) during gestational days 4-21 decreased dopamine content in the cerebral cortex of male
and female pups (Navarro et al., 1988). This effect was evident immediately following birth and until postnatal day 20. In contrast, exposure to this dose of nicotine from gestational days 12 through 18 to 19 increased forebrain dopamine content in both male and female rats at postnatal day 15 (Ribary and Lichtensteiger, 1989). That study examined the forebrain content of dopamine and its metabolites, dihydroxyphenyl acetic acid and homovanillic acid, in separate groups of male and female rats at gestational day 18, postnatal day 15 and 2.5 months. In males, a reduction in dopamine turnover was observed at postnatal day 15 and 2.5 months, but in females reduced turnover was not apparent until 2.5 months. The disparate findings reported in these two studies may reflect differences in the rat strains and/or the onset and duration of gestational nicotine exposure. A more recent study showed that postnatal day 22 males exposed to either 6 mg/kg/day or 3 mg/kg/day nicotine (via miniosmopump from gestational days 4-21) had decreased dopamine content in both the nucleus accumbens and striatum (Richardson and Tizabi, 1994). This suggests that early gestational exposure to nicotine is pivotal in determining its developmental effects on dopaminergic systems.

Previous studies, examining the effects of gestational nicotine on dopamine content in various brain regions, utilized an animal model that was established to study the effects of prenatal nicotine exposure on fetal development (Slotkin, 1998). In this model, osmotic minipumps continuously deliver nicotine during gestation, thereby reducing the potential for confounding effects due to the placental hypoxia induced by intermittently high blood levels of nicotine from maternal injections (Slotkin et al., 1987). In light of these findings, the present study used osmotic minipumps to deliver nicotine or vehicle to Lewis dams during gestational days 4 through 21. Lewis rats were studied
because of their known susceptibility to a variety of drugs of abuse including nicotine, which they have a propensity to self-administer (Brower et al., 2002; Kosten and Ambrosio, 2002). Acute administration of nicotine has been reported to increase dopamine release in the nucleus accumbens, with more robust effects evident in the nucleus accumbens shell (Nisell, Marcus, Nomikos and Svensson, 1997). Therefore, microdialysis was performed to characterize the dopamine response of the nucleus accumbens shell to acute injections of nicotine (IV) in both male and female adolescent Lewis rats that had been gestationally exposed to nicotine or vehicle. Cross-fostered and non-cross-fostered offspring were evaluated in order to distinguish the effects of gestational nicotine exposure on dopamine responsiveness in the offspring from the effects of altered maternal care due to nicotine treatment during pregnancy.
Methods and Materials

Gestational Treatment: Under isoflurane anesthesia, timed pregnant Lewis rats (n=54) were implanted with 2ML2 osmotic minipumps (Durect Co., Cupertino, CA) on gestational day 4. Nicotine treated dams received a constant subcutaneous infusion of nicotine 3 mg/kg/day (free base of nicotine bitartrate; Sigma, St. Louis, MO) for 16 days. Control animals received continuous infusions of equivalent concentrations of sodium bitartrate. Gestational treatment was maintained from gestational day 4 to gestational day 21, and pups were born on gestational day 22. There was no difference in the number of pups born to dams receiving gestational treatment with nicotine versus vehicle (8±1 and 9±1 pups, respectively; p>0.05).

