

# Enhanced Amphetamine Sensitivity and Increased Expression of Dopamine D2 Receptors in Postpubertal Rats after Neonatal Excitotoxic Lesions of the Medial Prefrontal Cortex

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Functional and structural abnormalities in the medial prefrontal cortex (MPFC) and overactive dopamine (DA) neurotransmission are thought to be the key pathologies in schizophrenia. To understand the role of MPFC in the pre- and postpubertal development of the subcortical DA system, the effects of neonatal [postnatal day 7 (PD7)] MPFC excitotoxic lesions on locomotor behaviors and the expression of DA receptor subtypes and DA transporter were investigated in Sprague Dawley rats at PD35 and PD56, respectively. No significant differences in the novelty or *d*-amphetamine-induced locomotion were observed between sham-operated and ibotenic acid-lesioned rats at PD35. Postpubertally (at PD56), however, the locomotor activity of lesioned rats in the novel environment and after *d*-amphetamine administration was enhanced significantly compared with controls. The expressions of DA D1, D2, D3, and D4 receptors and DA transporter were then estimated in

MPFC-lesioned and sham-operated rats at PD39 and PD60. The levels of DA D2 receptors, measured using [<sup>3</sup>H]-YM-09151-2 binding, and its mRNA by *in situ* hybridization, were observed to be significantly increased at PD60 in striatal and limbic areas of lesioned rats. Levels of other DA receptor subtypes were not significantly affected at any time points. Lesioned rats at PD39 show a small increase in DA transporter level in the shell of nucleus accumbens; however, this effect seems to wear off at PD60. The data suggest that neonatal MPFC lesions may alter the functional development and maturation of mesolimbic/nigrostriatal DA systems in that neonatally lesioned rats grow into a behavioral/neurochemical deficit.

*Key words:* prefrontal cortex; schizophrenia; animal model; nucleus accumbens; ibotenic acid; dopamine receptors; neurodevelopment

Considerable evidence from clinical, neuropsychological, brain imaging, and postmortem neuroanatomical studies strongly implicates the medial prefrontal cortex (MPFC) in the pathophysiology of schizophrenia (for review, see Weinberger et al., 1994). MPFC neuronal activity exerts an important regulatory control on the subcortical dopamine (DA) system, the overactivity of which is believed to underlie some of the psychotic symptoms of the disease (for review, see Goldstein and Deutch, 1992). The importance of the MPFC in regulating the action of the limbic system, especially the mesolimbic DA system, can be recognized by examining its connectivity. First, the neurons of the MPFC are interconnected with the limbic cortex directly through intracortical projections (Goldman-Rakic et al., 1984; Jay and Witter, 1991). In addition, the MPFC projects to the substantia nigra pars compacta and ventral tegmental area (VTA), the sources of striatal and mesocorticolimbic DAergic projections, respectively (Sesack and Pickel, 1992). Furthermore, MPFC efferents to the VTA control the DA output to the nucleus accumbens (Taber et

al., 1995; Karreman and Moghaddam, 1996). Additional MPFC connections include direct glutamatergic excitatory projections to the caudate-putamen and nucleus accumbens (Sesack et al., 1989; Deutch, 1992), which may offer further control of DA release (Grace, 1991; Karreman and Moghaddam, 1996).

Biochemical studies have indicated that lesion of the MPFC or activation of its pathways alters the function of the subcortical DA systems. For example, excitotoxic lesions of the adult rat MPFC have been associated with a transiently enhanced DA turnover in limbic regions (Christie et al., 1986), and 6-hydroxydopamine lesions of MPFC have been reported to increase limbic DA transmission (Pycocock et al., 1980; Leccese and Lyness, 1987) as well as enhance the responsiveness of the mesolimbic DA neurons to stress (Deutch et al., 1990; Doherty and Gratton, 1996). Chemical and electrical stimulation of the MPFC have been shown to increase DA release in the striatum (Murase et al., 1993; Taber and Fibiger, 1993). Furthermore, adult rats with MPFC excitotoxic lesions exhibit higher locomotor response to novelty, a transitory increase in amphetamine-induced locomotor activity (Jaskiw et al., 1990), swim-stress-induced locomotion (Jaskiw and Weinberger, 1992), and a decrease of the cataleptogenic effect of haloperidol (Worms et al., 1985), all of which are behaviors related to DA transmission.

In recent years, various evidence has accumulated that implicates cortical neuronal maldevelopment in schizophrenia (for review, see Weinberger and Lipska, 1995). Although studies on MPFC lesions induced in the adult rat provide important insights into the regulatory functions of this structure on DA systems, they do not address the consequences of lesions to the MPFC on the

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development of subcortical DAergic activity. Because schizophrenia symptoms typically appear after puberty, i.e., in adolescence or early adulthood, it is important to understand the role of early neurodevelopmental lesions on the maturation of DA systems before and after puberty. Recently, the developmental aspects of disrupting the prefrontal-temporal connectivity were addressed by performing neonatal lesions of the ventral hippocampus (VH) (Lipska et al., 1992, 1993a,b). These studies have shown that neonatal lesions of the VH result in a delayed, postpubertal onset of behavioral hyperactivity (Lipska et al., 1993a,b), which may be associated with decreased DA D3 receptors in the nucleus accumbens (Flores et al., 1996). In the present investigation, we have assessed the developmental consequence of neonatal ibotenic acid-induced lesions of MPFC in postnatal day (PD) 7 rats. At pre- and postpubertal ages, animals were tested in behavioral paradigms commonly used to assess the functioning of the mesolimbic DA system, which demonstrated a postpubertal onset of increased amphetamine-induced locomotion in the neonatally lesioned rats. DA receptor subtypes and the DA transporter were also measured at both ages and revealed an increase in the DA D2 receptors that correlated with the onset of behavioral changes in the MPFC-lesioned animals. The results suggest an important role of developing MPFC in the functional maturation of subcortical DA activity.

## MATERIALS AND METHODS

**Materials.** *d*-Amphetamine sulfate, ibotenic acid, ketanserin, butaclamol, 8-OH-DPAT, GTP, GBR-12909, and DA were purchased from RBI (Natick, MA); 1,3-di(2-5-tolyl)guanidine (DTG) was from Sigma (St. Louis, MO). [<sup>3</sup>H]-SCH23390 (70 Ci/mmol), [<sup>3</sup>H]-YM-09151-2 (86 Ci/mmol), and [<sup>3</sup>H]-WIN-35428 (84 Ci/mmol) were obtained from DuPont NEN (Boston, MA). [<sup>3</sup>H]-7-OH-DPAT (139 Ci/mmol), [<sup>3</sup>H]-Hyperfilm, and microscale tritium standards were purchased from Amersham Canada (Toronto, Ontario). Oligonucleotides probes were synthesized by the Sheldon Biotechnology Facility of McGill University (Montreal, Quebec). 2-Methyl-butane was purchased from BDH (Montreal, Quebec), and EDTA from Boehringer Mannheim (Laval, Quebec). Gelatin and bovine serum albumin were purchased from Fisher Scientific (Montreal, Quebec) and Calbiochem (La Jolla, CA) respectively. All other chemical used were also of analytical reagent quality.

