

Plasticity of Dopamine D₄ Receptors in Rat Forebrain: Temporal Association with Motor Hyperactivity Following Neonatal 6-Hydroxydopamine Lesioning

Kehong Zhang, M.D., Ph.D., Frank I. Tarazi, Ph.D., Eugen Davids, M.D., and Ross J. Baldessarini, M.D.

Genetic studies suggest that dopamine D₄ 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 D₄ receptor-selective antagonists. In this study, effects of such lesions on D₄ as well as other dopamine receptors (D₁ and D₂) 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, D₄ 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 D₁ and D₂ receptor binding in various forebrain regions. However, the time-course of lesion-induced motor hyperactivity correlated only with changes in D₄, but not D₁ and D₂ receptors. These results further support the hypothesis that D₄ 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.

KEY WORDS: Attention deficit hyperactivity disorder (ADHD); Development; Dopamine D₄ receptors; 6-Hydroxydopamine; Motor hyperactivity; Neonatal lesions

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 D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, and D₄) families (Neve and Neve 1997). Among these, the D₄ receptors, uniquely, have been implicated in clinical

From the Mailman Research Center, McLean Division of Massachusetts General Hospital, 115 Mill Street, Belmont, MA 02478, USA, and Department of Psychiatry and Neuroscience Program, Harvard Medical School, Boston, MA 02115, USA.

Address correspondence to: Kehong Zhang, M.D., Ph.D., Mailman Research Center, McLean Hospital, 115 Mill Street, Belmont, MA 02478, Tel.: (617) 855-3222, Fax (617) 855-3479, E-mail: kz@mclean.harvard.edu

Received June 14, 2001; revised September 21, 2001; accepted October 29, 2001.

Online publication: 11/6/01 at www.acnp.org/citations/Npp110601201.

ADHD by genetic linkage studies (La Hoste et al. 1996). Human D₄ 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 D₄ but not D₂ receptors, and exacerbated by a D₄ agonist (Zhang et al. 2001b). In addition, motor hyperactivity correlated closely with the magnitude of increased D₄, but not D₂ receptor binding in basal forebrain. The present study further investigated the role of D₄ receptors in motor hyperactivity by studying temporal relationships between lesion effects on developmental expression of D₄ 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 D₄ 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

[³H]Nemonapride (R[+]-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; 85.5 Ci/mmol) and [³H]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). [³H]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 [³H]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 photo-beam 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 μ m) 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 [^3H] 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.

D₁-like receptor binding was determined with 1 nM [^3H]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 [^3H]SCH-23390 binds to both D₁ and D₅ receptors under these assay conditions, expression of D₅ receptors in rat forebrain is very limited (Meador-Woodruff et al. 1992), and the majority of the binding in brain regions examined represents D₁ receptors.

D₂-like receptor binding was assayed with 1 nM [^3H]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 D₂, D₃, and D₄ sites, most of the binding represents D₂ sites.

D₄ receptor binding was assayed using 1 nM [^3H]nemonapride, with 300 nM of the D₂/D₃-selective antagonist raclopride to fully occlude D₂ and D₃ 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. Catharines, Ontario), and converted to nCi/mg tissue using [^3H]reference standards, with specific binding ex-