Cross-fostering: A standard model of cross-fostering was used (Cabrera et al., 1999; Nyirenda et al., 2001; Ribary and Lichtensteiger, 1989). Cross-fostering was conducted in order to acquire the following experimental groups: (1) “control group”, in which pups from sodium bitartrate-treated dams remained with their own mothers, (2) “cross-fostered control group”, in which pups from sodium bitartrate-treated dams were fostered to nicotine-treated dams, (3) “nicotine group”, in which pups from nicotine treated dams remained with their own mothers, and (4) “cross-fostered nicotine group”, in which pups from nicotine treated dams were fostered to mothers who had received sodium bitartrate during their own pregnancy. Separate litters were used to form non-fostered and cross-fostered groups. In the cross-fostered nicotine and cross-fostered control groups, pups were cross-fostered within 48 hours of delivery. When pups reached 45-55 g, they were weaned from the dams, stereotaxically implanted with guide cannulae, and housed individually.
Animal Surgery: 24 control female, 26 control male, 24 nicotine female, 20 nicotine male, 8 cross-fostered nicotine female, 14 cross-fostered nicotine male, 9 cross-fostered control female, and 9 cross-fostered control male adolescent Lewis rats were anesthetized with ketamine-xylazine (13 plus 87 mg/kg body weight, respectively, IP) and a chronic guide cannula (20 gauge) was stereotaxically implanted into the nucleus accumbens shell, according to the atlas coordinates of Paxinos and Watson (Paxinos and Watson, 1986). The coordinates for nucleus accumbens were AP +1.0 mm; DV –6.0 mm; ML, 0.4 mm, from bregma, with a flat skull. Immediately following stereotaxic surgery, animals received antibiotic injections of Baytril (9.1 mg/kg IM; Bayer Corp., Shawnee Mission, KS). Three days later, jugular catheters, constructed from 10 mm of silastic tubing attached to PE 50, were implanted under ketamine-xylazine and exited through the animal’s back. Thereafter, rats received an injection of Baytril (7.6 mg/kg IV).

Microdialysis: The microdialysis method and probes have been described previously (Fu et al., 1997). Briefly, each 1 mm concentric microdialysis probe was constructed from cellulose fiber tubing (MW cutoff, 13,000 daltons; outer diameter, 235 µm; Spectrum, Laguna Hills, CA) and silica tubing (outer diameter, 148 µm; inner diameter, 73 µm; Polymicron Technologies Inc., Phoenix, AZ). The recovery efficiency of individual probes was determined by *in vitro* dialysis for 60 min at 22 °C in a solution containing dopamine 100 pg/8 µl. The mean efficiency was 3.7 ± 0.5% (n=6).

Animals were acclimated to a 12/12 h reversed light cycle (lights off at 11 AM) before the microdialysis experiments, to facilitate experiments during an animal’s active (dark) phase. On the day of microdialysis, offspring, ranging in age from 29-36 days, were housed in alert-rat microdialysis chambers (CMA, Acton, MA) in an isolated
darkroom lit with a red safe-light. Microdialysis probes were perfused at 1 µl/min with a solution of Kreb’s Ringer Buffer (KRB: 147 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, and 0.85 mM MgCl₂, in polished water; 0.2 µm filter sterilized and degassed) containing 5 µM nomifensine. Nomifensine was added to the perfusate to ensure the detection of basal dopamine levels.

Microdialysis began 150 min after insertion of the probes through the guide cannulae; consecutive 15 min samples were collected into vials containing 1 µl of 5% perchloric acid. Three baseline samples were collected for analysis prior to an initial IV injection of either 0.07 mg/kg nicotine or 0.105 mg/kg nicotine in 100 µl of 0.9% sodium chloride over 70 sec. The dose of 0.07 mg/kg nicotine, defined as maximally stimulative (see Results), produced maximal dopamine secretion in the nucleus accumbens of adolescent non-cross-fostered control rats without adverse behavioral effects. In this treatment group, 0.105 mg/kg nicotine did not stimulate adverse behaviors nor did it induce a greater dopamine response than 0.07 mg/kg nicotine. However, higher doses were not used because they elicited adverse behavioral effects. Following the first injection of 0.07 mg/kg, nicotine, six samples were collected, and then a second identical injection was delivered and four additional samples were obtained. Following 0.105 mg/kg nicotine, four samples were collected to determine the peak response to a single injection at this dose. At the end of the experiments, the position of each probe was verified histologically. Only data from animals with probes located in the shell of the nucleus accumbens were utilized.

**HPLC-electrochemical analysis:** Chromatographic separation of dopamine was achieved with a 150 x 2 mm ODS C18 column (ESA) connected to an ESA model 580
HPLC pump. The mobile phase (0.5 ml/min) contained 50 mM sodium dihydrogen phosphate monohydrate, 2.0 mM decanesulfonic acid (DSA), 0.7 mM EDTA, 11% acetonitrile, and 11% methanol, pH 6.0. Samples (15 µl) were automatically injected by a CMA 200 refrigerated autosampler, and were analyzed by an ESA Coulochem II 5200A electrochemical detector with an ESA 5041 high sensitivity analytical cell and an ESA 5020 guard cell (ESA, Inc., Chelmsford, MA). Electrochemical detection was performed at 220 mV and 10 nA, with the guard cell at 350 mV. Under these conditions, the limit of detection for dopamine was 100 fg per injection.

