Plasticity of Dopamine D_4 Receptors in Rat Forebrain: Temporal Association with Motor Hyperactivity Following Neonatal 6-Hydroxydopamine Lesioning

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Genetic studies suggest that dopamine D_4 receptor polymorphism is associated with attention deficit hyperactivity disorder (ADHD). We recently reported that motor hyperactivity in juvenile male rats with neonatal 6-hydroxydopamine lesions of the central dopamine system can be reversed by dopamine D_4 receptor-selective antagonists. In this study, effects of such lesions on D_4 as well as other dopamine receptors (D_1 and D_2) were autoradiographically quantified at selected developmental stages. Neonatal lesions resulted in motor hyperactivity at postnatal day (PD) 25, but not at PD 37 or 60. Correspondingly, D_4 receptor levels in lesioned rats were substantially increased in caudate-putamen and decreased in nucleus accumbens at PD 25, but not at PD 37 or 60. Neonatal lesions also led to relatively minor changes in D_1 and D_2 receptor binding in various forebrain regions. However, the time-course of lesion-induced motor hyperactivity correlated only with changes in D_4, but not D_1 and D_2 receptors. These results further support the hypothesis that D_4 receptors may play a pivotal role in lesion-induced hyperactivity, and possibly in clinical ADHD. [Neuropsychopharmacology 26:625–633, 2002] © 2002 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

Attention-deficit hyperactivity disorder (ADHD) is a common neuropsychiatric condition characterized by hyperactivity, inattention and impulsivity, typically in school-aged boys (Barkley 1990). Abnormal dopamine (DA) neurotransmission has long been considered to underlie the disorder since most symptoms of ADHD can be alleviated by psychostimulant drugs, notably methylphenidate and amphetamines, that release DA among other actions. Increased radioligand binding to dopamine transporters (DAT) in patients with ADHD identified in recent brain imaging studies further implicates deficient DA functioning in the disorder (Dougherty et al. 1999; Dresel et al. 2000).

DA modulates physiological processes through activation of five G-protein coupled receptors classified into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) families (Neve and Neve 1997). Among these, the D_4 receptors, uniquely, have been implicated in clinical

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ADHD by genetic linkage studies (La Hoste et al. 1996). Human D_4 receptors occur in multiple forms with 2–11 copies of a 16-amino acid sequence in the putative third intracellular loop of the peptide (Van Tol et al. 1992; Lichter et al. 1993; Asghari et al. 1994). One such allele is the $D_{4.7}$ receptor, containing seven repeats of this sequence. It has repeatedly been associated with ADHD, as well as related behavioral traits such as novelty-seeking and impulsivity (Benjamin et al. 1996; Ebstein et al. 1996; La Hoste et al. 1996; Bailey et al. 1997; Rowe et al. 1998; Swanson et al. 1998; Faraone et al. 1999; Barr et al. 2000).

Some features of ADHD are simulated in several laboratory models, including: (1) rats with neonatal lesions of the central DA system induced by the neurotoxin 6-hydroxydopamine (6-OHDA; Shaywitz et al. 1976); (2) the spontaneously hypertensive Kyoto-Wistar rat (Tucker and Johnson 1981); (3) nonhuman primates treated with the DA neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Roeltgen and Schneider 1991); and (4) genetic knock-out mice lacking functional DAT (Giros et al. 1996). Juvenile male rats with neonatal 6-OHDA lesions are a particularly appropriate model for the hyperactivity of ADHD in that lesion-induced motor hyperactivity is most prominent at an age corresponding to human periadolescence (Shaywitz et al. 1976; Erinoff et al. 1979), and is dose-dependently antagonized by stimulants used to treat clinical ADHD (Heffner and Seiden 1982). In addition, the model is associated with learning deficits that are also antagonized by stimulants (Takasuna and Iwasaki 1996; Wool et al. 1987).