**Neonatal MPFC lesions.** Pregnant Sprague Dawley rats were obtained at gestational day 14–17 from Charles River Canada (St. Constant, Quebec). Animals were housed individually in a temperature- and humidity-controlled environment on a 12 hr light/dark cycle with free access to food and water. The day after birth, litters of six to eight male pups were formed, and on PD7 each pup (weighing 15–17 gm) was assigned to either a sham ( $n = 16$ ) or a lesion ( $n = 20$ ) group. All surgical procedures described in this study have been approved previously by McGill University Animal Care Committee in accordance with the guidelines of the Canadian Council for Animal Care. After the induction of anesthesia by hypothermia, pups were positioned on a platform fixed to a stereotaxic Kopf instrument. An incision was made over the skull and 0.3  $\mu$ l ibotenic acid (10  $\mu$ g/ $\mu$ l) or an equal volume of vehicle (0.1 M PBS, pH 7.4) was injected in each side of the MPFC during a 2 min period through a 30 gauge stainless steel cannulae positioned at the following coordinates: anteroposterior, +2.5 mm; mediolateral,  $\pm$  0.4 mm to bregma; ventrodorsal –2.2 mm from dura. The cannulae remained in place for 5 min after completion of the infusion. After the procedure, the pups were placed under a warming lamp for recovery and then returned to their mothers. On PD21 animals were weaned and grouped two or three animals per cage.

**Behavioral testing.** Four (PD35) or 7 weeks (PD56) after neonatal lesions, the locomotor activity of sham-operated and ibotenic acid-lesioned rats was assessed in two-photocell activity boxes connected to an IBM computer equipped with a software (ACTANAL) developed by Concordia University (Montreal, Quebec). The locomotor activity of each animal was assessed under the following testing conditions. (1) Locomotion after exposure to a novel environment: unacclimatized rats were placed in the activity boxes, and the beam crosses were recorded for 60 min. (2) Locomotion after *d*-amphetamine injection:

2 d after the first test, rats were again placed in the activity boxes, and basal locomotor activity was recorded for 60 min. Animals were injected first with 0.9% NaCl, and then after 60 min of locomotor assessment with *d*-amphetamine sulfate (1 mg/kg, s.c., dissolved in 0.9% NaCl); locomotion was monitored further for the next 120 min. Locomotor activity results were analyzed by applying two-way ANOVA followed by Newman-Keuls tests for *post hoc* comparisons, with lesion and age as independent factors ( $p < 0.05$  was considered significant).

**Brain processing.** Four sham-operated and five lesioned rats per group were selected at random and killed by rapid decapitation 48 hr after the last testing day (PD39 and PD60, respectively). Brains were removed rapidly, frozen in isopentane ( $-40^{\circ}\text{C}$ ), and stored at  $-80^{\circ}\text{C}$  until use. For assessment of lesion size, coronal sections at the level of the MPFC were stained with 0.5% cresyl violet and examined using a light microscope where lesions and probe placement could be visualized. Frozen brains were sectioned at 15  $\mu$ m thickness on the coronal plane using a Leitz cryostat. Sections were collected on cleaned, gelatin-coated microscope slides (four sections/slides), thaw-mounted, desiccated under vacuum at  $4^{\circ}\text{C}$  overnight, and then stored at  $-80^{\circ}\text{C}$  until the day of the experiment.

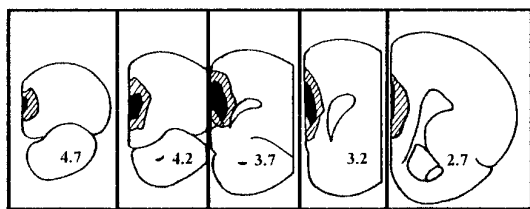
**Receptor autoradiography.** Coronal brain sections taken at the level of the nucleus accumbens and caudate-putamen [plate 10–11 of Paxinos and Watson atlas (1986)] were used in the following protocols. For D1-like receptor binding, sections were first preincubated for 10 min at room temperature in buffer containing 50 mM Tris-HCl, pH 7.4, 154 mM NaCl, 1 mM EDTA, and 0.1% bovine serum albumin. Sections were then incubated for 90 min at room temperature in the same buffer with the addition of 2 nM [<sup>3</sup>H]-SCH-23390 (74 Ci/mmol) and 30 nM ketanserin (to mask possible binding of ligand to serotonergic 5-HT<sub>2</sub> sites). Nonspecific binding was determined on adjacent brain sections by adding 1  $\mu$ M (+)-butaclamol to the buffer. Incubations were terminated by dipping the slides in ice-cold buffer followed by two consecutive 10 min washes in buffer. After a final dipping in ice-cold distilled water, slides were dried at room temperature and apposed to [<sup>3</sup>H]-Hyperfilm for 5 d, alongside microscale-calibrated tritium standards.

For D2-like and D4 receptor binding, the slides were first preincubated for 10 min at room temperature in buffer containing 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl<sub>2</sub>, and 4 mM MgCl<sub>2</sub>. Sections were then incubated for 2 hr at room temperature in the same buffer containing 1 nM [<sup>3</sup>H]-YM-09151-2 (86 Ci/mmol) with or without 100 nM raclopride, for assessment of D4 or D2 receptor bindings, respectively. 8-OH-DPAT (50 nM) was added in each case to mask possible binding of the ligands to serotonergic 5-HT<sub>1a</sub> sites. Nonspecific binding was determined on adjacent brain sections by adding 1  $\mu$ M (+)-butaclamol in the buffer. Incubations were terminated by dipping the slides in ice-cold buffer followed by two consecutive 10 min washes in the same buffer. After a final dipping in ice-cold distilled water, slides were dried at room temperature and apposed to [<sup>3</sup>H]-Hyperfilm for 12 d, alongside microscale-calibrated tritium standards.

[<sup>3</sup>H]-7-OH-DPAT binding to D3 receptor was assessed according to the procedure of Lévesque et al. (1992), with minor modifications (Flores et al., 1996). Tissue sections were first preincubated for 30 min in buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 300  $\mu$ M GTP. Sections were then incubated for 2 hr at room temperature with 2 nM [<sup>3</sup>H]-7-OH-DPAT (50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 300  $\mu$ M GTP, and 5  $\mu$ M DTG (to block binding to  $\sigma$  site). 1  $\mu$ M DA was used in adjacent sections to determine nonspecific labeling. Incubations were terminated by washing the brain sections twice for 10 min each in ice-cold 50 mM Tris-HCl, pH 7.4. After a brief dipping in ice-cold distilled water, brain sections were dried rapidly and apposed to [<sup>3</sup>H]-Hyperfilm for 6 weeks, alongside microscale-calibrated tritium standards.