**Analysis of nucleus accumbens dopamine content:** Twenty-eight day old female Lewis rats, treated during gestation with nicotine (n=8) or vehicle (n=8), were used to obtain nucleus accumbens tissue samples for dopamine and protein content. The methods used were similar to those described previously (Bergstrom et al., 2001). Briefly, brains were removed and placed in cold saline. The brains were then aligned on a tissue slicer (Stoelting, Wood Dale, IL), using the anterior optic chiasm and middle meningeal arteries as reference points, shifted 6 mm forward, and sliced into 1.5 mm sections with a razor blade. The nucleus accumbens tissue was microdissected, using a punch with an internal diameter of 2 mm, placed in a vial containing 500 µl of perchloric acid, homogenized, and then centrifuged. Dopamine levels in the supernatants were measured by HPLC with electrochemical detection, as described above. The precipitates were dissolved in NaOH and assayed for protein content, using a BCA kit (Pierce, Rockford, IL).

**Data analysis and statistics:** Chromatographic data were collected and analyzed with the PowerChrom system (AD Instruments, Castle Hill, NSW, Australia). Data (mean ± sem) were expressed as a percentage of basal dopamine levels. Basal values
were defined as the average dopamine levels detected in the samples obtained prior to administering each injection of nicotine (3 or 2 samples prior to the first or second injection, respectively). Peak responses were detected in the samples collected 15 min after each injection of nicotine. Statistical analysis was performed based upon the number of litters. Each litter was represented by a single value, which was the average of 1 or more pups. Data were analyzed by analysis of variance and unpaired t-tests, using SPSS software (version 8.0; Prentice Hall, Chicago, IL). The dependent variable was the peak dopamine increment expressed as a percentage of basal dopamine.
Results

Figure 1 shows a representative histological section of dialysis probe placement in the nucleus accumbens of an adolescent rat. The membranous tip of the probe, illustrated by the dashed lines, is located in the shell of the nucleus accumbens; similar probe placements were consistently obtained in both male and female rats exposed to gestational nicotine or vehicle.

**Females:** The first experiment, shown in figure 2, determined the levels of nucleus accumbens dopamine in adolescent female offspring in response to sequential treatments with nicotine (0.07 mg/kg). Comparison of the dopamine responses in the non-cross-fostered control groups shown in figures 2 (left panel) and 3 demonstrates that nicotine 0.07 and 0.105 mg/kg stimulated similar changes in dopamine release. Thus, 0.07 mg/kg nicotine was maximally stimulative. Prenatal nicotine exposure attenuated nucleus accumbens dopamine secretion in response to the first injection of 0.07 mg/kg nicotine [F(1,19)=16.47, p<0.01; figure 2, left panel]. In non-cross-fostered offspring, gestational nicotine attenuated the dopamine response; peak dopamine levels were 35±6% above baseline in the non-cross-fostered gestational nicotine cohort (see Table 1 for a summary of peak dopamine levels) compared to 71±9% in the non-cross-fostered gestational control group [F(1,11)=9.79, p<0.05]. Similarly, in cross-fostered offspring gestational nicotine attenuated the dopamine response. Cross-fostered prenatal nicotine offspring showed only a 15±10% increase in peak dopamine levels compared to 49±8% in the cross-fostered controls [F(1,8)=6.91, p<0.05]. In summary, gestational nicotine attenuated dopamine release in both non-cross-fostered and cross-fostered female offspring: in non-cross-fostered cohorts, there was a 50% reduction, whereas in cross-
fostered groups a 70% reduction was observed. Thus, in both non-cross-fostered and cross-fostered adolescent female offspring, gestational nicotine depressed the nucleus accumbens dopamine response to the first injection of a maximally stimulative dose of nicotine.

Figure 2 (left panel) also shows that cross-fostering per se reduced nucleus accumbens dopamine responses to 0.07 mg/kg nicotine in females [F(1,19)=5.11, p<0.05]. Cross-fostered adolescent rats that received gestational vehicle had a dopamine increase of 48.8±8.4% above baseline while the increase was 71±9% in non-cross-fostered offspring. Moreover, cross-fostered offspring that had gestational nicotine treatment failed to release dopamine in response to the acute injection of nicotine (15±9%; p>0.05), whereas the non-cross-fostered gestational nicotine females showed an increase of 35±6% (p<0.005). No interaction between cross-fostered and gestational treatment was observed for dopamine secretion in response to an acute injection of nicotine.

