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# Medial-to-Lateral Gradient of Neostriatal NGF Receptors: Relationship to Cholinergic Neurons and NGF-like Immunoreactivity

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**High-affinity binding sites for recombinant human NGF (rhNGF) were studied in the caudate-putamen of the adult rat and rabbit. Displaceable $^{125}$I-rhNGF binding sites were densely distributed throughout the caudate-putamen and were 2–3-fold more prevalant in the ventrolateral and lateral than in the medial caudate-putamen. The amount of nondisplaceable binding did not vary throughout the caudate-putamen. The medial-to-lateral receptor gradient was correlated ($r = +0.99$) with a 2–3-fold medial-to-lateral increase in ChAT activity. In contrast, NGF-like immunoreactivity (NGF-LI) was prevalent but uniformly distributed in the caudate-putamen. Lesions of intrinsic cholinergic neurons by quinolinic acid produced extensive gliosis in the medial, central, and lateral caudate-putamen, yet $^{125}$I-rhNGF binding was decreased in each of these regions. The activity of ChAT and $^{125}$I-rhNGF binding throughout the caudate-putamen were each decreased by 40% following quinolinic acid. Binding was not changed after 70–77% dopamine nerve terminal depletions induced by 6-hydroxydopamine, demonstrating a nonglial, nondopaminergic locus for striatal NGF binding sites. The cholinergiclike topography of NGF binding sites throughout the intact caudate-putamen, the parallel decreases of cholinergic neurons and NGF binding sites following intrinsic neuronal loss, and the uniform neostriatal gradient of NGF-LI are consistent with the trophic role of endogenous NGF for cholinergic interneurons of the caudate-putamen.**

Recent studies have revealed a trophic role of NGF for cholinergic interneurons of the caudate-putamen of neonatal rats (Mobley et al., 1985, 1989; Aloe, 1987; Vantini et al., 1989) and adult rats (Gage et al., 1989; Hagg et al., 1989). These studies are consistent with the presence of NGF protein (Korsching et al., 1985) and mRNA (Shelton and Reichardt, 1986) in the neostriatum. However, the topography, neuronal locus, and even the presence of binding sites for NGF in the neostriatum have been unclear. A mouse monoclonal antibody, 192 IgG, recognizes both the low- and the high-affinity forms of the NGF receptor. In most studies, these sites were not found in the rat or human caudate-putamen (Hefti et al., 1986; Taniuchi et al., 1986; Batchelor et al., 1988; Pioro and Cuello, 1988; Schatteman et al., 1988; Gage et al., 1989; Gomez-Pinilla et al., 1989; Hefti and Mash, 1989). A few immunostaining studies identified neostriatal NGF receptors but only in the ventrolateral rat caudate-putamen (Kordower et al., 1988; Gibbs et al., 1989; Kiss and Patel, 1989; Woolf et al., 1989) or a reduced number of sites in the medial caudate-putamen (Pioro and Cuello, 1990). These NGF-receptor-positive neurons are colocalized with a small proportion of neurons that contain the definitive ACh neuronal marker ChAT (Kordower et al., 1988; Kiss and Patel, 1989; Woolf et al., 1989).

The binding of iodinated murine NGF ($^{125}$I-muNGF) to a high-affinity site has also been detected in homogenates of rat neostriatum (Riopelle et al., 1987; Mobley et al., 1989). The anatomical mapping of these sites has been achieved with tissue sections labeled with $^{125}$I-muNGF, washed in ethanol and organic solvents, and immersed in a liquid photoemulsion coating (Richardson et al., 1986; Raivich and Kreutzberg, 1987; Riopelle et al., 1987). This procedure has identified sparse populations of high-affinity NGF binding sites that are uniformly distributed in the neostriatum (Richardson et al., 1986; Raivich and Kreutzberg, 1987). Although $^{125}$I-muNGF binding sites measured in a small portion of the neostriatum are colocalized with cholinergic neurons (Riopelle et al., 1987), the uniform distribution of muNGF binding sites is not consistent with the 2–3-fold medial-to-lateral gradient for cholinergic markers in the neostriatum (Guyenet et al., 1977; Rea and Simon, 1981; Marien et al., 1987). Emulsion autoradiography with muNGF may not have detected such a gradient for a variety of technical reasons.

We have used $^{125}$I-labeled recombinant human NGF ($^{125}$I-rhNGF) and quantitative dry-film autoradiography with computerized image analysis to localize high-affinity NGF binding sites throughout the rat brain (Altar et al., 1991). These procedures avoid the losses of bound ligand that typically occur with the ethanol and organic solvent washes used with emulsion autoradiography (Kuhar and Unnerstall, 1982). In contrast to prior immunohistochemistry or emulsion autoradiography studies, a dense population of saturable $^{125}$I-rhNGF binding sites was found in the neostriatum (Altar et al., 1991). These sites bound rhNGF with high and equal affinity in the lateral and medial thirds of the neostriatum ($K_d$ values of 52 and 70 pM, respectively), and the maximal density ($B_{max}$) of $^{125}$I-rhNGF sites was about 2-fold greater in the lateral than in the medial caudate-putamen. The present study quantified the topography of NGF binding sites throughout the intact neostriatum and their relationship to ChAT and NGF-like immunoreactivity (NGF-LI). The response of NGF receptors and cholinergic neurons to neurotoxin-induced degeneration of striatal interneurons or dopamine nerve terminals was also investigated.

