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Hyperactivity in the Offspring of Nicotine-Treated Rats: Role of the Mesolimbic and Nigrostriatal Dopaminergic Pathways

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RICHARDSON, S. A. AND Y. TIZABI. *Hyperactivity in the offspring of nicotine-treated rats: Role of the mesolimbic and nigrostriatal dopaminergic pathways.* PHARMACOL BIOCHEM BEHAV 47(2) 331-337, 1994.—To evaluate the involvement of the mesolimbic and nigrostriatal dopaminergic systems in hyperactivity in offspring of nicotine-treated dams, timed-pregnant Sprague-Dawley rats were implanted SC on gestational day 4 with osmotic minipumps to receive saline or nicotine (3 or 6 mg/kg/day) for 16 days. Hyperactive and nonhyperactive male offspring of nicotine-treated dams as well as nonhyperactive offspring of saline-treated dams were selected and sacrificed at day 22 postnatally. Discrete brain areas (the nucleus accumbens [NAcc], striatum [STR], frontal cortex [FC], ventral tegmental area [VTA], and substantia nigra [SN]) were microdissected for the evaluation of dopamine (DA) concentration and/or the D_2 receptor subtype. Dopamine concentration was decreased in the VTA and STR but was increased in the SN of the hyperactive offspring. The reduction in striatal DA level was associated with a reduction in the number of D_2 receptors in that area. The data suggest a role for the VTA and striatal dopaminergic system in offspring hyperactivity.

Nicotine Hyperactivity Dopamine D_2 receptor Mesolimbic Nigrostriatal

UNDERSTANDING the impact of in utero nicotine exposure via maternal cigarette smoking continues to be of substantial importance. Interestingly, the increase in the prevalence of hyperactivity in children during the past 40 years has coincided with an increase in the frequency of cigarette smoking by women (7,32). Thus, a number of studies have suggested that cigarette smoking during pregnancy may be one of the causes of hyperactivity in children (2,5,6,25).

Since chronic exposure of pregnant rats to nicotine, the principle active component of tobacco, results in hyperactive offspring (8,19,28,30), such an animal model may be utilized to investigate the biochemical basis of this behavioral disorder.

Several lines of evidence suggest that activation of the mesolimbic dopaminergic pathway underlies the locomotor stimulant action of nicotine in adult rats (4,9,14,20). However, information on involvement of the mesolimbic dopaminergic system in the offspring hyperactivity following prenatal exposure to nicotine is lacking. Thus, utilizing the model of chronic administration of nicotine to pregnant rats, we have investigated the mesolimbic as well as the nigrostriatal dopaminergic system's involvement in offspring hyperactivity.

METHOD

Timed-pregnant Sprague-Dawley rats weighing between 200 and 250 g were purchased from Charles River (Kingston, NY). The animals were maintained in an environmentally controlled room with a 12-h light/dark cycle, temperature range of 22-24°C, and relative humidity of 40-70%. They were allowed free access to food and water.

On the fourth day of gestation rats were randomly divided into two groups and were anesthetized with halothane. An incision was made in the scapula, and an osmotic minipump (model 2002, Alza Corp., Palo Alto, CA) containing either nicotine dihydrochloride (J. T. Baker Chemical Co., Phillipsburg, NJ) or sterile physiological saline was implanted SC. The incision was closed with wound clips and covered with

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betadine ointment to prevent infection. Animals were permitted to recover in their home cages.

The osmotic minipumps were filled with nicotine dihydrochloride, which was dissolved in sterile physiological saline. The minipumps, with a flow rate of 12.1 μl/day and total delivering capacity of 16 days, were filled such that either 3 or 6 mg/kg/day of nicotine was delivered during fetal development up to 2 days prior to parturition (day 22 of gestation).

Activity Test

The male pups were tested for their locomotor behavior on days 19 and 21, the age when activity differences become readily apparent (33). The activity test was conducted in an automated "open field" photocell cage (Omnitech Electronics Inc., Columbus, OH) between 0800 and 1600. Spontaneous locomotor activity, determined by the number of horizontal beam interruptions, was recorded immediately following placement of animals in the recording cages for a total duration of 60 min. Nicotine- and saline-treated offspring were tested simultaneously during the behavioral trial.

Based on pilot studies, the offspring with cumulative scores of 3000 or higher for both tests were selected as hyperactive and offspring with cumulative scores of 2400 or less were selected as nonhyperactive.