Figure 2 (right panel) shows that prenatal control female offspring had a diminished dopamine response to the second injection of nicotine. However, this did not occur in any of the other groups, in that the dopamine response to the second injection was not attenuated in comparison to the first injection.

In order to identify the main effects and interactions of treatments, basal dopamine levels were compared between all treatment groups that received acute injections of nicotine (see Table 2 for a summary of basal dopamine values). In female offspring that had received gestational nicotine, baseline dopamine levels were unaffected (1.6±0.2 pg/10 µl vs. 1.5±0.2 pg/10 µl for gestational nicotine vs. control, This article has not been copyedited and formatted. The final version may differ from this version.
respectively; F(1,49)=0.21, p>0.05). In contrast, cross-fostering reduced baseline dopamine levels in offspring, regardless of prenatal treatment (0.9±0.1 pg/10 µl compared to 1.7±0.2 pg/10 µl in non-cross-fostered offspring; F(1,49)=7.12, p<0.05). No interaction between cross-fostering and gestational treatment was observed [F(1,49)=1.16, p>0.05]. Nucleus accumbens dopamine content, determined in a separate group of non-cross-fostered offspring that had been exposed to gestational vehicle vs. nicotine, was also unaffected by prenatal treatment [93±19 (N=8) vs. 94±13 ng/mg protein (N=8)]. This analysis of nucleus accumbens dopamine content was limited to non-cross-fostered offspring because (i) cross-fostering did not interact with gestational treatment on basal dopamine levels or nicotine-induced dopamine secretion, and (ii) the primary purpose of these investigations was to determine the effects of gestational exposure to nicotine. In summary, gestational nicotine had no effect on baseline extracellular nucleus accumbens dopamine levels or on total dopamine content.

In adolescent females, the absence of an interaction between gestational nicotine treatment and cross-fostering (figure 2) makes it unlikely that cross-fostering would alter the peak response to a higher dose of acute nicotine. Therefore, further experiments evaluated the acute stimulation of dopamine release by a higher dose of nicotine in non-cross-fostered offspring exposed to gestational nicotine. Figure 3 demonstrates nucleus accumbens dopamine release in response to an acute injection of 0.105 mg/kg nicotine. In contrast to nicotine 0.07 mg/kg, there was no difference in dopamine release between female offspring that had received gestational control vs. gestational nicotine. In these groups, dopamine release increased by 63±9% and 82±10% above baseline levels, respectively. Moreover, these dopamine responses to 0.105 mg/kg nicotine were no
different than those observed to 0.07 mg/kg nicotine in non-cross-fostered gestational control animals (fig. 2). Thus, a higher dose of nicotine (0.105 mg/kg) is required to stimulate maximal nucleus accumbens dopamine release in female offspring that were exposed to nicotine during gestation. The dopamine response to this dose of nicotine was not accompanied by adverse behavioral responses in females, but higher doses were and, therefore, could not be tested.

**Males:** In male adolescent offspring, baseline dopamine levels were unaffected by prenatal nicotine treatment (1.6±0.4 pg/10 µl vs. 2.0±0.5 pg/10 µl for gestational nicotine vs. control, respectively). Furthermore, in contrast to the data obtained with females, cross-fostering did not affect baseline dopamine levels in males. Cross-fostered males had baseline dopamine levels of 1.6±0.4 pg/10 µl compared to 1.9±0.4 pg/10 µl in non-cross-fostered males. No interaction was observed between cross-fostering and gestational treatment.