DA transporter binding was assayed essentially according to the method of Kaufman et al. (1991). The sections were first preincubated for 20 min at  $4^{\circ}\text{C}$  in 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl. Sections were then incubated for 2 hr at  $4^{\circ}\text{C}$  in the same buffer containing 10 nM [<sup>3</sup>H]-WIN-35428 (84 Ci/mmol). Nonspecific binding was determined on adjacent brain sections by adding 1  $\mu$ M GBR-12909 in the buffer. Incubations were terminated by washing the brain sections twice for 1 min each in ice-cold 50 mM Tris-HCl, pH 7.4. After a brief dipping in ice-cold distilled water, brain sections were dried rapidly and apposed to [<sup>3</sup>H]-Hyperfilm for 6 weeks.

**In situ hybridization.** The probe for D2 receptor was a 39-mer oligonucleotide complementary to nucleotides 937–975 of the coding region of rat D2 receptor cDNA (5'-GGT CCGGGTTTTGCCATTGGGCATG-GTCTGGATCTCAAA) (Bunzow et al., 1988) and recognizes both alternatively spliced isoforms (D2L and D2S) of the receptor mRNA. The



**Figure 1.** Schematic drawing of coronal sections (modified from Paxinos and Watson, 1986) illustrating lesion boundaries and the areas of neural loss and gliosis determined from Nissl-stained coronal sections from PD60 rats with ibotenic acid lesion of the neonatal MPFC. The stippled lines and solid black areas indicate the largest and smallest lesions, respectively. Coordinates refer to distance in millimeters anterior to bregma (Paxinos and Watson, 1986).

probe for D1 receptor was a 39-mer oligonucleotide complementary to nucleotides 1240–1278 of the coding region of rat D1 receptor cDNA (5'-ATAGTCCAATATGACCGATAAGGCTGGGGACAGCTTCTC) (Zhou et al., 1990). The oligonucleotides were 3'-end-labeled with [ $\alpha$ - $^{35}$ S] dATP and terminal transferase and purified through an NACS column (Bethesda Research Labs, Mississauga, ON). *In situ* hybridization was performed on 15  $\mu$ m coronal sections, essentially according to previously described procedure (Srivastava et al., 1992). The sections were fixed in 4% paraformaldehyde, and after acetylation (triethanolamine and acetic anhydride) and defatting the sections were hybridized by placing 80–100  $\mu$ l of hybridization mixture containing [ $\alpha$ - $^{35}$ S]-labeled oligonucleotide probe ( $3 \times 10^5$  cpm),  $5 \times$  SSC, 50% formamide,  $2 \times$  Denhardt's solution, 10% dextran sulfate, 100  $\mu$ g/ml salmon sperm DNA, and 50  $\mu$ g/ml yeast tRNA at 42°C for 15–18 hr in a humidified box. They were washed  $4 \times 30$  min in  $2 \times$  SSC at room temperature,  $2 \times 30$  min in  $0.1 \times$  SSC at 45°C, and finally  $1 \times 15$  min in  $0.1 \times$  SSC at room temperature. After a brief rinse in ethanol containing 0.3 M ammonium acetate, pH 7.0, sections were air-dried and apposed to [ $^3$ H]-Hyperfilm.

**Data analysis.** Films from autoradiography and *in situ* assays were analyzed using a computerized image analysis system (MCID-4, Imaging Research, St. Catherine, Ontario). The binding data were analyzed in brain subregions according to Paxinos and Watson (1986), namely, the dorsolateral, dorsomedial, and ventral caudate-putamen, the fundus striatum, the shell and core of the nucleus accumbens, olfactory tubercles, and the islands of Calleja, and are expressed as femtomole per milligram wet tissue. Optical density of *in situ* films was analyzed in the same regions, and the results are expressed in random optical density units. Comparison between groups was carried out by applying two-way ANOVA and *post hoc* Newman-Keuls, with lesion and age as independent factors and  $p < 0.05$  considered significant.

## RESULTS

### Verification of the lesion

Bilateral reduction in the size of the frontal cortex was seen in the neonatally lesioned rats. Cresyl violet-stained sections obtained from neonatal lesioned animals, at both PD39 and PD60, revealed moderate bilateral damage to the MPFC, with neuronal loss, atrophy, and apparent retraction of the MPFC. Small cavities in the MPFC also were seen occasionally (Figs. 1 and 2).

### Behavioral testing

The prepubertal (PD35) and postpubertal (PD56) effects of neonatal MPFC lesions on locomotor activity in a novel environment are illustrated in Figure 3. In the two age groups, both lesioned and sham-operated animals exhibited active exploratory behavior when placed in a novel environment. Although no potentiation of novelty-induced locomotion was seen in neonatally lesioned rats at PD35, a significant increase in exploratory behavior was observed at PD56 in neonatally MPFC-lesioned rats during the first 10 min period (Fig. 3).

Amphetamine administration induced a marked increase in the locomotor activity at PD35 and PD56 in neonatally lesioned rats



**Figure 2.** Photomicrograph of a coronal section of MPFC stained with cresyl violet showing retraction of the tissue, gliosis, and neural loss in PD60 neonatally lesioned rats. Scale bar, 160  $\mu$ m.

(Fig. 4). Analysis of the entire period of *d*-amphetamine effect revealed no significant differences in the locomotor activity between sham-operated and MPFC-lesioned rats at PD35. Locomotor activity, however, was significantly increased (83%) at PD56 in neonatally MPFC-lesioned animals when compared with sham-operated controls ( $F_{(3,32)} = 6.035$ ;  $p = 0.0022$ ) (Fig. 4). No significant effect of saline injection (vehicle) was observed in any of the groups (Fig. 4).

### [ $^3$ H]-SCH-23390 binding

D1-like receptors, as measured by [ $^3$ H]-SCH-23390 binding, are distributed throughout the dorsal and ventral regions of the striatum. The distribution and density of [ $^3$ H]-SCH-23390 binding did not differ significantly between the sham-operated and neonatal MPFC-lesioned animals at either PD39 or PD60 (Table 1).