Tissue Collection and Dopamine Measurement

Twenty-four hours after the last activity test, the pups were sacrificed by decapitation. The brains were rapidly removed and frozen by immersion in powdered dry ice. Discrete brain areas (nucleus accumbens [NAcc], striatum [STR], frontal cortex [FC], ventral tegmental area [VTA], and substantia nigra [SN]) were microdissected according to the method of Palkovits and Brownstein (26). The samples were sonicated and assayed for dopamine (DA) according to a modified radioenzymatic assay method of Peuler and Johnson (29).

D_2 Binding Assay

For measurement of D_2 receptor, the NAcc and STR were dissected on ice according to the method of Heffner et al. (12). Tissues were homogenized in 5 ml of ice cold 50 mM Tris-HCl buffer containing 0.1% ascorbic acid, 0.01 mM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ at pH 7.4. The homogenate was centrifuged at 27 000 × *g* for 20 min at 4°C, and the supernatant discarded. The pellet was washed twice by adding 5 ml of the above buffer, followed by homogenization and centrifugation at 20 000 × *g* for 30 min. The final pellet was suspended at a concentration of ~50–100 μg/ml of protein in a final volume of 0.45 ml of the above buffer.

The receptor assay was carried out according to a modified procedure described by Burt et al. (3) and Lau et al. (18). The assay tubes (final volume of 0.45 ml) contained six concentrations of [^{3}H]spiperone (91 Ci/mmol; Amersham, Arlington Heights, IL) ranging from 0.1 to 3.2 nM, 10 μM sulpiride, or buffer, and 50–100 μg protein. Both spiperone and sulpiride are selective D_2 antagonists. The reaction was initiated by adding 100-μl pellet suspension to the tubes containing the radiolabeled ligand and incubating the tubes for 15 min at 37°C in a shaking water bath. The reaction was terminated by adding 5 ml of ice-cold buffer to the tubes. This mixture was poured immediately onto a GF/B glass microfiber filter disc (Whatman Inc., Clifton, NJ) that had been equilibrated with 0.1% polyethyleneamine for 1 h and was placed on a filter manifold under constant vacuum. Each filter was washed three times with 5 ml of ice-cold buffer.

After drying, filters were placed in 10 ml of scintillant and tritium activity was determined in a Beckman liquid scintillation spectrometer following 6 h equilibration. Nonspecific [^{3}H]spiperone binding was determined in the presence of 10 μM sulpiride. Specific binding was determined by subtracting the nonspecific from the total binding. Scatchard plots to calculate the B_{max} and K_d were obtained using the nonlinear least-squares regression LIGAND analysis (21). Protein concentration in the resuspended pellet was determined by Bradford method (1).

TABLE 1

PLASMA LEVELS OF NICOTINE AND COTININE IN PREGNANT RATS

Treatment	Gestation Day 12		Gestation Day 20	
	Nicotine (ng/ml)	Cotinine (ng/ml)	Nicotine (ng/ml)	Cotinine (ng/ml)
Saline	<1	<10	<1	<10
Nicotine				
3 mg/kg/day	38 ± 3	398 ± 23	37 ± 2	350 ± 24
6 mg/kg/day	107 ± 11*	750 ± 71*	102 ± 10*	602 ± 48*

Results are the mean ± SE of four to five determinations. *$p < 0.01$, significantly different from dams infused with 3 mg/kg/day.

Determination of Nicotine and Cotinine in Plasma

To determine the nicotine and cotinine concentration in the plasma, separate groups of dams receiving 3 or 6 mg/kg/day of nicotine were decapitated at 12 and 20 days of pregnancy and approximately 6 ml trunk blood was collected. Plasma levels of nicotine and cotinine were measured by gas chromatography and mass spectrometry at the Division of Clinical Pharmacology, University of California San Francisco Medical Center according to the method of Jacob et al. (15).

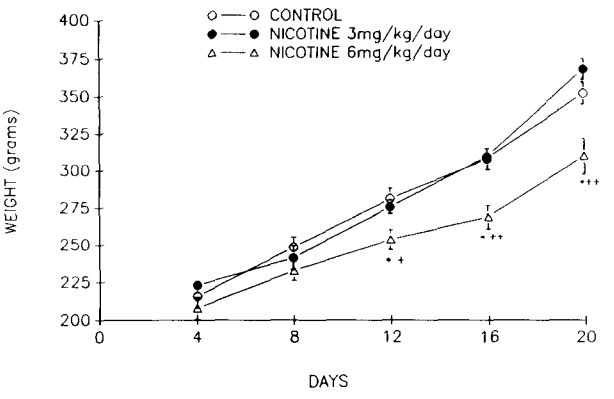

FIG. 1. Maternal weight during gestation. Dams were implanted with osmotic minipumps containing saline or nicotine (3 or 6 mg/kg/day) on day 4 of gestation. Data represent the means ± SE of 20–25 dams. *$p < 0.05$ compared to saline control. +$p < 0.05$ compared to nicotine 3 mg/kg/day. ++$p < 0.01$ compared to nicotine 3 mg/kg/day.