Figure 4 shows that gestational nicotine exposure resulted in a reduction in the dopamine response to a maximally stimulative injection of 0.07 mg/kg nicotine in adolescent male offspring, similar to that seen in their female counterparts (figure 2). In non-cross-fostered male adolescents, the first nicotine injection induced peak dopamine levels that were 39±10% above baseline in offspring exposed to gestational nicotine compared to 86±11% in controls [F(1,14)=9.73, p<0.01]. Similarly, in cross-fostered males, peak responses were 38±8% in gestational nicotine versus 83±20% in controls (N=5) [F(1,9)=5.26, p<0.05]. Thus, gestational nicotine treatment consistently reduced the acute dopamine response by approximately 55%. As observed in females, there were no differences across all treatment groups in the nucleus accumbens dopamine responses.
to a second injection of 0.07 mg/kg nicotine. Moreover, within treatments, the dopamine responses to the first and second injections were not significantly different. In summary, gestational nicotine treatment attenuated nucleus accumbens dopamine release to an acute injection of 0.07 mg/kg nicotine in both female and male adolescent rats. However, cross-fostering \textit{per se} failed to attenuate dopamine release in male (figure 4) compared to female offspring (figure 2).

To evaluate the effects of a higher dose of nicotine on dopamine release in male offspring exposed to prenatal nicotine, experiments were again focused on non-cross-fostered offspring, since no interaction between gestational nicotine and cross-fostering was demonstrated. Figure 5 demonstrates that, similar to the observations made in adolescent females, gestational nicotine treatment did not affect the dopamine response in male offspring to the higher 0.105 mg/kg dose of nicotine (increase above baseline: 49±16\% vs. 71±11\% in gestational nicotine vs. control, respectively). Moreover, these dopamine responses were similar to those observed with 0.07 mg/kg nicotine in non-cross-fostered gestational control males (fig. 4). As with females, no adverse behavior responses were elicited by this higher dose.
**Discussion**

Prenatal exposure to nicotine reduced the accumbal dopamine response to a maximally stimulative dose of nicotine (0.07 mg/kg) in adolescent male and female Lewis rats, regardless of cross-fostering. Neither dopamine content nor basal dopamine secretion in the nucleus accumbens shell was affected by gestational nicotine treatment in female rats. Similarly, basal dopamine secretion was unaffected in male offspring exposed to nicotine during gestation. Gestational nicotine did not diminish the nucleus accumbens dopamine response to an acute injection of 0.105 mg/kg nicotine in either gender, and the dopamine response to this dose was not different from the response to 0.07 mg/kg nicotine observed in non-cross-fostered offspring treated with vehicle during gestation. These observations are consistent with reduced sensitivity to acute nicotine, in that higher doses of the drug were required to elicit equivalent levels of nucleus accumbens dopamine release in gestational nicotine-exposed females vs. control females.

In the present study, cross-fostering also affected nucleus accumbens dopamine secretion in adolescent females but not in males. In adolescent females, the effect of cross-fostering *per se* on the nucleus accumbens dopamine response to the lower dose of acute nicotine was similar to that seen with gestational nicotine treatment. Thus, cross-fostering reduced the dopamine response to 0.07 mg/kg nicotine. In fact, with the combination of cross-fostering and gestational nicotine exposure, adolescent females failed to show any dopamine response to 0.07 mg/kg nicotine. It should be noted that nucleus accumbens dopamine baseline levels were lower in cross-fostered vs. non-cross-fostered females. Such a reduction may reflect an increase in inhibitory tone, affecting the activity of dopaminergic neurons, which project from the VTA to the nucleus.
accumbens. Increased inhibitory tone, perhaps due to developmental effects of cross-
fostering on interactions between GABAergic and dopaminergic neurons (Erhardt et al.,
2002; Ikemoto et al., 1997), might reduce both basal and nicotine-stimulated dopamine
release in the nucleus accumbens of cross-fostered female adolescents.

Exposure to nicotine throughout gestation decreased the sensitivity to nicotine-
stimulated nucleus accumbens dopamine during adolescence, after a nicotine-free interval
of approximately 35 d. Although the mechanism(s) underlying this reduced sensitivity to
nicotine is unknown, basal dopamine secretion was unaffected by gestational nicotine in
both male and female rats. Furthermore, nucleus accumbens dopamine content, tested in
females only, was also unaffected by gestational nicotine treatment. These findings
suggest that the dopamine secretory pool in nucleus accumbens dopaminergic neurons
was unaffected. Similarly, both the activity of basal inputs and the intrinsic activity of
these dopaminergic neurons appear to be unaltered, insofar as this can be inferred from
the basal nucleus accumbens dopamine levels.