We found recently that motor hyperactivity following 6-OHDA lesioning of neonatal rats can be reversed dose-dependently by selective antagonists for D_4 but not D_2 receptors, and exacerbated by a D_4 agonist (Zhang et al. 2001b). In addition, motor hyperactivity correlated closely with the magnitude of increased D_4, but not D_2 receptor binding in basal forebrain. The present study further investigated the role of D_4 receptors in motor hyperactivity by studying temporal relationships between lesion effects on developmental expression of D_4 receptors and lesion-induced motor hyperactivity. In a pilot experiment, we found that lesion-induced motor hyperactivity reached peak levels at postnatal day (PD) 25, and disappeared by PD 35. Consequently, PD 25, 37 and 60 were chosen to represent three critical developmental stages: juvenile rats with motor hyperactivity (PD 25), juvenile rats lacking hyperactivity (PD 37), and rats in early adulthood (PD 60). We hypothesize that up-regulation of D_4 receptors in lesioned rats occurs selectively during the periadolescent period when hyperactivity is present, but normalizes with further maturation as motor activity of lesioned rats returns to control level.

MATERIALS AND METHODS

Radioligands and Chemicals

[^{3}H]Nemonapride (R[+]-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; 85.5 Ci/mmol) and [^{3}H]SCH-23390 (R[+]-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol; 81.4 Ci/mmol) were from New England Nuclear (NEN; Boston, MA). [^{3}H]β-CIT ([−]-2-β-carbomethoxy-3-β-[4-iodophenyl]-tropane; 64.7 Ci/mmol) was from Tocris-Cookson (Bristol, UK). Tritium-sensitive Hyperfilm, D-19 developer and fixative were from Eastman-Kodak (Rochester, NY). 1,3-Ditolylguanidine (DTG), *cis*-flupenthixol dihydrochloride, desipramine hydrochloride, 6-OHDA hydrobromide, ketanserin tartrate, S(−)-pindolol, S(−)-raclopride tartrate, and S(−)-sulpiride were from Sigma-RBI (Natick, MA). Other chemicals were from Fisher Scientific (Dallas, TX) or Sigma Chemicals (St. Louis, MO).

Neonatal Lesioning

Uses of animals were approved by the Institutional Animal Care and Use Committee (IACUC) of McLean Hospital, in compliance with applicable federal and local guidelines for ethical use of experimental animals. Sprague-Dawley rats (Charles River Labs; Wilmington, MA) were maintained under a 12/12-h artificial daylight/dark schedule (on at 7 A.M.), with free access to tap-water and standard rat chow. On PD 1, male pups were randomly assigned to lactating dams (10/dam). On PD 5, pups received a subcutaneous (s.c.) injection of desipramine hydrochloride (25 mg/kg), followed by randomized intracisternal (i.c.) injections of either 6-OHDA hydrobromide (equivalent to 100 μg free base) or vehicle (320 mM NaCl containing 0.1% ascorbic acid) under hypothermal anesthesia 60 min later (Shaywitz et al. 1976; Zhang et al. 2001b). Pups were returned to nursing dams after regaining consciousness. The extent of lesioning was verified by quantifying DAT binding autoradiographically with [^{3}H]β-CIT as a specific indicator of DA nerve terminals at the completion of behavioral experiments (Kula et al. 1999; Zhang et al. 2001b).

Behavioral Experiments

Motor activity was quantified with an infrared photobeam activity monitoring system (San Diego Instruments; San Diego, CA) controlled by a microcomputer, as detailed previously (Zhang et al. 2001b). Individual rats were tested in a novel environment in the absence of food and water (17 × 8 × 8 inch transparent plastic cages in a 4 × 8 horizontal grid of infrared beams), between 10:00 and 16:00 h on PD 24, 36, or 59. Scores were collected at 5-min intervals for 2.5 h. Locomotor activity was defined as breaking of consecutive photobeams.