TABLE 2

EFFECTS OF NICOTINE INFUSION ON MATERNAL DELIVERY, LITTER SIZE, AND SEX AND WEIGHT OF OFFSPRING

Treatment	Percent Delivery in Dams	Litter Birth Weight (g)	Litter Size	# Male Pups	# Female Pups	Male Body Weight (g) at Day 7	Female Body Weight (g) at Day 7
Saline control	83 ± 5 n = 17	6.7 ± 0.2	12 ± 0.6	6 ± 0.5	6 ± 0.4	15.4 ± 0.7	15 ± 0.6
Nicotine (3 mg/kg/day)	76 ± 9 n = 20	7.2 ± 0.2	12 ± 0.8	6 ± 0.7	5 ± 0.5	15.6 ± 0.8	17 ± 1.8
Nicotine (6 mg/kg/day)	67 ± 10 n = 20	6.4 ± 0.1*	11 ± 0.6	7 ± 0.8	6 ± 0.5	14.3 ± 0.5	14 ± 0.5*

Results represent mean ± SE of 20–25 litters. *$p < 0.05$ compared to nicotine 3 mg/kg/day.

Statistical Analysis

For statistical analysis the general linear model procedure was applied and significant changes were determined by the least squares-means post hoc test (11). Acceptable statistical significance was determined at $p < 0.05$.

RESULTS

Plasma Levels of Nicotine and Cotinine in Pregnant Rats

Table 1 shows the plasma levels of nicotine and its major metabolite cotinine in pregnant dams at days 12 and 20 of gestation following 3- or 6-mg/kg/day nicotine administration. At both time points plasma levels of both nicotine and cotinine were considerably higher (1.7- to 3.6-fold) in dams receiving 6 mg/kg/day nicotine. There were no significant differences in the plasma levels of nicotine or cotinine between days 12 and 20 of gestation in either dose regimen. The plasma nicotine levels obtained in this study following 3- or 6-mg/kg/day nicotine administration are equivalent to plasma nicotine levels of humans smoking approximately two or five packs of cigarettes per day, respectively (13,22).

Effects of Chronic Nicotine Infusion on Pregnant Dams

Figure 1 shows the effect of chronic nicotine infusion on dams' weight during various gestation periods. Dams receiving 6 mg/kg nicotine had a lower weight gain throughout gestation compared to dams receiving 3 mg/kg nicotine or saline. Dams receiving 3 mg/kg nicotine did not show any variation in their weight gain compared to saline-treated rats.

Table 2 shows the effects of maternal nicotine infusion on the percentage of pregnant dams delivering successfully at term, litter birth weight, litter size, and sex and weight of offspring. Nicotine treatment resulted in a dose-dependent decrease in the number of dams which delivered successfully at term. The offspring of dams infused with 6 mg/kg/day nicotine had an 11.1% reduction in their birth weight compared to offspring of dams infused with 3 mg/kg/day nicotine, $F(2, 8) = 2.16$, $p < 0.05$. It is interesting to note that at day 7 postnatally only the female offspring of dams infused with 6 mg/kg/day nicotine showed a reduction (17.6%) in their body weight compared to female offspring of dams infused with 3 mg/kg/day nicotine, $F(2, 15) = 3.19$, $p < 0.05$. Whether this weight change is sustained at a later age or whether it is associ-