pressed as mean \pm 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

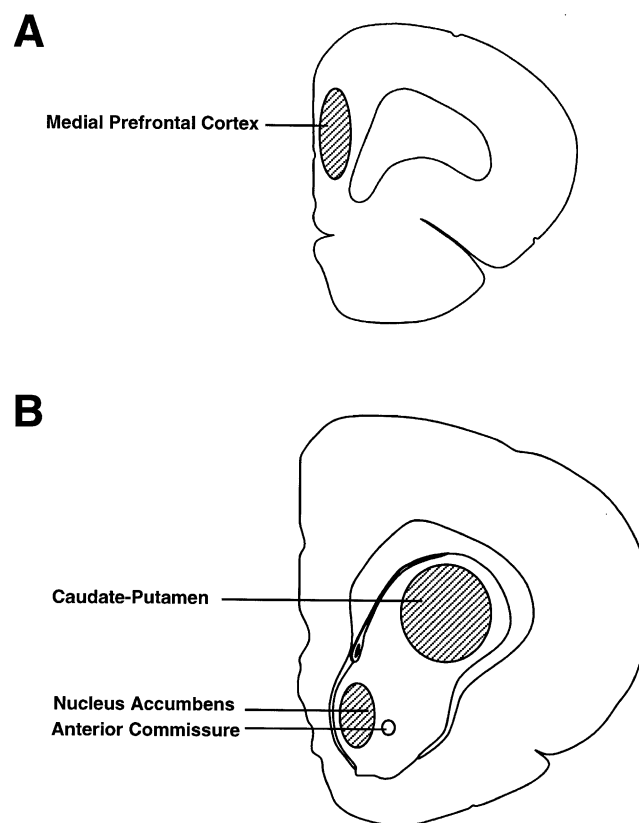


Figure 1. Schematic representation of brain regions included in autoradiographic analyses. A. 2.7–3.2 mm anterior to bregma; B. 1.2–1.6 mm anterior to bregma.

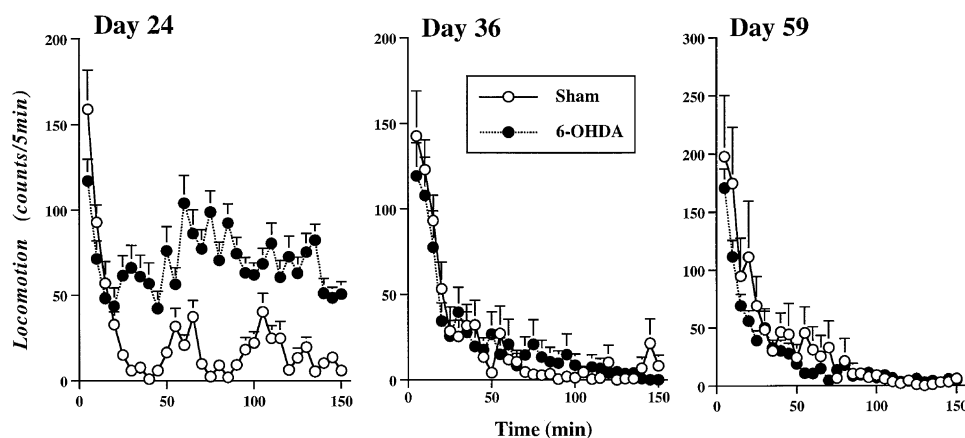


Figure 2. Effects of neonatal 6-OHDA lesioning on spontaneous locomotor activity in a novel environment at PD 24, 36, or 59. Activity (mean \pm S.E.M.) were accumulated every 5 min over 2.5 h at 10:00–16:00 h in the absence of food and water ($n = 9$ –11 rats/group).

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; $F_{2,18\text{ df}} = 15.1$, $p < .01$).

D₁ Receptors

In sham-control rats, there were moderate and statistically significant maturational losses of D₁ receptor bind-

ing between PD 25 and 60 across all brain regions evaluated ($F_{2,18\text{ df}} = 4.28$, $p < .05$; Table 1). Neonatal 6-OHDA lesions resulted in a small decrease in D₁ receptor binding in CPu at PD 60 (by 8.8%; $p < .05$), but not at PD 25 or 37. D₁ receptor binding in NAc was unaffected by the lesions at any age. In mPFC, the lesions led to a significant increase of D₁ receptor binding at PD 37 (26.2%; $p < .05$), but not at PD 25 and 60.

D₂ Receptors

Across three brain regions examined, there were significant maturational losses of D₂ receptor binding in both controls ($F_{2,18\text{ df}} = 30.3$, $p < .001$) and lesioned rats ($F_{2,18\text{ df}} = 20.4$, $p < .01$; Table 1). Neonatal 6-OHDA lesions increased D₂ receptor binding in CPu by 20.6%, 13.2%, and 14.9% at PD 25, 37 and 60, respectively, whereas D₂ receptor binding in NAc and mPFC was not affected.