DA Transporter and Receptor Autoradiography

Rats were sacrificed by rapid decapitation one day after behavioral testing. Brains were quickly removed and frozen in prechilled isopentane. Coronal sections (10 μm) were prepared in a cryostat at −17°C, thaw-mounted on gelatin-coated microscopic slides, and stored at −80°C until used in quantitative autoradiographic assays. For each assay, data from three contiguous brain sections were pooled to yield an average result for each of 9–11 subjects/experimental group.

For DAT binding assays, tissue sections were preincubated for 60 min at room temp. in 50 mM Tris-citrate buffer (pH 7.4) containing (mM): NaCl (120), and $MgCl_2$ (4). Sections were then incubated for another 60 min in fresh buffer containing 2 nM [^{3}H]β-CIT. Specific binding was defined with excess GBR-12909 (1 μM). Sections were then washed twice (5 min in ice-cold buffer), rinsed in deionized water, and air-dried.

All DA receptor binding assays were carried out at room temp. in 50 mM Tris-HCl buffer (pH 7.4) containing (mM): NaCl (120), KCl (5), $CaCl_2$ (2), and $MgCl_2$ (1). After preincubation for 60 min, brain sections were transferred to fresh buffer containing radioligand of specified concentration, and incubated for 60 min. Sections were then washed twice (5 min in ice-cold buffer), rinsed in deionized water, and air-dried.

D_1-like receptor binding was determined with 1 nM [^{3}H]SCH-23390 (Tarazi et al. 1998a; Zhang et al. 2001b). 5-$HT_{2A/2C}$ binding sites were masked with 100 nM ketanserin. Nonspecific binding was determined with 1 μM *cis*-flupenthixol. Although [^{3}H]SCH-23390 binds to both D_1 and D_5 receptors under these assay conditions, expression of D_5 receptors in rat forebrain is very limited (Meador-Woodruff et al. 1992), and the majority of the binding in brain regions examined represents D_1 receptors.

D_2-like receptor binding was assayed with 1 nM [^{3}H]nemonapride, with 0.5 μM DTG and 0.1 μM pindolol included to block σ and 5-HT_{1A} binding sites, respectively (Tarazi et al. 1998a; Zhang et al. 2001b). Nonspecific binding was determined with 10 μM S(−)-sulpiride. Although the signal includes binding to D_2, D_3, and D_4 sites, most of the binding represents D_2 sites.

D_4 receptor binding was assayed using 1 nM [^{3}H]nemonapride, with 300 nM of the D_2/D_3-selective antagonist raclopride to fully occlude D_2 and D_3 sites, as well as 0.5 μM DTG and 0.1 μM pindolol to block σ and 5-HT_{1A} binding sites (Tarazi et al. 1998a; Zhang et al. 2001b). Nonspecific binding was determined with 10 μM S(−)-sulpiride.

Dried sections were exposed to tritium-sensitive Kodak Hyperfilm for 2–6 weeks before standard photographic processing (Tarazi et al. 1998a; Zhang et al. 2001b). Radioligand binding was quantified with a computerized image analyzer (Image Research Inc.; St. Catherines, Ontario), and converted to nCi/mg tissue using [^{3}H]reference standards, with specific binding expressed as mean ± S.E.M. in fmol/mg tissue. Radioligand density was quantified in caudate-putamen (CPu), nucleus accumbens septi (NAc), and medial prefrontal cortex (mPFC) as outlined in Figure 1.

Data Analysis

Lesion effects on DA transporter and receptor density were analyzed by two-way analysis of variance (ANOVA) for overall effects of treatment in various brain regions, followed by post-hoc Dunnett's *t*-tests for planned comparisons. Two-tailed probability (*p*) of < .05 indicated statistically significant differences. Behavioral data were analyzed using Mann-Whitney non-parametric analysis.