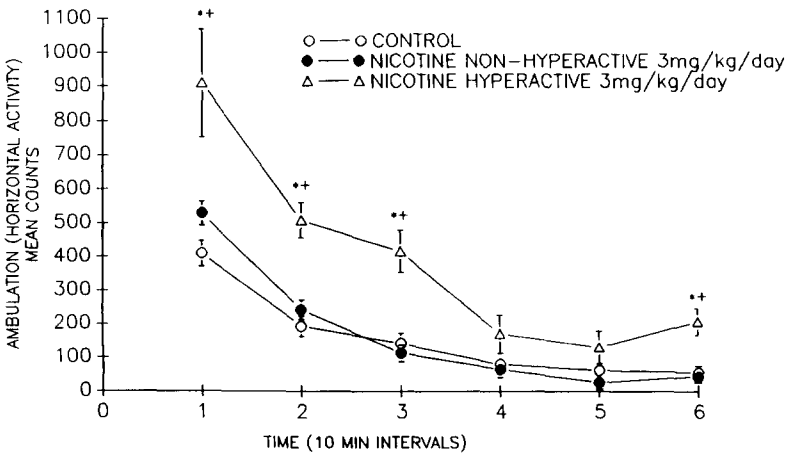

FIG. 2. Ambulation (horizontal activity) mean cumulative counts at 10-min intervals for a total of 60 min for two tests. Offspring prenatally exposed to saline or nicotine at 3 mg/kg/day were tested on days 19 and 21 for locomotor activity. Data represent the means ± SE of 20–25 litters. *$p < 0.01$ compared to saline control. +$p < 0.01$ compared to nicotine nonhyperactive.

ated with behavioral changes in this sex remain to be elucidated. The litter size and number of male or female pups born per litter were indistinguishable between the three treatment groups.

Behavioral Effects of Nicotine

Figures 2 and 3 illustrate the means of cumulative horizontal activity scores at 10-min intervals for a total of 60 min in offspring of dams treated with 3 or 6 mg/kg/day nicotine, respectively. Although the hyperactive offspring were selected on the basis of their higher cumulative scores, only the hyperactive offspring of dams treated with the higher dose of nicotine had sustained hyperactivity throughout the test periods. In addition, the mean cumulative scores of this group were at least 60% higher than with the 3-mg dose.

The weights of the offspring at the time of activity testing (day 19) were not significantly different among the three groups (control = 35.2 ± 1.0 g, n = 28; 3-mg offspring = 37.7 ± 1.4 g, n = 26; 6 mg offspring = 34.7 ± 1.0 g, n = 25; values are means ± SE).

The Effects of Maternal Nicotine Exposure on Dopamine Levels in Discrete Brain Regions of Offspring

Tables 3 and 4 summarize DA levels in discrete brain regions in male offspring of dams treated with 3 or 6 mg/kg/day nicotine, respectively. With the 3-mg dose (Table 3), the NAcc and STR of the offspring had significantly lower DA concentrations than control offspring. In addition, the SN of the hyperactive offspring had elevated DA concentration (44%) compared to control offspring, $F(8, 218) = 4.25$, $p < 0.05$. With the 6-mg dose (Table 4), the NAcc of the nonhyperactive offspring had lower DA concentration compared to both saline control and hyperactive offspring (15% and 23%, respectively). In the STR, both hyperactive and nonhyperactive offspring of nicotine-treated dams had significantly lower DA concentrations (33% and 13%, respectively) than control offspring. However, when compared to the nicotine nonhyperactive offspring, the hyperactive offspring had a significantly lower (25%) DA concentration. Likewise, in the VTA the hyperactive offspring of dams treated with 6 mg/kg/day nicotine had a lower DA concentration compared to either saline control or nicotine nonhyperactive offspring (44% and 51%, respectively). In the SN, the hyperactive offspring had higher DA concentration (43%) than saline control, $F(8, 155) = 9.60$, $p < 0.05$. Dopamine levels in the FC were not significantly altered between the three groups of offspring in either dose regimen (Tables 3 and 4).

The Effects of Maternal Nicotine Exposure on D_2 Receptors in Discrete Brain Regions of Offspring

Tables 5 and 6 summarize the binding capacity (B_{max}) and affinity constant (K_d) values for the D_2 receptor in the NAcc and STR in male offspring of dams infused with 3 or 6 mg/kg/day nicotine, respectively. With the 3-mg dose (Table 5), the NAcc of the offspring had a higher binding capacity than control offspring. In the STR, only the hyperactive offspring had a significant reduction (16%) in B_{max} compared to control offspring, $F(2, 20) = 5.92$, $p < 0.05$. The binding affinity (K_d) was not significantly altered among the three groups.

With the 6-mg dose (Table 6), the NAcc of the nonhyperactive offspring had a significantly higher B_{max} compared to both saline and hyperactive offspring (118% and 90%, respectively), $F(2, 20) = 2.86$, $p < 0.05$. In the STR, no significant changes in either B_{max} or K_d were observed.