### [ $^3$ H]-YM-09151-2 binding

[ $^3$ H]-YM-09151-2 binds DA receptor subtypes belonging to the D2 family (D2, D3, and D4). Total D2-like receptor binding shows a dorsoventral density gradient maximal in the caudate-putamen region. The distribution and level of D2-like binding sites did not differ significantly between the neonatally sham-operated and MPFC-lesioned animals at PD39 (Table 2, Fig. 5). At PD60, however, the level of D2-like binding sites was increased significantly in the nucleus accumbens shell of the MPFC-lesioned animals compared with sham controls (19%;  $F_{(3,14)} = 4.923$ ;  $p = 0.0159$ ) (Table 2, Fig. 5). Putative DA D4 receptor binding, measured by [ $^3$ H]-YM-01951-2 in the presence of raclopride, did not differ significantly between control and MPFC-lesioned rats (Table 2). The level of D2 and D3 receptors, measured by subtracting [ $^3$ H]-YM-01951-2 binding in the presence of raclopride from total [ $^3$ H]-YM-01951-2 binding, was significantly increased in the shell of the nucleus accumbens (35%;  $F_{(3,14)} = 4.598$ ;  $p =$

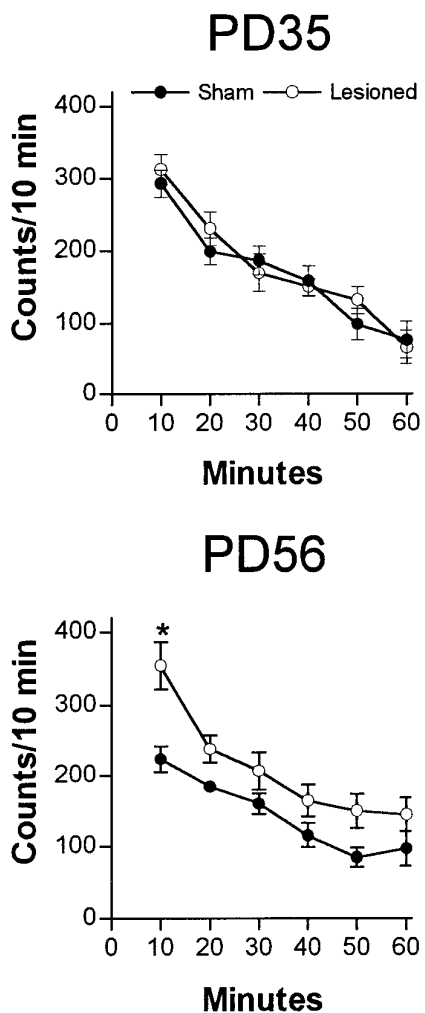


Figure 3. Locomotor activity (mean number of beam interruptions per 10 min  $\pm$  SEM;  $n = 8-10$  per group) in a novel environment of sham-operated or neonatal MPFC-lesioned animals tested either at PD35 or PD56. Locomotor activity was determined as described in Materials and Methods. At PD35, lesioned animals did not differ from sham-operated animals at any testing interval. In contrast, at PD56, lesioned rats were more active than the sham-operated rats during the first 10 min of exploration ( $*p < 0.05$ ).

0.0193) and the dorsolateral caudate-putamen (21%;  $F_{(3,14)} = 4.765$ ;  $p = 0.0172$ ) (Table 3).

**[<sup>3</sup>H]-7-OH-DPAT binding**

DA D3 receptors, measured by [<sup>3</sup>H]-7-OH-DPAT binding, were found principally in the islands of Calleja, the olfactory tubercle, and the shell of the nucleus accumbens (Table 4). A low level of D3 receptor expression was also detected in the striatum, particularly in its medial portion. D3 receptor levels in the fundus of caudate-putamen and the core of the nucleus accumbens were significantly higher at PD60 than PD39 in both sham-operated and lesioned groups and may indicate an age-related enhancement in D3 receptors. Compared with sham-operated controls, none of the MPFC-lesioned animals showed any significant differences in D3 binding levels.

**[<sup>3</sup>H]-WIN-35428 binding**

The DA transporters, as measured using [<sup>3</sup>H]-WIN-35428, are distributed throughout the dorsal and ventral part of the striatum.

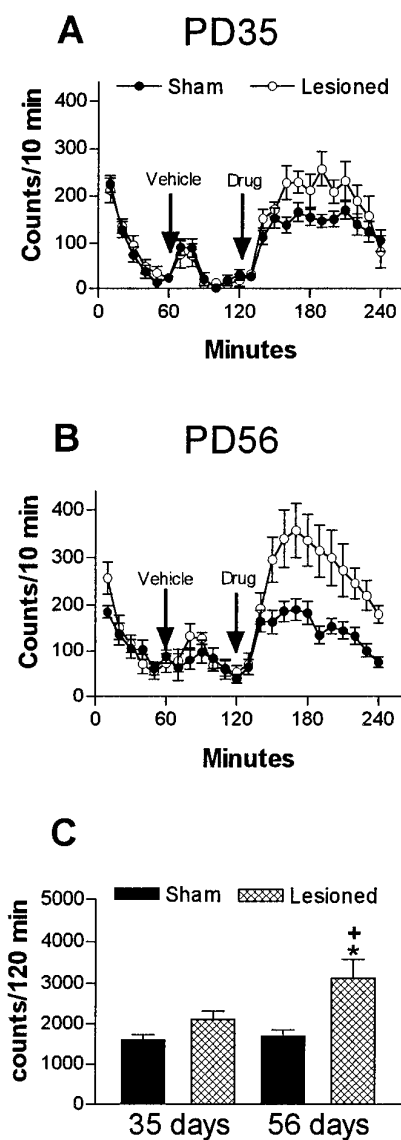


Figure 4. Locomotor activity after vehicle (saline) and *d*-amphetamine administration (1 mg/kg, s.c.) of sham-operated or ibotenic acid-lesioned groups (mean number of beam interruptions per 10 min  $\pm$  SEM;  $n = 8-10$  per group). *A*, Temporal profile of locomotor activity at PD35. *B*, Temporal profile of locomotor activity at PD56. *C*, Analysis of total activity scores after *d*-amphetamine reveals that lesioned rats are significantly more active than sham-operated rats only at PD56.  $*p < 0.01$ , significantly different from the sham-operated groups;  $+p < 0.05$ , significantly different from PD35 lesioned rats.

DA transporter levels in the shell of the nucleus accumbens are lower at PD60 than at PD39 in the sham group. The distribution and level of DA transporter binding sites do not differ significantly between the sham-operated and neonatally MPFC-lesioned animals at PD60 (Table 4). At PD39, however, the level of DA transporter binding sites was significantly decreased (22%) in the shell of the nucleus accumbens in the MPFC-damaged animals ( $F_{(3,14)} = 10.413$ ;  $p = 0.0007$ ) (Table 4).