RESULTS

Motor Activity

Neonatal 6-OHDA lesions resulted in robust spontaneous hyperactivity at PD 24 (Figure 2). Activity of lesioned rats was not different from that of sham controls for the first 10 min of testing, but declined much more
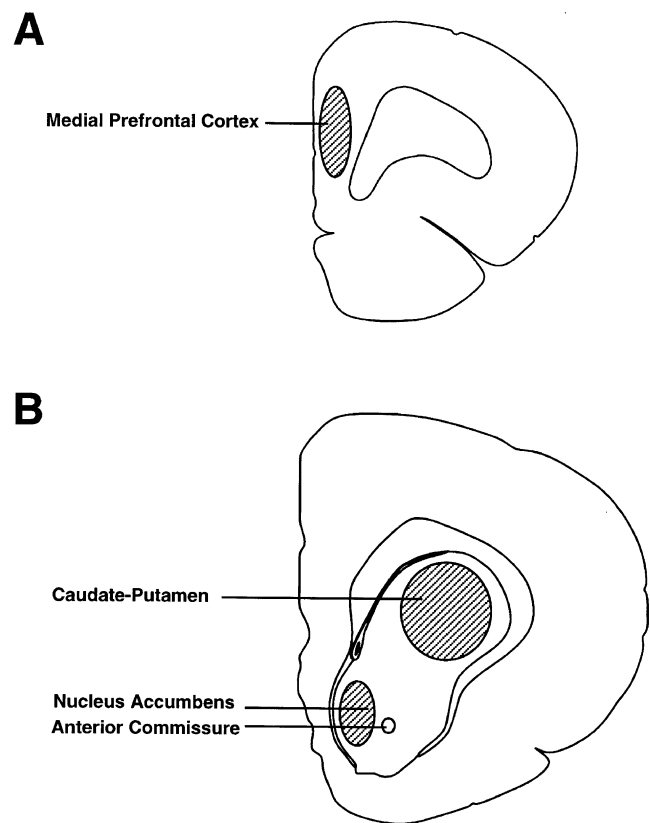

Figure 1. Schematic representation of brain regions included in autoradiographic analyses. A. 2.7–3.2 mm anterior to bregma; B. 1.2–1.6 mm anterior to bregma.
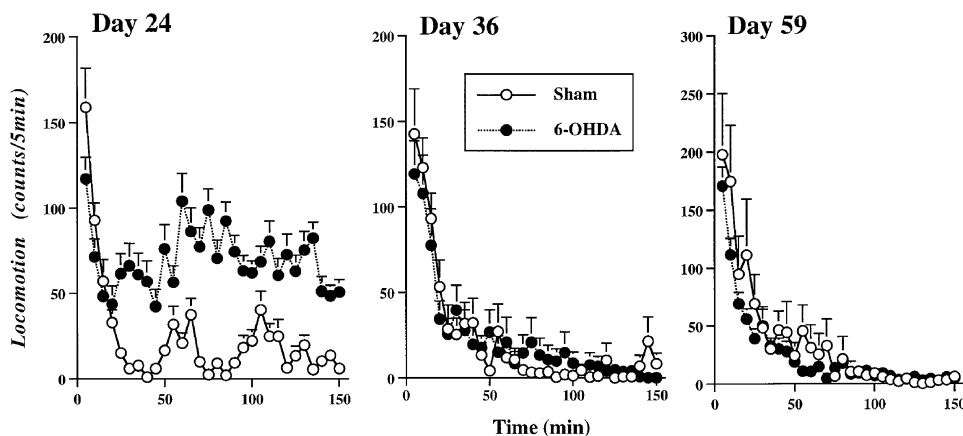
Figure 2. Effects of neonatal 6-OHDA lesioning on spontaneous locomotor activity in a novel environment at PD 24, 36, or 59. Activity (mean $\pm$ S.E.M.) were accumulated every 5 min over 2.5 h at 10:00–16:00 h in the absence of food and water (n = 9–11 rats/group).

slowly than in controls thereafter. At PD 36 and 59, neither total locomotor activity nor its distribution within 2.5 h of testing differed significantly between lesioned rats and sham controls.