D₄ Receptors

D₄ receptor binding decreased significantly with maturation in all brain regions in control rats ($F_{2,18\text{ df}} = 52.2$; $p < .001$; Figure 4). At PD 25, neonatal 6-OHDA lesions resulted in significantly increased D₄ receptor binding in CPu (by 33.3%; $p < .01$), and decreased D₄ 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. D₄ receptor binding in mPFC was not affected at any age.

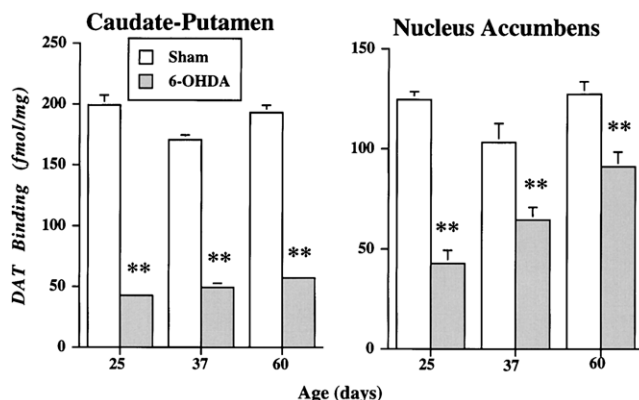


Figure 3. Effects of neonatal 6-OHDA lesions on DAT concentrations assayed with [³H]β-CIT at PD 25, 37, or 60. Data are means \pm S.E.M. (fmol/mg tissue) for $n = 9$ –11 rats/group; ** $p < .01$ vs. corresponding sham-lesioned controls.

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 D₁ and D₂ 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 (Hefner et al. 1983). Therefore, it is possible that post-lesioning 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.

D₁ 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.

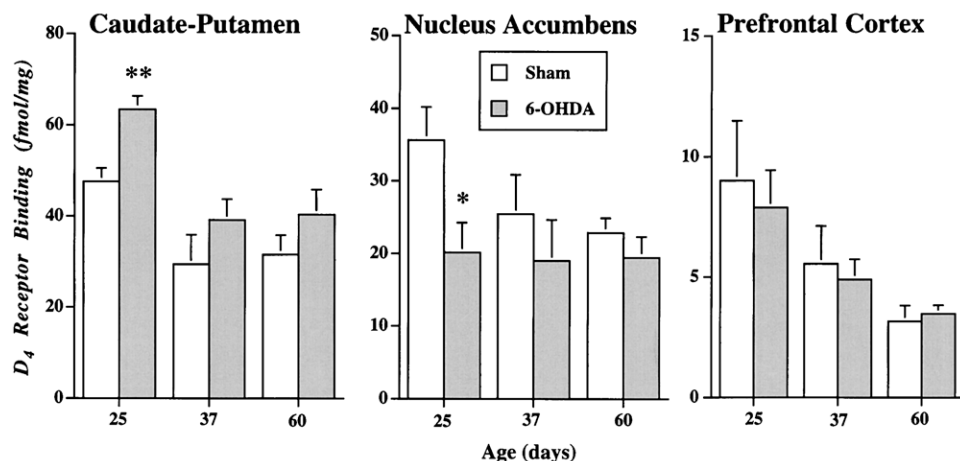


Figure 4. Effects of neonatal 6-OHDA lesioning on D₄ receptor levels (fmol/mg tissue) assayed at PD 25, 37, or 60 with [³H]nemonapride in the presence of raclopride to occlude D₂ and D₃ sites. Data are means ± S.E.M. for 9–11 rats/group; * $p < .05$, ** $p < .01$ vs. corresponding sham-lesioned controls.