**In situ hybridization**

The distribution and level of D1 mRNA did not differ significantly between the sham-operated and MPFC-lesioned animals in any age group (Table 1). At PD60, however, D2 receptor mRNA was significantly increased in the lesioned animals

**Table 1. Quantitative evaluation of D1-like receptors by autoradiography and D1 receptor mRNA by *in situ* hybridization in subregions of the striatum and in the olfactory tubercles**

	D1-like [ <sup>3</sup> H]-SCH-23390			D1 <i>in situ</i> hybridization		
	Sham (n = 4)	Lesion (n = 5)	% change	Sham (n = 4)	Lesion (n = 5)	% change
<b>PD 39</b>						
CP-dorsolateral	410 ± 25	386 ± 36	-6.7	0.90 ± 0.02	0.87 ± 0.07	-3.7
CP-dorsomedial	380 ± 23	350 ± 38	-7.9	0.84 ± 0.01	0.78 ± 0.04	-7.8
CP-ventral	404 ± 23	387 ± 38	-4.3	0.86 ± 0.02	0.87 ± 0.03	1.0
Fundus-CP	423 ± 30	399 ± 34	-5.5	0.94 ± 0.04	0.90 ± 0.03	-4.8
Accumbens-core	379 ± 18	312 ± 37	-17.6	0.76 ± 0.05	0.8 ± 0.07	-5.7
Accumbens-shell	378 ± 20	324 ± 36	-14.2	0.90 ± 0.03	0.84 ± 0.05	-6.2
Olfactory tubercle	339 ± 15	296 ± 31	-12.5	1.20 ± 0.04	1.13 ± 0.12	-6.4
<b>PD 60</b>						
CP-dorsolateral	388 ± 9.2	383 ± 6.6	-1.5	0.74 ± 0.09	0.87 ± 0.1	17.4
CP-dorsomedial	366 ± 5.5	356 ± 10.2	-2.7	0.71 ± 0.06	0.73 ± 0.1	1.9
CP-ventral	387 ± 5.2	394 ± 11.5	1.6	0.72 ± 0.08	0.82 ± 0.1	13.4
Fundus-CP	387 ± 14	405 ± 6.9	4.7	0.77 ± 0.09	0.94 ± 0.06	22.0
Accumbens-core	357 ± 9.1	368 ± 16.5	3.1	0.60 ± 0.06	0.65 ± 0.08	6.82
Accumbens-shell	359 ± 6.4	376 ± 11.1	4.8	0.69 ± 0.06	0.70 ± 0.12	1.7
Olfactory tubercle	321 ± 13.1	347 ± 4.5	8.1	1.09 ± 0.14	1.19 ± 0.1	9.2

Receptor levels are expressed in fmol/mg wet tissue, and *in situ* hybridization data are expressed as random optical density and represent the mean ± SEM of pooled values obtained from four sections per animal. Striatal subregions are defined according to Paxinos and Watson atlas (1986). CP, Caudate-putamen.

**Table 2. Quantitative evaluation of D2-like DA receptors in subregions of the striatum and in the olfactory tubercles**

	D2-like [ <sup>3</sup> H]-YM-09151-2			D4-like [ <sup>3</sup> H]-YM-09151-2 + raclopride		
	Sham (n = 4)	Lesion (n = 5)	% change	Sham (n = 4)	Lesion (n = 5)	% change
<b>PD 39</b>						
CP-dorsolateral	190 ± 3.6	192 ± 4.0	1.0	76 ± 2.0	73 ± 2.8	-3.2
CP-dorsomedial	152 ± 2.9	140 ± 4.01	-2.5	59 ± 2.9	58 ± 2.5	-2.9
CP-ventral	138 ± 3.9	140 ± 6.67	1.7	58 ± 2.2	57 ± 2.5	-2.3
Fundus-CP	120 ± 3.4	126 ± 4.2	-3.0	55 ± 2.0	52 ± 1.8	-6.8
Accumbens-core	92 ± 3.2	89 ± 3.9	-2.7	41 ± 1.7	38 ± 0.8	-6.7
Accumbens-shell	91 ± 3.4	84 ± 3.92	-8.2	40 ± 2.0	36 ± 0.7	-9.3
Olfactory tubercle	85 ± 6.2	85 ± 4.5	-0.3	42 ± 1.6	48 ± 3.3	11.4
<b>PD 60</b>						
CP-dorsolateral	173 ± 1.7 <sup>†</sup>	187 ± 6.7	8.0	73 ± 1.3	67 ± 2.1	-9.3
CP-dorsomedial	132 ± 4.0	140 ± 2.7	6.5	56 ± 1.2	53 ± 1.8	-6.3
CP-ventral	125 ± 2.0	138 ± 1.8	10.0	55 ± 1.0	54 ± 3.2	-2.5
Fundus-CP	107 ± 3.4	117 ± 7.3	9.8	50 ± 1.2	52 ± 4.2	4.0
Accumbens-core	84 ± 3.0	94 ± 1.9	11.5	41 ± 2.7	39 ± 2.6	-5.4
Accumbens-shell	85 ± 3.7	101 ± 3.4 <sup>*†</sup>	18.7	42 ± 4.2	44 ± 4.8	2.9
Olfactory tubercle	89 ± 4.3	92 ± 3.5	3.5	49 ± 1.9	53 ± 1.3	8.9

Receptor levels are expressed in fmol/mg wet tissue and represent the mean ± SEM of pooled values obtained from four sections per animal. Striatal subregions are defined according to Paxinos and Watson atlas (1986). CP, Caudate-putamen.

\* $p < 0.05$  compared with age-matched sham group; <sup>†</sup> $p < 0.05$  compared with corresponding PD39 group.

compared with sham controls in the shell of the nucleus accumbens (24%;  $F_{(3,14)} = 36.065$ ;  $p < 0.0001$ ), dorsomedial caudate-putamen (28%;  $F_{(3,14)} = 9.164$ ;  $p = 0.0013$ ), and fundus caudate-putamen (36%;  $F_{(3,14)} = 10.553$ ;  $p = 0.0007$ ) (Table 3, Fig. 6). D2 mRNA levels in the nucleus accumbens were higher at PD60 than at PD39 in both the sham and lesioned group, whereas in the caudate-putamen of the le-

sioned group there were also higher D2 mRNA levels at PD60 compared with PD39.