DISCUSSION

Consistent with previous reports (24,31), the results of this study demonstrate that nicotine-infused dams have a reduced weight gain during gestation and deliver less successfully at term. In addition, it was demonstrated that prenatal nicotine exposure causes a dose-dependent stimulation of locomotor activity in selective offspring. Thus, identification of the hyperactive and nonhyperactive offspring following prenatal nicotine exposure is essential in the interpretation of biochemical findings.

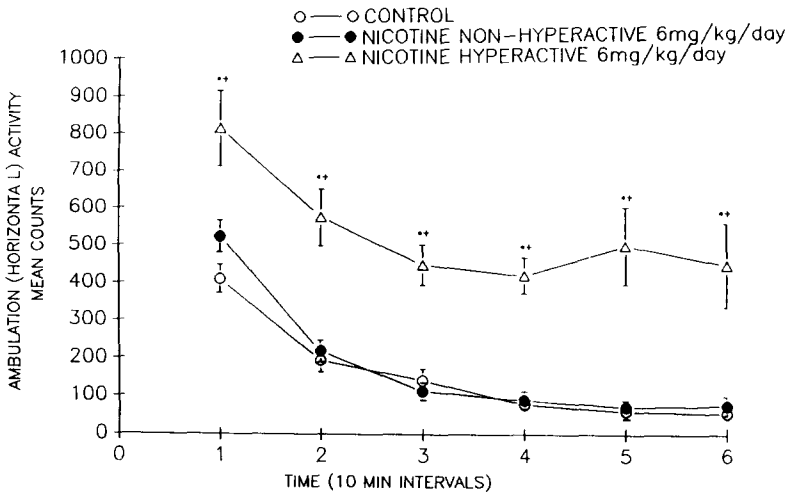

FIG. 3. Ambulation (horizontal activity) mean cumulative counts at 10-min intervals for a total of 60 min for two tests. Offspring prenatally exposed to saline or nicotine at 6 mg/kg/day were tested on days 19 and 21 for locomotor activity. Data represent the mean ± SE of 20–25 litters. *$p < 0.01$ compared to saline control. +$p < 0.01$ compared to nicotine nonhyperactive.

TABLE 3

EFFECTS OF MATERNAL NICOTINE (3 mg/kg/day) EXPOSURE ON DOPAMINE LEVELS IN DISCRETE BRAIN REGIONS OF MALE OFFSPRING

Offspring Group	Nucleus Accumbens	Striatum	Frontal Cortex	Ventral Tegmental Area	Substantia Nigra
Saline control	77 ± 6 (18)	95 ± 7 (17)	1 ± 0.1 (17)	31 ± 4 (12)	18 ± 2 (11)
Nicotine nonhyperactive	51 ± 7 (16)*	56 ± 5 (19)*	1 ± 0.1 (15)	26 ± 2 (17)	23 ± 3 (15)
Nicotine hyperactive	61 ± 8 (14)†	70 ± 8 (17)*‡	1 ± 0.1 (15)	30 ± 5 (18)	26 ± 3 (14)*

Dopamine concentrations are in pg/μg protein. Parentheses indicate the number of animals in each group. Values represent the mean ± SE. *$p < 0.01$ compared to saline control. †$p < 0.05$ compared to saline control. ‡$p < 0.01$ compared to nicotine nonhyperactive.

TABLE 4

EFFECTS OF MATERNAL NICOTINE (6 mg/kg/day) EXPOSURE ON DOPAMINE LEVELS IN DISCRETE BRAIN REGIONS OF MALE OFFSPRING

Offspring Group	Nuculeus Accumbens	Striatum	Frontal Cortex	Ventral Tegmental Area	Substantia Nigra
Saline control	80 ± 5 (10)	113 ± 9 (8)	1.2 ± 0.1 (12)	34 ± 5 (7)	21 ± 4 (12)
Nicotine nonhyperactive	68 ± 5 (12)*	98 ± 5 (8)*	1.8 ± 0.2 (12)	39 ± 4 (12)	25 ± 3 (14)
Nicotine hyperactive	88 ± 4 (11)†	76 ± 4 (12)†‡	1.8 ± 0.1 (15)	19 ± 2 (12)†‡	30 ± 2 (13)*

Dopamine concentrations are in pg/μg protein. Parentheses indicate the number of animals in each group. Values represent the mean ± SE. *$p < 0.05$ compared to saline control. †$p < 0.01$ compared to nicotine nonhyperactive. ‡$p < 0.01$ compared to saline control.