DA Transporters

Neonatal 6-OHDA lesions resulted in large reductions in DAT binding in CPu and NAc (Figure 3). At PD 25, average losses of DAT binding were 80.1% in CPu and 65.7% in NAc. With further maturation, loss of DAT binding in CPu (71.2% and 70.4% at PD 37 and 60, respectively) was not statistically different from that of PD 25, whereas DAT binding in NAc recovered substantially (37.5% and 28.6% loss at PD 37 and 60; $F_{2,18\,df}$ = 15.1, $p < .01$).

D$_1$ Receptors

In sham-control rats, there were moderate and statistically significant maturational losses of D$_1$ receptor bind-

Figure 3. Effects of neonatal 6-OHDA lesions on DAT concentrations assayed with [^{3}H]β-CIT at PD 25, 37, or 60. Data are means $\pm$ S.E.M. (fmol/mg tissue) for n = 9–11 rats/group; ** $p < .01$ vs. corresponding sham-lesioned controls.

ing between PD 25 and 60 across all brain regions evaluated ($F_{2,18\,df}$ = 4.28, $p < .05$; Table 1). Neonatal 6-OHDA lesions resulted in a small decrease in D$_1$ receptor binding in CPu at PD 60 (by 8.8%; $p < .05$), but not at PD 25 or 37. D$_1$ receptor binding in NAc was unaffected by the lesions at any age. In mPFC, the lesions led to a significant increase of D$_1$ receptor binding at PD 37 (26.2%; $p < .05$), but not at PD 25 and 60.

D$_2$ Receptors

Across three brain regions examined, there were significant maturational losses of D$_2$ receptor binding in both controls ($F_{2,18\,df}$ = 30.3, $p < .001$) and lesioned rats ($F_{2,18\,df}$ = 20.4, $p < .01$; Table 1). Neonatal 6-OHDA lesions increased D$_2$ receptor binding in CPu by 20.6%, 13.2%, and 14.9% at PD 25, 37 and 60, respectively, whereas D$_2$ receptor binding in NAc and mPFC was not affected.

D$_4$ Receptors

D$_4$ receptor binding decreased significantly with maturation in all brain regions in control rats ($F_{2,18\,df}$ = 52.2; $p < .001$; Figure 4). At PD 25, neonatal 6-OHDA lesions resulted in significantly increased D$_4$ receptor binding in CPu (by 33.3%; $p < .01$), and decreased D$_4$ binding in NAc (by 43.6%; $p < .05$). Differences between lesioned and control rats were not statistically significant at later ages, when motor activity of lesioned rats returned to control levels. D$_4$ receptor binding in mPFC was not affected at any age.
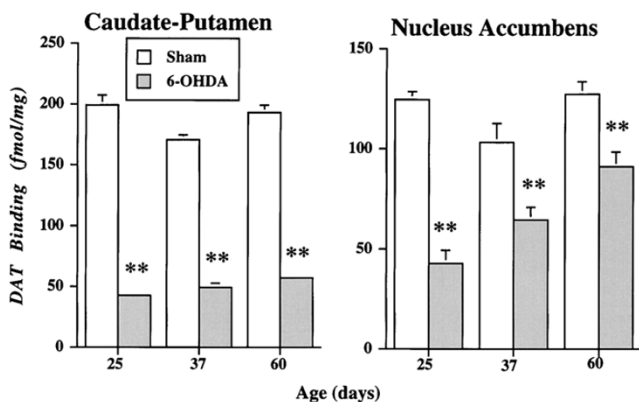
DISCUSSION

In agreement with previous reports (Shaywitz et al. 1976; Heffner and Seiden 1982; Zhang et al. 2001b), neonatal 6-OHDA lesioning of developing DA projections in rat forebrain resulted in robust motor hyperactivity. This behavioral response appears to represent deficient

Table 1. Effects of Neonatal 6-OHDA Lesions on Radioligand Binding to D_1 and D_2 Receptors in Rat Forebrain