## DISCUSSION

The present study demonstrates the age-dependent nature of the effects of neonatal MPFC excitotoxic lesion on behaviors linked to the mesolimbic DA system. Neonatally induced MPFC damage by

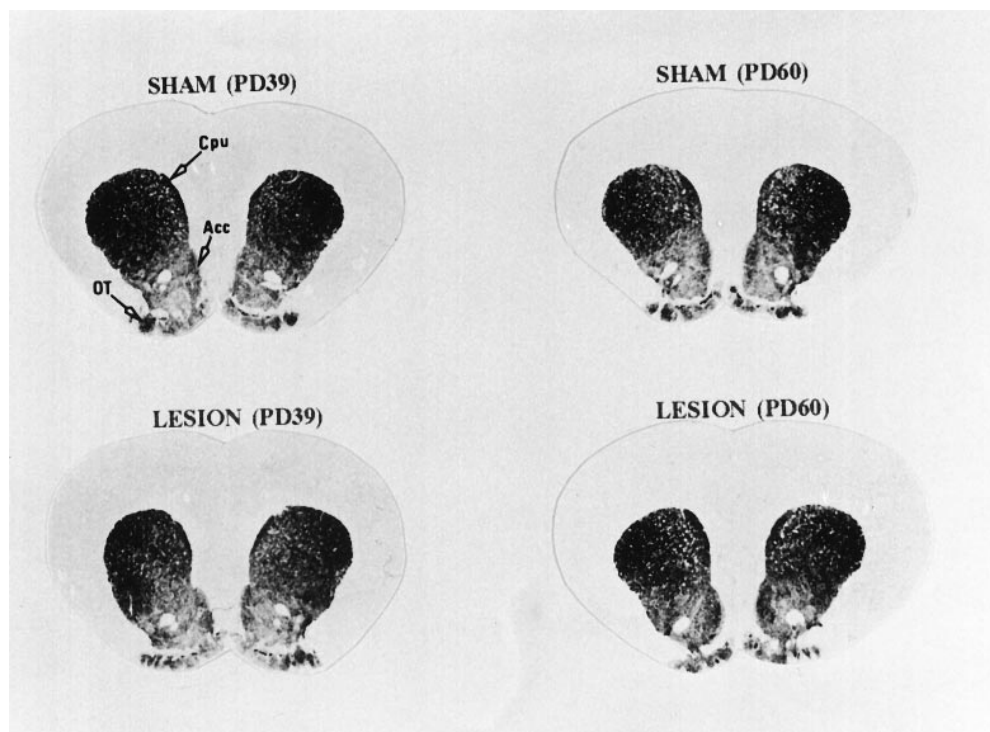


Figure 5. Effect of neonatal MPFC lesions on D2 DA receptor binding in the striatum and nucleus accumbens. Coronal sections were incubated, as described in Materials and Methods, in the presence of 1 nM [<sup>3</sup>H]-YM-09151-2. Results from the quantitative analysis of [<sup>3</sup>H]-YM-09151-2 binding are presented in Table 2. *Cpu*, Caudate-putamen; *Acc*, nucleus accumbens; *OT*, olfactory tubercles; *PD39*, postnatal day 39; *PD60*, postnatal day 60.

Table 3. Quantitative evaluation of D2–D3 DA receptors by autoradiography and D2 mRNA by *in situ* hybridization in subregions of the striatum and in the olfactory tubercles

	D2 + D3 [ <sup>3</sup> H]-YM-09151-2 – ([ <sup>3</sup> H]-YM-09151-2 + raclopride)			D2 <i>in situ</i> hybridization		
	Sham (n = 4)	Lesion (n = 5)	% change	Sham (n = 4)	Lesion (n = 5)	% change
<b>PD 39</b>						
CP-dorsolateral	114 ± 5.6	119 ± 3.1	3.8	1.47 ± 0.27	1.63 ± 0.27	11.2
CP-dorsomedial	92 ± 2.1	83 ± 4	–10.4	1.17 ± .2	1.32 ± 0.2	13.1
CP-ventral	80 ± 5.8	83 ± 5.5	4.7	1.21 ± 0.22	1.36 ± 0.2	12.4
Fundus-CP	65 ± 4.9	74 ± 5.0	14.5	1.24 ± 0.22	1.39 ± 0.21	12.7
Accumbens-core	51 ± 4.1	52 ± 2.8	0.5	0.90 ± 0.12	0.92 ± 0.13	2.8
Accumbens-shell	51 ± 4.6	47 ± 3.2	–7.4	0.78 ± 0.1	0.75 ± 0.1	–3.1
Olfactory tubercle	43 ± 5.6	38 ± 3.2	–8.8	2.08 ± 0.22	2.5 ± 0.3	20.1
<b>PD 60</b>						
CP-dorsolateral	99 ± 1.5 <sup>†</sup>	120 ± 5.4 <sup>**</sup>	21.0	2.24 ± 0.08	2.74 ± 0.11 <sup>††</sup>	22.2
CP-dorsomedial	75 ± 4.1	87 ± 2.78	16.3	1.71 ± 0.06	2.19 ± 0.09 <sup>*†</sup>	27.8
CP-ventral	69 ± 2.2	83 ± 2.6	20.1	1.87 ± 0.04	2.28 ± 0.09 <sup>††</sup>	22.3
Fundus-CP	57 ± 2.6	65 ± 4.5	14.9	1.76 ± 0.03	2.4 ± 0.1 <sup>**††</sup>	35.8
Accumbens-core	43 ± 2.9	55 ± 1.4	27.7	1.4 ± 0.06 <sup>††</sup>	1.57 ± 0.02 <sup>††</sup>	12.3
Accumbens-shell	43 ± 2.3	58 ± 1.6 <sup>*</sup>	34.9	1.27 ± 0.07 <sup>††</sup>	1.6 ± 0.08 <sup>**††</sup>	24.3
Olfactory tubercle	40 ± 3.5	39 ± 2.5	–2.8	2.94 ± 0.03	2.78 ± 0.12	–5.27

Receptor levels are expressed in fmol/mg wet tissue, and *in situ* hybridization data are expressed as random optical density and represent the mean ± SEM of pooled values obtained from four sections per animal. Striatal subregions are defined according to Paxinos and Watson atlas (1986). CP, Caudate-putamen.

\**p* < 0.05; \*\**p* < 0.01 compared with age-matched sham group; <sup>†</sup>*p* < 0.05; <sup>††</sup>*p* < 0.01 compared with corresponding PD39 group.

ibotenic acid produced an increase in locomotor behavior at PD56, evident during the exploratory period as well as after *d*-amphetamine administration. Furthermore, as demonstrated by autoradiography and *in situ* hybridization, these behavioral changes at PD56 occur concomitantly with an increase in the expression of DA D2 receptors in the shell of the nucleus accumbens and caudate-putamen (dorsomedial and fundus). Similarly lesioned rats tested at PD35, however, did not differ significantly

from sham-operated animals either in terms of behavior or DA receptor expression.