Nicotine exposure has been shown to alter the number and relative expression
levels of nicotinic receptor subtypes in both the prenatal human brain in vitro and the
perinatal monkey brain in vivo (Hellstrom-Lindahl et al., 2001; Slotkin et al., 2002).
Prenatal human cortical neurons displayed increased (³H)-epibatidine (0.2 nM) and (³H)-
cytisine (2 nM) binding following 3 days of nicotine in vitro (Hellstrom-Lindahl et al.,
2001). In addition, increased expression of α3 and α7 mRNAs, but not α4, was reported.
In Rhesus monkeys, perinatal exposure to nicotine selectively upregulated nicotinic
receptors in the brainstem and cerebral cortex. These findings support the hypothesis that
nicotinic cholinergic receptor expression may be developmentally modified in the present
model of chronic gestational nicotine treatment. For example, if gestational nicotine reduced the number of functional nicotinic receptors (i.e., upregulated the desensitized fraction) and/or altered the ratio of the various nicotinic cholinergic receptor holoproteins expressed by mesolimbic dopaminergic neurons (Hsu et al., 1996; Klink et al.), reduced sensitivity to nicotine might be observed during adolescence. Moreover, similar developmental changes in receptor subunit expression might be related to the difference in dopamine responsiveness to the second vs. the first injection of nicotine that was observed in females in the gestational control, but not the gestational nicotine group (figure 2).

It may seem paradoxical that an animal model of human vulnerability to nicotine dependence, as reported in adolescent daughters of mothers who smoked during pregnancy, would demonstrate reduced sensitivity to nicotine-induced dopamine secretion. This appears paradoxical in view of the fact that nucleus accumbens dopamine secretion is directly associated with the rewarding properties of addictive drugs (Di Chiara, 1999). However, it has been suggested that lower levels of nucleus accumbens dopamine are related to drug abuse (Gardner, 1999). Blum and colleagues postulated the existence of a reward deficiency syndrome linking addictive, impulsive and compulsive disorders to the basal dysfunction of dopamine reward systems (Blum et al. 1995, 1196). This hypothesis was, in part, based on animal studies showing that dopamine agonists decreased self-administration and reduced drug seeking (Dyr et al. 1993, Pulvirenti & Koob 1994). In addition, alcohol preference in rats has been linked to low levels of nucleus accumbens dopamine (McBride et al., 1995). Therefore, it is possible that a lesser dopamine response to nicotine would require higher doses of the drug in order to
drive the mesolimbic system and obtain adequate dopamine-dependent reinforcement. Thus, a human subject with reduced dopamine responsiveness would probably obtain sufficient dopamine-dependent reinforcement from nicotine by smoking more.

An increase in cigarette smoking would be expected to lead to stronger behavioral conditioning which may underlie the vulnerability of gestational nicotine exposed female adolescents to become dependent on cigarette smoking. Viewed in terms of learning theory, higher fixed ratio schedules (ratio of smoking responses to nicotine reinforcement) would result in increased difficulty in achieving extinction. Thus, gestational nicotine exposure would compromise the ability of female adolescents to quit smoking.

Figure 2 shows the reduction in dopamine responsiveness to a second dose of nicotine in the gestational control females; in contrast, reduced responsiveness was not evident in the gestational nicotine group. Thus, regardless of gestational treatment, the dopamine responses to the second dose of acute nicotine were the same. This lack of a difference may suggest that an increased number of responses (e.g. cigarette smoking) would not be required to achieve reinforcement in individuals gestationally exposed to nicotine. However, there are two facts that are likely to invalidate this conjecture: (1) in the present study, nicotine was delivered by forced injections, rather than self-administered; and (2) the second injection was delivered 1.5 h following the first.

During acquisition of cigarette smoking, many adolescents are likely to have large time intervals between cigarette use due to factors such as school, parental restrictions and the irregular occurrence of social interactions that stimulate smoking. In fact, the initial symptoms of nicotine dependence can develop without daily episodes of cigarette
smoking (DiFranza et al., 2002), and decreased responsiveness is unlikely to be present at the initiation of the next episode, which often occurs a day or more later. As proposed in the preceding discussion, the increased strength of the stimulus-response (i.e., cigarette presentation-cigarette smoking) association would enhance the susceptibility to becoming dependent on cigarette smoking.