	PD 25		PD 37		PD 60	
	Sham	6-OHDA	Sham	6-OHDA	Sham	6-OHDA
D_1 Receptor Density						
CPu	255.6 ± 20.5	265.6 ± 13.1	237.3 ± 10.0	230.3 ± 8.2	230.8 ± 4.3	**210.5 ± 4.3***
NAc	233.1 ± 21.4	214.2 ± 14.3	191.1 ± 17.9	179.9 ± 6.2	208.8 ± 5.5	94.0 ± 9.2
mPFC	48.9 ± 3.4	51.5 ± 3.1	37.3 ± 2.4	**47.1 ± 2.0***	43.8 ± 1.1	42.8 ± 2.2
D_2 Receptor Density						
CPu	222.5 ± 7.2	**268.4 ± 4.9****	166.1 ± 9.5	**188.1 ± 7.2***	155.7 ± 4.0	**178.9 ± 7.1***
NAc	108.8 ± 8.9	96.1 ± 6.6	85.7 ± 10.8	75.8 ± 6.4	81.8 ± 4.8	75.0 ± 3.5
mPFC	15.3 ± 3.4	13.4 ± 2.6	9.4 ± 2.7	8.6 ± 1.5	7.8 ± 1.5	7.7 ± 0.7

Data are specific binding, as mean fmol/mg tissue ± S.E.M. Brain regions are caudate-putamen (CPu), nucleus accumbens septi (NAc), and medial prefrontal cerebral cortex (mPFC).

*By ANOVA: $p < .05$, significantly different (boldface) from sham-lesioned control littermates (N = 9–11).

**By ANOVA: $p < .01$, significantly different (boldface) from sham-lesioned control littermates (N = 9–11).

habituation to a novel environment, since motor activity in the initial testing period did not differ appreciably between lesioned rats and sham controls (Figure 2, PD 24). Also in accord with previous studies (Shaywitz et al. 1976; Erinoff et al. 1979), lesion-induced motor hyperactivity was evident only during early development (PD 24 in this study), and no longer present at PD 36 or 59 (Figure 2).

Neonatal 6-OHDA lesions resulted in a substantial and sustained decrease in DAT binding in CPu. In contrast, loss of DAT binding in NAc (66%) at PD 25 was less than that in CPu (80%; $p < .05$), and recovered substantially at later ages (29% loss at PD 60). Recovery of DAT binding in NAc was temporally paralleled by normalization of motor behavior with maturation. The mesolimbic DA pathway to NAc plays an important role in exploratory behavior (Le Moal and Simon 1991), including in 6-OHDA lesion-induced hyperactivity (Heffner et al. 1983). Therefore, it is possible that postlesioning plasticity involving DA neurotransmission in NAc may contribute to normalization of motor activity in early adulthood.

Developmental studies on DA innervation to NAc after site-specific injection of 6-OHDA into CPu have yielded contradictory results. Whereas gradual recovery of tyrosine hydroxylase (TH) immunoreactivity has been reported (Frohna et al. 1997), slowly progressive loss of DA content was noted after more complete lesions (Teicher et al. 1998). Recovery of DAT levels in NAc at PD 37 and 60 in the present study (Figure 3) may indicate repair or regrowth of DA projections from ventral tegmental area to NAc. Parallel developmental studies of DA tissue content, extracellular DA concentration, and density of DA terminals after 6-OHDA lesioning of neonates might further clarify adaptive changes in DA innervation to the NAc after varying degree of lesioning.