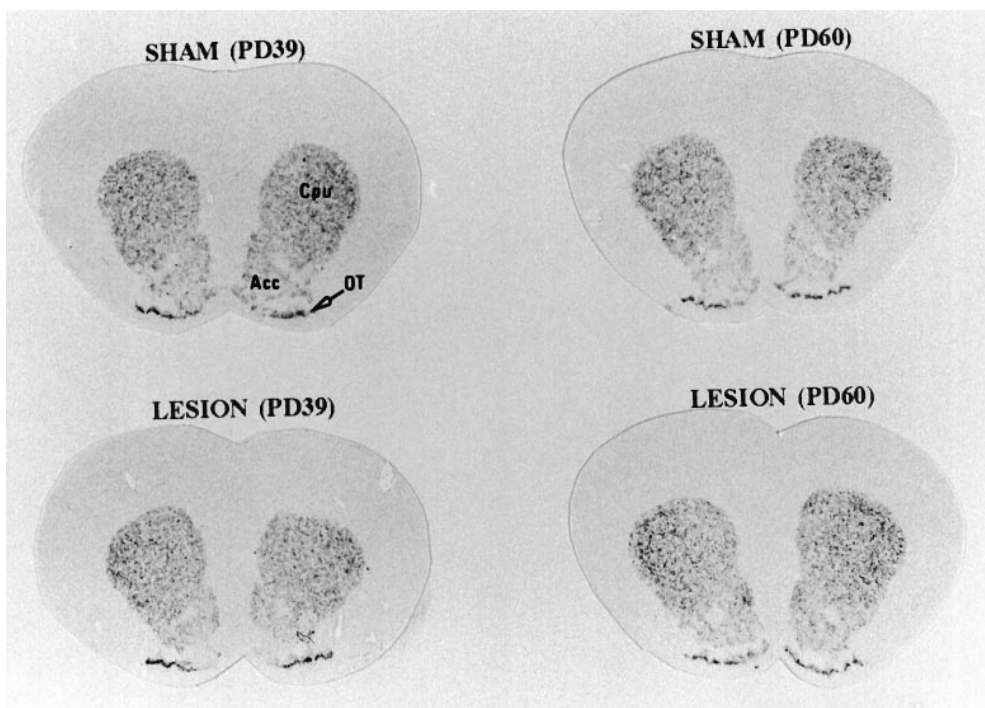
In contrast to neonatal lesions, excitotoxic lesions of the adult rat MPFC result in a transient (present at 2 but not 4 weeks postlesion) increase in locomotion in response to a novel environment (Jaskiw and Weinberger, 1990) or *d*-amphetamine (Jaskiw et al., 1990). We have also observed that novelty and *d*-amphetamine effects were not significant 4 or 7 weeks after

**Table 4. Quantitative evaluation of D3 receptors and dopamine transporter levels in subregions of the striatum and in the olfactory tubercles**

	D3 [ <sup>3</sup> H]-7-OH-DPAT			DA transporter [ <sup>3</sup> H]-WIN-35428		
	Sham (n = 4)	Lesion (n = 5)	% change	Sham (n = 4)	Lesion (n = 5)	% change
<b>PD 39</b>						
CP-dorsolateral	0.93 ± 0.16	1.09 ± 0.13	16.7	18.3 ± 0.7	18.5 ± 1.3	1.0
CP-dorsomedial	1.05 ± 0.20	1.37 ± 0.14	30.4	16.7 ± 1.2	17.1 ± 0.8	2.1
CP-ventral	2.02 ± 0.26	2.05 ± 0.19	1.7	16.5 ± 0.8	16.9 ± 0.4	2.2
Fundus-CP	2.87 ± 0.61	2.39 ± 0.57	-16.9	16.9 ± 0.5	17.0 ± 0.4	0.7
Accumbens-core	5.28 ± 0.49	4.30 ± 0.39	-18.6	15.4 ± 0.4	13.6 ± 0.7	-11.6
Accumbens-shell	7.74 ± 0.81	7.81 ± 0.59	0.9	15.3 ± 0.4	11.9 ± 0.6*	-22.2
Olfactory tubercle	5.10 ± 0.36	5.83 ± 0.47	14.2	17.2 ± 0.2	15.2 ± 1.0	-11.7
Island of Calleja	32.86 ± 3.42	29.69 ± 2.01	-9.64	n.d.	n.d.	
<b>PD 60</b>						
CP-dorsolateral	0.92 ± 0.17	1.00 ± 0.13	8.2	19.5 ± 1.1	19.6 ± 0.6	0.3
CP-dorsomedial	1.51 ± 0.18	1.73 ± 0.15	14.5	15.9 ± 1.6	19.6 ± 0.3	23.8
CP-ventral	2.85 ± 0.15	2.86 ± 0.13	0.41	16.6 ± 1.2	18.9 ± 1.1	14.1
Fundus-CP	5.50 ± 0.41	5.35 ± 0.56	-2.7	16.9 ± 0.7	16 ± 0.7	-5.8
Accumbens-core	7.69 ± 0.59 <sup>††</sup>	8.24 ± 0.74 <sup>††</sup>	7.14	12.5 ± 0.9	15 ± 1.0	19.7
Accumbens-shell	9.46 ± 0.40 <sup>††</sup>	9.84 ± 0.26 <sup>††</sup>	4.0	10.2 ± 1.0 <sup>††</sup>	12.5 ± 0.4	21.5
Olfactory tubercle	5.42 ± 0.22	5.14 ± 0.28	-1.9	11.7 ± 1.4	13.0 ± 0.4	11.6
Island of Calleja	30.87 ± 1.27	33.46 ± 0.61	8.4	n.d.	n.d.	

Receptor levels are expressed in fmol/mg wet tissue and represent the mean ± SEM of pooled values obtained from four sections per animal. Striatal subregions are defined according to Paxinos and Watson atlas (1986). CP, Caudate-putamen; n.d., not determined.

\* $p < 0.05$  compared with age-matched sham group; <sup>††</sup> $p < 0.01$  compared with corresponding PD39 group.



**Figure 6.** Effect of neonatal MPFC lesions on the expression of D2 receptor mRNA in the striatum and nucleus accumbens assessed by *in situ* hybridization. Coronal sections were fixed and hybridized with a [<sup>35</sup>S]-labeled D2R probe. Results from the quantitative analysis of *in situ* hybridization are presented in Table 3. *Cpu*, Caudate-putamen; *Acc*, nucleus accumbens; *OT*, olfactory tubercles; *PD39*, postnatal day 39; *PD60*, postnatal day 60.

MPFC lesion of adult rats, time periods that correspond to the postoperative intervals of the present study (unpublished results). It is interesting to note that lesions in the adult rat MPFC have been associated with an increase in the swim-stress-induced locomotion (Jaskiw and Weinberger, 1992). Furthermore, 6-hydroxydopamine lesions of the MPFC also enhance responsiveness to stress (Deutch, 1992; Doherty and

Gratton, 1996). Thus, it is possible that our observation of an increased locomotor activity in neonatally MPFC-lesioned rats at PD56 present within the first 10 min after placing the animals in the locomotor activity boxes could be a result of both previous handling stress and a novel environment.