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 (Hefner 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 D₄ receptors (time-limited increases in CPU, losses in NAc) were much larger than those of D₁ or D₂ receptors. Moreover, behavioral hyperactivity most closely paralleled the temporal pattern of D₄ receptor expression in basal forebrain after the lesions, as D₄ receptor changes in both CPU and NAc were maximal at PD 25. These relationships add support to the hypothesis that D₄ 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

This work was supported by NIH grants MH-34006 (RJB), MH-47370 (RJB), a grant from the Bruce J. Anderson Foundation (RJB), the McLean Hospital Private Donors Neuropsychopharmacology Research Fund (RJB), NARSAD Young Investigator Award (FIT), the Deutsche Forschungsgemeinschaft (DA 516/1-1; ED), and the Livingston Fellowship from Harvard Medical School (KZ).

REFERENCES

- Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR (1997): Cellular distribution of the rat D₄ dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res* 752:26–34
- Asghari V, Schoots O, Van Kats S, Ohara K, Jovanovic V, Guan H-G, Bunzow JR, Petronis A, Van Tol HHM (1994): Dopamine D₄ receptor repeat: Analysis of different native and mutant forms of the human and rat genes. *Mol Pharmacol* 46:364–373
- Bailey HN, Palmer CG, Ramsey C, Cantwell D, Kim K, Woodward JA, McGough J, Asarnow RF, Nelson S, Smalley SL (1997): DRD4 gene and susceptibility to attention deficit hyperactivity disorder. *Am J Med Genet Neuropsychiat Genet* 74:623–624
- Barkley RA (1990): *Attention Deficit Hyperactivity Disorder: A Handbook For Diagnosis And Treatment*. New York: Guilford Press
- Barkley RA (1997): Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychol Bull* 121:65–94
- Barkley RA, Grodzinsky G, DuPaul GJ (1992): Frontal lobe functions in attention deficit disorder with and without hyperactivity: a review and research report. *J Abnorm Child Psychol* 20:163–188
- Barr CL, Wigg K, Bloom S, Schachar R, Tannock R, Roberts W, Malone M, Vandenberg DJ, Kennedy JL (2000): Further evidence from haplotype analysis for linkage to the dopamine D₄ receptor gene and attention-deficit hyperactivity disorder. *Am J Med Genetics* 96:262–267
- Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH (1996): Population and familial association between D₄ dopamine receptor gene and measures of novelty-seeking. *Nat Genet* 12:81–84
- Breese GR, Baumeister AA, McCown TJ, Emerick SG, Frye GD, Crotty K, Mueller RA (1984): Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. *J Pharmacol Exp Ther* 231:343–354
- Breese GR, Baumeister AA, Napier TC, Frye GD, Mueller RA (1985): Evidence that D₁ dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxy-phenylalanine in rats treated neonatally with 6-hydroxydopamine. *J Pharmacol Exp Ther* 235:287–295
- Breese GR, Duncan GE, Napier TC, Bondy SC, Iorio LC, Mueller RA (1987): 6-Hydroxydopamine treatments enhance behavioral responses to intracerebral microinjection of D₁ and D₂-dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonists binding. *J Pharmacol Exp Ther* 240:167–191
- Bubser M, Schmidt WJ (1990): 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behav Brain Res* 37:157–168
- Caboche J, Rogard M, Besson MJ (1991): Comparative development of D₁-dopamine and μ opiate receptors in normal and in 6-hydroxydopamine-lesioned neonatal rat striatum: Dopaminergic fibers regulate μ but not D₁ receptor distribution. *Dev Brain Res* 58:111–122
- Cromwell HC, Levine MS, King BH (1999): Cortical damage enhances pemoline-induced self-injurious behavior in prepubertal rats. *Pharmacol Biochem Behav* 62:223–227
- De la Garza II R, Madras BK (2000): [³H]PNU-101958, a D₄ dopamine receptor probe, accumulates in prefrontal cortex and hippocampus of non-human primate brain. *Synapse* 37:232–244
- Dewar KM, Soghomonian JJ, Bruno JP, Descarries L, Reader TA (1990): Elevation of dopamine D₂ but not D₁ receptors in adult rat striatum after neonatal 6-hydroxydopamine denervation. *Brain Res* 536:287–297
- Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fishman AJ (1999): Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet* 354:2132–2133
- Dresel S, Krause J, Krause KH, La Fougere C, Brinkbaumer K, Kung HF, Hahn K, Tatsch K (2000): Attention deficit hyperactivity disorder: binding of [^{99m}Tc]TRODAT-1 to the dopamine transporter before and after methylphenidate treatment. *Eur J Nucl Med* 27:1518–1524
- Erstein RP, Novik O, Umansky R, Priel B, Osher Y, Blaine D, Bennett ER, Nemanov L, Katz M, Belmaker RH (1996): Dopamine D₄ receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. *Nat Genet* 12:78–80
- Erinoff L, MacPhail RC, Heller A, Seiden LS (1979): Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. *Brain Res* 164:195–205
- Ernst M, Zametkin A (1995): The interface of genetics, neuroimaging, and neurochemistry in attention-deficit hyperactivity disorder. In Bloom FE, Kupfer DJ (eds),