D_1 receptors in neostriatum have been reported, inconsistently, to be unaffected by moderate or severe neonatal 6-OHDA lesions (Dewar et al. 1990; Caboche et al. 1991; Radja et al. 1993; Frohna et al. 1995; Zhang et al. 2001b), or decreased by nearly complete lesions (Gelbard et al. 1989). This inconsistency may reflect differences in the extent or timing of lesioning or sampling.
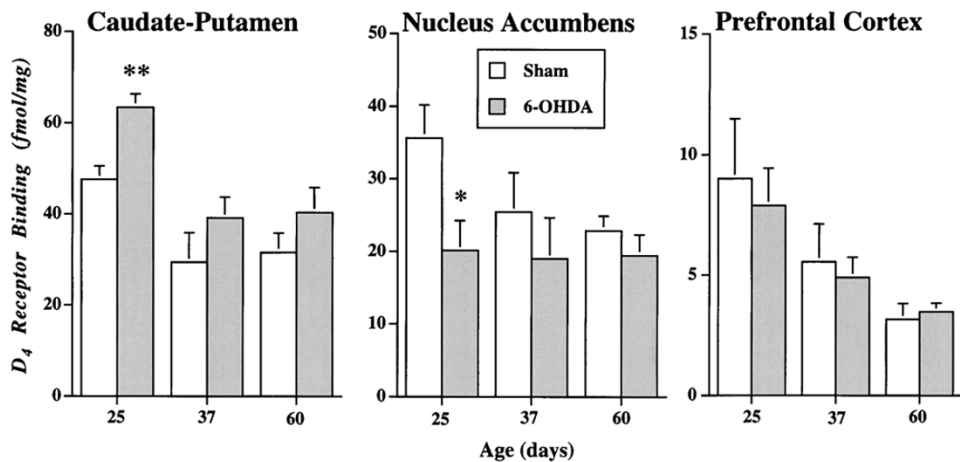

Figure 4. Effects of neonatal 6-OHDA lesioning on D_4 receptor levels (fmol/mg tissue) assayed at PD 25, 37, or 60 with [^{3}H]nemonapride in the presence of raclopride to occlude D_2 and D_3 sites. Data are means ± S.E.M. for 9–11 rats/group; *$p < .05$, **$p < .01$ vs. corresponding sham-lesioned controls.

In the present study, we found a small, but statistically significant late decrease in D_1 receptor binding in CPu at PD 60, but not PD 25 or 37 (Table 1). The timing of this change does not readily account for motor hyperactivity that was present only at PD 25. We also found a significant increase in D_1 receptor binding in mPFC at PD 37 (Table 1). In view of the proposed role of frontal cortex in self-injurious behavior that follows the lesioning, this relatively late increase in D_1 expression may contribute to self-injurious behavior induced by D_1 agonists that is particularly prominent in rats at such later ages following neonatal 6-OHDA lesions (Breese et al. 1984, 1985; Cromwell et al. 1999).

D_2 receptors in neostriatum have been found to be unchanged or slightly increased by neonatal 6-OHDA lesions (Breese et al. 1987; Dewar et al. 1990; Radja et al. 1993; Frohna et al. 1995; Zhang et al. 2001b). We found that such lesions resulted in small increases of D_2 receptor binding in CPu at all developmental stages evaluated, regardless of whether motor hyperactivity was present or not (Table 1). A lack of temporal correlation between D_2 receptor changes and motor hyperactivity, together with previous pharmacological evidence that D_2-selective antagonists do not affect lesion-induced hyperactivity (Heffner and Seiden 1982; Zhang et al. 2001b), suggest that D_2 receptors are not critically involved in the hyperactivity.

Consistent with our earlier observation of maturational pruning of D_4 receptors at puberty (Tarazi et al. 1998b), D_4 receptor binding in CPu, NAc, and mPFC of sham-control rats was highest at PD 25, and decreased significantly with further maturation (Figure 4). After lesioning at PD 25, D_4 receptor binding was significantly increased in the CPu, whereas in the NAc, D_4 receptor binding was substantially decreased. The extent of these changes (33% increase in CPu and 44% decrease in NAc) was much greater than the observed upregulation of D_2 receptors. More importantly, changes in D_4 receptors were detected only when lesioned rats exhibited hyperactivity (PD 25), and not with further maturation (PD 37 and 60) when motor activity of lesioned rats had returned to control levels. In contrast to the temporary effects of neonatal 6-OHDA lesions on D_4 receptors, effects of such lesions in adult rats follow a different pattern. Selective destruction of nigrostriatal DA projections in adult rats increased D_4 receptor binding in CPu at five weeks but not one week after lesioning (Tarazi et al. 1998a; Zhang et al. 2001a), suggesting mechanisms for D_4 receptor regulation differ at specific developmental stages.