These investigations also demonstrated a similar degree of reduced nucleus accumbens dopamine responsiveness to nicotine in male adolescent offspring with gestational nicotine exposure. This may suggest that dopamine neurotransmission in nucleus accumbens is tangential to the predisposing influence of gestational nicotine exposure on adolescent female nicotine dependence. However, based on the sexually dimorphic development of the brain, gestational exposure to nicotine may have different neuroanatomical and behavioral consequences. Thus, attenuated nucleus accumbens dopamine responsiveness may be responsible for the following gender-specific consequences: the predisposition to dependence on smoking in female adolescents and the prevalence of the attention deficit hyperactivity disorder (ADHD) in adolescent males exposed to nicotine in utero (Millberger et al. 1996).

During adolescence, sexual dimorphism in dopaminergic systems is evident in the overproduction and pruning of D1 and D2 receptors that is much more prevalent in striatum and accumbens of male than female rats (Andersen et al., 1997). This sex difference, which peaks at the onset of puberty, may be associated with gender differences in the prevalence of ADHD (Andersen et al., 2000). Using an animal model of ADHD, differences have been observed in distribution, affinity and plasticity of D1 and D2 receptors in the nucleus accumbens, caudate-putamen, and olfactory tubercle.
Using this model, a reduction in electrically-stimulated dopamine release in caudate-putamen and prefrontal cortex was also associated with ADHD (Russell et al., 1995). These and other studies indicate that altered dopamine responsiveness may contribute to ADHD (for a review see Biederman & Faraone 2002); therefore, the reduced dopamine secretion observed herein could underlie the increase in ADHD in males with gestational nicotine exposure. The reduced nicotine-induced dopamine release during adolescence suggests that a reduction in dopamine responsiveness to other stimuli (e.g. electrical stimulation) may be induced by gestational exposure to nicotine.

In summary, both males and females exposed to nicotine during gestation show diminished nucleus accumbens dopamine responsiveness to nicotine during adolescence. Based on the sexually dimorphic development of the brain and of dopamine neurotransmission, we postulate that the diminished nucleus accumbens dopamine responsiveness, observed herein, may have gender-specific effects on dopamine-dependent brain functions related to motivation. This may lead to different behavioral consequences: ADHD in males and a predisposition to dependence on smoking in females.
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Footnotes

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Corresponding author:

Burt M. Sharp, M.D.
Department of Pharmacology
University of Tennessee Health Science Center
874 Union Ave.
Memphis, TN 38163
Phone: 901-448-6000
FAX: 901-448-7206
E-mail: bsharp@utmem.edu
Figure 1. A representative photomicrograph of dialysis probe placement in the nucleus accumbens of an adolescent rat (45-55 g). Rats were intracardially perfused on ice with 4% paraformaldehyde in 0.05 M phosphate buffered saline, pH 6.8. Brains were removed, infused with sucrose, cryosectioned at 20 µm and stained with cresylviolet. The location of the dialysis probe membrane is indicated by the dotted line. ac=anterior commissure; magnification bar= 600 µm

Figure 2. Prenatal nicotine and cross-fostering affected nucleus accumbens dopamine release in response to nicotine in adolescent females. The initial nicotine injection (0.07 mg/kg i.v.) was delivered at 0 min (arrow in left panel) following three baseline samples, and an identical injection of nicotine was also administered 90 min thereafter (arrow in right panel). Dopamine levels are expressed as a percentage of the average baseline value. In each treatment group, the numbers of litters used for each injection are presented as (x:y). Peak responses (15 min after each injection of nicotine) were analyzed by two-way ANOVA: for the first injection, F(prenatal nicotine)=16.47, p=0.001; F(cross-fostering)=5.11, p<0.05; F(nicotine x fostering)=0.00; for the second injection, F(prenatal nicotine)=0.00; F(cross-fostering)=0.00; F(nicotine x fostering)=0.356, p>0.05. Baseline dopamine levels were 1.6±0.4 and 1.6±0.3 pg/10 μl for prenatal nicotine and controls, respectively. In adolescent female offspring with gestational nicotine exposure, the nucleus accumbens dopamine response to the first nicotine injection was attenuated, regardless of postnatal fostering condition, whereas responses to the second injection were not different from respective controls.
Figure 3. Nucleus accumbens dopamine release in response to 0.105 mg/kg IV nicotine was unaffected by prenatal nicotine exposure in adolescent females. The arrow indicates the delivery of 0.105 mg/kg nicotine at 0 min. Dopamine baseline levels were unaffected by gestational nicotine (1.5±0.3 pg/10 µl compared to 1.8±0.3 pg/10 µl in controls; p>0.05). An independent t-test showed no significant difference in peak dopamine responses.