- Psychopharmacology: The Fourth Generation Of Progress. New York: Raven Press, pp. 1643–1652
- Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M, Sakai J (1999): Dopamine D₄ gene 7-repeat allele and attention deficit hyperactivity disorder. *Am J Psychiatry* 156:768–770
- Flores G, Wood GK, Liang JJ, Quirion R, Srivastava LK (1996): Enhanced amphetamine sensitivity and increased dopamine D₂ receptors in postpubertal rats after neonatal excitotoxic lesions of the medial prefrontal cortex. *J Neurosci* 16:7366–7375
- Frohna PA, Neal-Beliveau BS, Joyce JN (1995): Neonatal 6-hydroxydopamine lesions lead to opposing changes in the levels of dopamine receptors and their messenger RNAs. *Neuroscience* 68:505–518
- Frohna PA, Neal-Beliveau BS, Joyce JN (1997): Delayed plasticity of the mesolimbic dopamine system following neonatal 6-OHDA lesions. *Synapse* 25:293–305
- Gelbard HA, Teicher MH, Baldessarini RJ, Gallitano AL, Marsh ER, Zorc J, Faedda G (1989): Dopamine D₁ receptor development depends on endogenous dopamine. *Dev Brain Res* 56:137–140
- Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996): Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 379:606–612
- Heffner TG, Heller A, Miller FE, Kotake C, Seiden LS (1983): Locomotor hyperactivity in neonatal rats following electrolytic lesions of mesocortical dopamine neurons. *Dev Brain Res* 9:29–37
- Heffner TG, Seiden LS (1982): Possible involvement of serotonergic neurons in the reduction of locomotor hyperactivity caused by amphetamine in neonatal rats depleted of brain dopamine. *Brain Res* 244:81–90
- Kula NS, Baldessarini RJ, Tarazi FI, Fisser R, Wang S, Trometer J, Neumeyer JL (1999): [³H]β-CIT: a radioligand for dopamine transporter in rat brain tissue. *Eur J Pharmacol* 385:291–294
- La Hoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL (1996): Dopamine D₄ receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol Psychiatry* 1:128–131
- Lacroix L, Broersen LM, Weiner I, Feldon J (1998): The effects of excitotoxic lesion of the medial prefrontal cortex on latent inhibition, prepulse inhibition, food hoarding, elevated plus maze, activity avoidance and locomotor activity in the rat. *Neuroscience* 82:431–442
- Le Moal M, Simon H (1991): Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol Rev* 71:155–234
- Lichter JB, Barr CL, Kennedy JL, Van Tol HHM, Kidd KK, Livak KJ (1993): A hypervariable segment in the human dopamine receptor D₄ (DRD4) gene. *Hum Mol Genet* 6:767–773
- Meador-Woodruff JH, Mansour A, Grandy DK, Damask SP, Civelli O, Watson SJ Jr (1992): Distribution of D₅ dopamine receptor mRNA in rat brain. *Neurosci Lett* 145:209–212
- Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS (1996): Localization of dopamine D₄ receptors in GABAergic neurons of the primate brain. *Nature* 381:245–248
- Neve KA, Neve RL (1997): Molecular biology of dopamine receptors. In Neve KA, Neve RL (eds), *The Dopamine Receptors*. Totowa, New Jersey: Humana Press. pp. 27–76
- Oak JN, Oldenhof J, Van Tol HHM (2000): The dopamine D₄ receptor: One decade of research. *Eur J Pharmacol* 405:303–327