Previous reports have suggested that behavioral outcome of frontal lesion depends on the age at which lesions are made as

well as the age when behavioral assessments are done. For example, animals receiving early neonatal lesion (PD1-4) show more severe cognitive behavioral deficits than similarly lesioned adult animals. In contrast, PD7-10 lesioned animals show a sparing or recovery of behavioral deficits (related to memory and learning) when tested as adults (Kolb and Whishaw, 1981; de Brabander et al., 1991a; Freeman and Stanton, 1992; Kolb and Gibb, 1993). In the light of evidence of functional recovery after neonatal frontal lesions, our findings support the opposite; i.e., the behavioral deficit (or at least a behavioral effect) is dormant prepubertally and seems to express at approximately postpubertal age. Our results thus are more consistent with early frontal damage in nonhuman primates, which shows sparing of memory functions in infants that later becomes a deficit in adults (Goldman-Rakic et al., 1983). Interestingly, our findings in neonatal MPFC-lesioned rats are similar to previous reports on neonatal excitotoxic lesion of the VH that also demonstrated postpubertal onset of enhanced *d*-amphetamine- and stress-induced hyperlocomotion (Lipska et al., 1993a,b; Flores et al., 1996). It is not clear whether there are common mechanisms in the postpubertal onset of behavioral changes in neonatal MPFC and VH lesions; however, it is known that the VH sends excitatory projections to regions of the MPFC (Jay and Witter, 1991). Therefore, a neonatal lesion of the VH (deafferenting MPFC) may affect medial prefrontal cortical functions during neonatal development and consequently alter DA-related behaviors, as suggested previously (Lipska et al., 1993a,b, 1994; Weinberger and Lipska, 1995).

It has been suggested that changes in cortical dendritic arborization (dendritic branching or spine density) may be responsible for behavioral sparing or recovery after frontal cortical lesions (Kolb and Whishaw, 1989; Kolb and Gibb, 1993). Integrity of cortical noradrenergic afferents to cortex seems necessary for behavioral sparing, because cortical 6-OHDA blocks the behavioral sparing and alters cortical morphogenesis after neonatal frontal cortex damage in rats (Kolb and Sutherland, 1992). Furthermore, de Brabander et al. (1991b) showed an increase of the DA innervation to the cortex after neonatal MPFC lesions in rats. In view of the complex neuroanatomical and neurochemical reorganization in the frontal cortex after neonatal injury, the delayed behavioral changes seen in the neonatal ibotenic acid-lesioned animals may be attributed to neurodevelopmental disturbances and compensatory changes, such as sprouting and rerouting, that could cause changes in the pattern of neural projections, including changes in mesocortical DAergic projections (Kolb et al., 1994). Consistent with the evidence of neural reorganization after frontal cortical damage, there are reports of alterations in cell adhesion and extracellular matrix molecules in the rat and mouse striatum (Poltorak et al., 1993; Butler et al., 1994) after frontal cortex lesions.

Another aim of the present study was to evaluate the pre- and postpubertal expressions of D1-like, D2, D3, and D4 DAergic receptors and DA transporter in rats that had undergone bilateral ibotenic acid lesions of the MPFC at PD7. Significant increases in the expression of D2 receptors were observed at PD60 in the shell of the nucleus accumbens and dorsomedial and fundus areas of the caudate-putamen. In contrast, the overall expression of the D1-like, D3, and D4 receptors did not change significantly after neonatal MPFC lesioning. The neonatal MPFC lesions also induced a small but significant reduction of the DA transporter in the shell of the nucleus accumbens at PD39; however, there were not significant differences at PD60. The reduction in the prepubertal level of DA transporter seems to be without significant

behavioral consequence, insofar as novelty- and amphetamine-induced locomotion are concerned. It may be indicative, however, of the beginning of postlesion compensatory changes in the mesolimbic DA system occurring prepubertally, which eventually results in overt behavioral changes at pubertal age, possibly mediated by increased levels of D2 receptors. Previous studies in MPFC-lesioned adult rats have not shown significant changes in the effects of quinpirole, a D2 agonist, on locomotor behavior (Braun et al., 1993). Our studies on neonatal MPFC lesion thus suggest a critical difference between the consequences of lesions in adult and neonatal animals insofar as D2 receptors are concerned, albeit indirectly. It is also possible that the changes in the D2 receptor levels reported here may not be a consequence of the loss of direct MPFC projection but may relate to neurodevelopmental disturbances secondary to an early MPFC damage.

A key finding of the present study, namely the increase in D2 receptors in the shell of the nucleus accumbens associated with potentiated amphetamine-induced locomotion, is particularly interesting in light of our previous report of a decrease in D3 receptors in the same area of neonatal VH-lesioned rats (Flores et al., 1996). Neonatal VH lesions have been reported to result in postpubertal increase in novelty-, stress-, and amphetamine-induced locomotor activity in rats (Lipska et al., 1993a,b; Flores et al., 1996). Thus, the behavioral consequences of both neonatal VH and MPFC lesions appear to emerge postpubertally, although differential changes in DA receptor subtypes accompany the behavioral changes. The reason for a differential effect on DA receptor subtypes in these two lesion paradigms is not clear. MPFC projects to both substantia nigra pars compacta and VTA (Sesack and Pickel, 1992). Stimulation of MPFC leads to increased DA release within the nucleus accumbens through the VTA (Taber et al., 1995; Karreman and Moghaddam, 1996), whereas stimulation of the VH produces a triphasic DA release via direct connections to the nucleus accumbens (Fibiger and Phillips, 1996). VH lesions, on the other hand, might not affect DA release through VTA but might directly alter the level of D3 receptors in the accumbens.

Previous studies in patients with schizophrenia have suggested an aberration during development of the prefrontal-temporolimbic cortices (for review, see Weinberger and Lipska, 1995). Morphometric studies have reported cytoarchitectural disorganization of the MPFC in schizophrenic brains (Benes et al., 1986, 1991; Raine et al., 1992), and positron emission tomography studies in schizophrenic patients have demonstrated a reduced blood flow and metabolic activity in the prefrontal cortex (popularly termed hypofrontality) (Ingvar and Franzen, 1974; Buchsbaum et al., 1990; Andreasen et al., 1992; Berman et al., 1992). Increased neuronal density without cell loss has been reported in prefrontal area 9 and occipital area 17 in the schizophrenic brain, suggesting neuronal atrophy and loss of dendritic arborization in these areas (Selemon et al., 1995). Along with these anatomical and functional alterations, increments in the levels of D2-like DA receptors in the caudate-putamen and nucleus accumbens of schizophrenics have been reported in many studies, although some of these changes could be attributed to increases in the D4 receptor (Seeman and Niznik, 1990; Seeman et al., 1993). Our findings in neonatal MPFC-lesioned rats agree with some of the features of human schizophrenics: for example, postpubertal increase in mesolimbic DA-related behavior associated with an increment in the level of D2 receptor in the nucleus accumbens.

In summary, increases in spontaneous and *d*-amphetamine-induced locomotor activity are evident in the PD56 neonatal



MPFC-lesioned rats, which may be attributable in part to increased expression of the DA D2 receptor in the nucleus accumbens. These data are also consistent with previous studies implicating MPFC neurons in the modulation of subcortical DA activity in the adult rat.

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