Previously, we found no differences in D_4 receptor binding in NAc between sham-control and lesioned rats using procedures identical to those employed in the present study (Zhang et al. 2001b). These seemingly inconsistent results may reflect antemortem exposure to D_4-selective drugs in the previous study. Additional experiments are needed to verify the potential effects of such drug exposure.

To recapitulate, DA receptor subtypes were found to be

differentially regulated in response to neonatal 6-OHDA lesions of rat forebrain DA systems. Close examination of these changes in the context of motor hyperactivity revealed that, in addition to plasticity of presynaptic DA terminals in NAc, changes of D_4 receptors in CPu or NAc are also likely to be involved in motor hyperactivity in lesioned rats. In view of our previous finding that lesion-induced motor hyperactivity was reversed by D_4-selective antagonists, the present results further suggest a pivotal role of D_4 receptors.

Mechanisms by which D_4 receptor plasticity may contribute to motor hyperactivity remain obscure, particularly due to limited information about the physiological role of these receptors (Tarazi and Baldessarini 1999; Oak et al. 2000). PFC is likely to be a crucial site due to its relative abundance of D_4 receptors (Mrzljak et al. 1996; Ariano et al. 1997; Tarazi et al. 1998a; De la Garza II and Madras 2000). Moreover, this brain region is proposed to be critically involved in the pathophysiology of ADHD, based on clinical neuropsychological and functional brain imaging studies (Barkley et al. 1992; Ernst and Zametkin 1995; Barkley 1997). In the present study, we did not find significant changes in D_4 receptor binding in mPFC (Figure 4). However, altered coupling of D_4 receptors to G-proteins, or other downstream molecular events require further consideration.

D_4 receptors are proposed to be present on glutamatergic terminals of corticostriatal projections from mPFC to CPu, based on studies of cortical ablation (Tarazi et al. 1998a). Glutamatergic efferents from mPFC are important in limiting behavioral responses to various stimuli (Bubser and Schmidt 1990; Flores et al. 1996; Wilkinson et al. 1997; Lacroix et al. 1998). Upregulation of D_4 receptors in CPu at PD 25 after lesioning (Figure 4), therefore, may contribute to behavioral hyperactivity, indirectly, by enhancing behaviorally inhibitory descending influences of cortex on lower limbic-motor centers.

The seemingly opposite effects of neonatal 6-OHDA lesions on D_4 receptors in NAc vs. CPu is particularly intriguing. D_4 receptors may reside on glutamatergic terminals in CPu (Tarazi et al. 1998a), but a subset of D_4 receptors in NAc has been localized to DA terminals (Svingos et al. 2000). Decreased D_4 receptor binding in NAc in 6-OHDA lesioned rats may represent loss of these presynaptic sites, perhaps leading to an increase in DA release from its remaining terminals. However, the precise manner in which adaptive changes in both CPu and NAc contribute to the age-limited expression of behavioral hyperactivity remains to be further clarified. Nonetheless, the striking developmental parallels between the behavioral hyperactivity and changes of D_4 expression in CPu and NAc after neonatal lesions suggest their involvement in hyperactivity and behavioral responses of these rats to D_4-selective agents.

In conclusion, we found that DA receptor subtypes in rat forebrain were differentially affected by removing

DA projections to basal forebrain during early postnatal development. Of the three DA receptors examined, changes in D_4 receptors (time-limited increases in CPu, losses in NAc) were much larger than those of D_1 or D_2 receptors. Moreover, behavioral hyperactivity most closely paralleled the temporal pattern of D_4 receptor expression in basal forebrain after the lesions, as D_4 receptor changes in both CPu and NAc were maximal at PD 25. These relationships add support to the hypothesis that D_4 receptors are involved in motor hyperactivity that follows neonatal lesions of DA neurons with 6-OHDA in rats, and potentially also in clinical ADHD.

ACKNOWLEDGMENTS

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