- Radja FR, El Mansari M, Soghomonian JJ, Dewar KM, Ferron A, Reader TA, Descarries L (1993): Changes of D₁ and D₂ receptors in adult rat neostriatum after neonatal dopamine denervation: quantitative data from ligand binding, in situ hybridization and iontophoresis. *Neuroscience* 57:635–648
- Roeltgen DP, Schneider JS (1991): Chronic low-dose MPTP in nonhuman primates: a possible model for attention deficit disorder. *J Child Neurol* 6:S82–S89
- Rowe DC, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, Terris ST, Mohr JH, Sherman S, Abramowitz A, Waldman ID (1998): Dopamine DRD4 receptor polymorphism and attention-deficit hyperactivity disorder. *Mol Psychiatry* 3:419–426
- Shaywitz RA, Yager RD, Klopfer JH (1976): Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. *Science* 191:305–308
- Svingos AL, Periasamy S, Pickel VM (2000): Presynaptic dopamine D₄ receptor localization in the rat nucleus accumbens shell. *Synapse* 36:222–232
- Swanson JM, Sunahara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, La Hoste GJ, Wigal S (1998): Association of the dopamine receptor D₄ (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD). *Mol Psychiatry* 3:38–41
- Tarazi FI, Campbell A, Yeghiayan SK, Baldessarini RJ (1998a): Localization of dopamine receptor subtypes in caudate-putamen and nucleus accumbens septi of rat brain: Comparison of D₁-, D₂-, and D₄-like receptors. *Neuroscience* 83:169–176
- Tarazi FI, Tomasini EC, Baldessarini RJ (1998b): Postnatal development of D₄-like receptors in rat forebrain: Comparison with D₂-like receptors. *Dev Brain Res* 110:227–233
- Tarazi FI, Baldessarini RJ (1999): Dopamine D₄ receptors: significance for molecular psychiatry at the millenium. *Mol Psychiatry* 4:529–538
- Takasuna M, Iwasaki T (1996): Active and passive avoidance learning in rats neonatally treated with intraventricular 6-hydroxydopamine. *Behav Brain Res* 74:119–126
- Teicher MH, Anderson SL, Campbell A, Gelbard HA, Baldessarini RJ (1998): Progressive accumbens degeneration after neonatal striatal 6-hydroxydopamine in rats. *Neurosci Lett* 247:99–102
- Tucker DC, Johnson AK (1981): Behavioral correlates of spontaneous hypertension. *Neurosci Biobehav Rev* 5:463–471
- Van Tol HHM, Wu CM, Guan H-C, Ohara K, Bunzow JR,

- Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V (1992): Multiple dopamine D₄ receptor variants in the human population. *Nature* 358:149–152
- Wilkinson LS, Dias R, Thomas KL, Augood SJ, Everitt BJ, Robbins TW, Roberts AC (1997): Contrasting effects of excitotoxic lesions of the prefrontal cortex on the behavioral response to d-amphetamine and presynaptic and postsynaptic measures of striatal dopamine function in monkeys. *Neuroscience* 80:717–730
- Wool RS, Weldon DA, Shaywitz BA, Anderson GM, Cohen DJ, Teicher MH (1987): Amphetamine reverses learning deficits in 6-hydroxydopamine-treated rat pups. *Dev Psychobiol* 20:219–232
- Zhang K, Tarazi FI, Baldessarini RJ (2001a): Dopamine denervation enhances D₄ receptor binding in rat caudate-putamen. *Pharmacol Biochem Behav* 69:111–116
- Zhang K, Tarazi FI, Baldessarini RJ (2001b): Role of dopamine D₄ receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats. *Neuropsychopharmacology* (in press)