TABLE 5

EFFECTS OF MATERNAL NICOTINE (3 mg/kg/day) EXPOSURE ON THE NUCLEUS ACCUMBENS AND STRIATAL D_2 RECEPTORS ([^{3}H]SPIPERONE BINDING) IN MALE OFFSPRING

	Nucleus Accumbens		Striatum	
Offspring Group	B_{max} (fmol/mg protein)	K_d (pM)	B_{max} (fmol/mg protein)	K_d (pM)
Saline control	35 ± 2	74 ± 15	89 ± 11	97 ± 17
Nicotine nonhyperactive	64 ± 9*	102 ± 6	79 ± 11	96 ± 15
Nicotine hyperactive	57 ± 3*	82 ± 9	75 ± 6*	98 ± 18

Values represent the mean ± SE of five independent experiments. Tissues from five rats were pooled in each experiment. *$p < 0.05$ compared to saline control.

TABLE 6

EFFECTS OF MATERNAL NICOTINE (6 mg/kg/day) EXPOSURE ON THE NUCLEUS ACCUMBENS AND STRIATAL D_2 RECEPTORS ([^{3}H]SPIPERONE BINDING) IN MALE OFFSPRING

	Nucleus Accumbens		Striatum	
Offspring Group	B_{max} (fmol/mg protein)	K_d (pM)	B_{max} (fmol/mg protein)	K_d (pM)
Saline control	34 ± 10	66 ± 22	91 ± 14	94 ± 6
Nicotine nonhyperactive	74 ± 3*	80 ± 19	77 ± 9	102 ± 7
Nicotine hyperactive	39 ± 12†	72 ± 4	81 ± 14	92 ± 9

Values represent the mean ± SE of four independent experiments. Tissues from five rats were pooled in each experiment. *$p < 0.05$ compared to saline control. †$p < 0.05$ compared to nicotine nonhyperactive.

Studies with adult rats suggest that activation of the mesolimbic dopaminergic pathway underlies the locomotor stimulant action of nicotine (4,9,14,16,20,23,27). Thus, direct infusion of nicotine or cytisine, a nicotinic agonist, into the VTA increases ambulation (16,23,27), which can be blocked by selective DA antagonists (16). Furthermore, it has been shown that nicotine-induced hyperactivity is associated with increase dihydroxyphenylalanine (DOPA)/DA ratio in the NAcc, which suggests an increase in DA utilization in this nucleus (4). This contention is supported by findings of increased dopamine release in the NAcc following direct nicotine infusion in that nucleus (20). In addition, using in vivo microdialysis technique it has been demonstrated that nicotine-induced hyperactivity is associated with increased DA release in the NAcc (14).

In our study, however, no consistent changes in either DA levels or D_2 receptor subtype were noted in the NAcc of the hyperactive offspring. This finding does not support an association between increased DA release in the NAcc and hyperactivity following prenatal exposure to nicotine. On the other hand, administration of a higher dose of nicotine did result in lower DA concentration in the VTA of hyperactive offspring, which suggests a role for VTA in offspring hyperactivity. This finding is also in contrast to hyperactivity in adult rats where nicotine administration does not significantly affect the DA concentration in the VTA (17). It should be noted that significant differences in the approaches of the studies to determine DA function (measurement of levels and receptors in our study vs. microdialysis or DOPA/DA ratio by others) preclude direct comparison of the results, particularly in reference to DA release or turnover. However, based on comparison of the DA levels as well, it may be suggested that the central biochemical changes in nicotine-induced hyperactive offspring differ from those in nicotine-induced hyperactive adult rats.

Our data also suggest a role for the nigrostriatal dopaminergic pathway in offspring hyperactivity. We found an increase in DA concentration in the SN and a reduction in DA concentration in the STR of hyperactive offspring. However, the reduction in striatal DA level was associated with a reduction in the number of D_2 receptors in that area. This downregulation of D_2 receptors in the presence of decreased DA levels suggests increased striatal DA release in hyperactive offspring. It is interesting to note that a decrease in the number of striatal D_2 rceptors in offspring of nicotine-treated dams has been reported (10).

In summary, this study supports the contention that in utero nicotine exposure has detrimental effects on pregnant dams as well as on their offspring. Furthermore, the data emphasize the need to differentiate between the hyperactive and nonhyperactive offspring in delineating the biochemical basis of this disorder. In addition, both mesolimbic and nigrostriatal dopaminergic pathways appear to be involved in offspring hyperactivity.

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