Figure 4. Prenatal nicotine attenuated the nucleus accumbens dopamine response to an acute injection of nicotine in adolescent males. The initial nicotine injection (0.07 mg/kg i.v.; left panel) was delivered at 0 min (arrow) following three baseline samples, and an identical injection of nicotine was administered 90 min thereafter (right panel). Dopamine levels are expressed as a percentage of the average baseline value. In each treatment group, the numbers of litters used for both injections are presented as (x:y). Peak responses (15 min after each injection of nicotine) were analyzed by two-way ANOVA: for the first injection, F(prenatal nicotine)=12.24, p=0.003; F(cross-fostering)=2.09, p>0.05; F(nicotine x fostering)=1.61, p>0.05; for the second injection, F(prenatal nicotine)=1.91, p>0.05; F(cross-fostering)=0.86, p>0.05; F(nicotine x fostering)=0.00. Baseline dopamine levels were 1.6±0.4 and 2.0±0.5 pg/10 µl in prenatal nicotine and controls, respectively. In adolescent male offspring with gestational nicotine exposure, the nucleus accumbens dopamine response to the first nicotine injection was attenuated, regardless of postnatal fostering condition.
Figure 5. Nucleus accumbens dopamine release in response to 0.105 mg/kg IV nicotine was unaffected by prenatal nicotine exposure in adolescent males. The arrow indicates the delivery of 0.105 mg/kg nicotine at 0 min. Dopamine baseline levels were unaffected by gestational nicotine (0.9±0.4 pg/10 µl and 2.2±0.5 pg/10 µl, respectively; p>0.05). An independent t-test showed no significant difference in peak dopamine responses.
Figure 1
Figure 2

Females
Nicotine (0.07 mg/kg IV)

- - prenatal control (7:5)
- - - prenatal CF control (6:6)
- - - - prenatal nicotine (6:4)
- - - - - prenatal CF nicotine (5:4)

DOPAMINE RESPONSE (% CHANGE)

MINUTES

(45) (30) (15) 0 15 30 45 60 75 90 105 120 135 150 165
Figure 3

Females
Nicotine (0.105 mg/kg IV)

DOPAMINE RESPONSE (% CHANGE)

MINUTES

prenatal control (8)
prenatal nicotine (6)
Figure 5

Males
Nicotine (0.105 mg/kg IV)

DOPAMINE RESPONSE (% CHANGE)

MINUTES

prenatal control (8)
prenatal nicotine (7)
### TABLE 1

Peak nucleus accumbens dopamine responses  
(Percentage above baseline)

<table>
<thead>
<tr>
<th>Acute injection</th>
<th>Gestational treatment</th>
<th>Female</th>
<th></th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cross-Fostered</td>
<td>Non-Fostered</td>
<td>Cross-Fostered</td>
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<tr>
<td>0.07 mg/kg nicotine</td>
<td>Nicotine</td>
<td>15±10%</td>
<td>35±6%</td>
<td>38±8%</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>49±8%</td>
<td>71±9%</td>
<td>83±20%</td>
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<tr>
<td>0.105 mg/kg nicotine</td>
<td>Nicotine</td>
<td>N/A</td>
<td>82±10%</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>N/A</td>
<td>63±9%</td>
<td>N/A</td>
</tr>
</tbody>
</table>
TABLE 2

Nucleus accumbens basal dopamine levels

(pg/10 µl)

<table>
<thead>
<tr>
<th>Acute injection</th>
<th>Gestational treatment</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07 mg/kg nicotine</td>
<td>Nicotine</td>
<td>0.7±0.2</td>
<td>2.3±0.6</td>
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<td>1.3±0.3</td>
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<tr>
<td></td>
<td>Vehicle</td>
<td>1.1±0.2</td>
<td>2.0±0.5</td>
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<td>1.9±0.8</td>
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<tr>
<td>0.105 mg/kg nicotine</td>
<td>Nicotine</td>
<td>N/A</td>
<td>1.5±0.3</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>N/A</td>
<td>1.8±0.3</td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>