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## Materials and Methods

*Animals and surgical treatments.* Male Sprague–Dawley rats (2–3 months of age) were provided by Simonsen (Gilroy, CA). Six animals received a left intrastriatal injection of 150 nmol quinolinic acid dissolved in 2 μl phosphate-buffered saline (PBS) at a pH of 7.3 (Schwarcz et al., 1983) infused 0.5 mm caudal to the bregma suture, 3.5 mm lateral to the midline, and 6.2 mm ventral to the surface of the skull. An additional 12 animals received an infusion into the left central caudate-putamen of 1.5 μl of the sterile 0.9% saline vehicle containing 0.1% ascorbic acid or an infusion into the left caudate-putamen of 25 μg 6-hydroxydopamine dissolved in the vehicle (all reagents from Sigma Chemical Co., St. Louis, MO, unless indicated otherwise). The coordinates for the 6-hydroxydopamine injection were 0.5 mm rostral to bregma, 3 mm lateral to the midline suture, and 6.2 mm ventral to the skull surface. Animals were allowed to survive for 1 week (quinolinic acid) or 1 or 4 weeks (6-hydroxydopamine).

Intact or surgically operated rats or 2 New Zealand albino adult male rabbits (Elkhorn Farms, Watsonville, CA) were killed by carbon dioxide asphyxiation or sodium pentobarbital overdose, respectively. For binding assays, the 5 or 6 brains in each group were frozen in isopentane at −15°C. Serial 12-μm-thick coronal sections of rat brain were collected between 1.0 mm rostral to the bregma suture and 0.26 mm caudal to bregma (Fig. 1, left side) and from homologous regions of the rabbit neostriatum. Sections were thaw-mounted onto gelatin-coated glass microscope slides and stored at −70°C for up to 1 month. For neurochemical measurements, each brain was removed from the calvarium and chilled for 2–3 min on ice. Coronal slices of 1 mm thickness were taken from the same region of the brain used for the binding studies with other animals (Fig. 1, right side). Neostriatal subregions from 4–5 brains were dissected on ice within 4 min of death, pooled, and homogenized in 4°C buffer (Korsching and Thoenen, 1983). Four of these pooled tissue homogenates were assayed for ChAT and NGF-LI.

*rhNGF.* Chinese hamster ovary-cell-derived rhNGF was provided by Dr. Gene Burton (Genentech, Inc.). The rhNGF was concentrated 20–50-fold and fractionated by both anion and cation exchange chromatography. The final purity of 98% was achieved employing reverse-phase HPLC (Petrides and Shooter, 1986) and identified by Coomassie-stained SDS gel scanning (Laemmli, 1970) to contain mostly rhNGF species of 118 and 120 amino acids (L. E. Burton, unpublished observations). The fractions were pooled, concentrated by an Amicon stirred cell (YM-10 membrane), and dialyzed overnight into 25 mM Tris, 0.1 M NaCl (pH, 7.0) buffer. Material was sterile filtered and stored at 4°C. The rhNGF aliquots were also monitored for bioactivity using the pheochromocytoma (PC12) culture system (Greene and Rukenstein, 1989), using muNGF as a standard (Collaborative Research, Bedford, MA). The PC12 assay is sensitive to variations in rhNGF concentration in the range of 6.25–100 pg rhNGF/ml with a coefficient of variation of 10–20%. The rhNGF showed stimulatory activity with an $EC_{50}$ of 39 and 52 pg/ml in 2 experiments conducted in separate laboratories.

*Iodination of rhNGF.* rhNGF was iodinated by a modification of the method of De Larco et al. (1981). Fifty microliters of 1.5 M potassium phosphate and 2 mCi per 20 μl of $Na^{125}I$ (Amersham, Arlington Heights, VA) were added to 20 μg/24 μl of rhNGF (average rhNGF concentration of 5.5 μM during iodination). Chloramine-T (0.1 mg/ml in PBS) was added in 3 sequential doses of 4 μg/40 μl followed by incubations of 2, 1.5, and 1 min, respectively. The reaction was quenched with 40 μl of 50 mM *N*-acetyl-L-tyrosine and 40 μl of 1 M potassium iodide. A 200-μl volume of 8 M urea-HCl (pH, 2.3) was added, and the mixture was loaded onto a Vydac C-18 reverse-phase HPLC column where the rhNGF was eluted with an acetonitrile/trifluoroacetic acid (TFA)/$H_2O$ gradient (20–45% acetonitrile in 0.1% TFA over 25 min). Peak fractions were pooled and neutralized with 50 μl of 1.5 M potassium phosphate (pH, 7.4). The $^{125}$I-rhNGF was labeled to a specific activity of 125 μCi/μg (3226 Ci/mmol rhNGF dimer), as determined with an ELISA for rhNGF (Bennett et al., 1990). The acetonitrile was evaporated under nitrogen, and the tracer solution was diluted 1000-fold for use in the binding assay. The $^{125}$I-rhNGF was used within 6 d because decreases in displaceable $^{125}$I-rhNGF binding were noted after this time.

The biological activity of 2- and 3-week-old labeled rhNGF was established with the PC12 assay (Greene and Ruckenstein, 1989), in which $^{125}$I-rhNGF (71.6 μCi/μg; 1850 Ci/mmol rhNGF dimer) stimulated neurite outgrowth with an $EC_{50}$ of 49 ± 19 pg/ml ($n$ = 4; 2-week-old tracer) and 49 ± 0.8 pg/ml ($n$ = 3; 3-week-old tracer). Without a biochemical separation of labeled and unlabeled species, we cannot exclude the possibility that biological activity is due to unlabeled rhNGF. However, the high specific activity of $^{125}$I-rhNGF and the demonstration in the rat brain of retrograde transport of $^{125}$I-rhNGF that can be blocked by rhNGF (Altar and Bakhit, 1991) also indicate that iodinated rhNGF is biologically active.

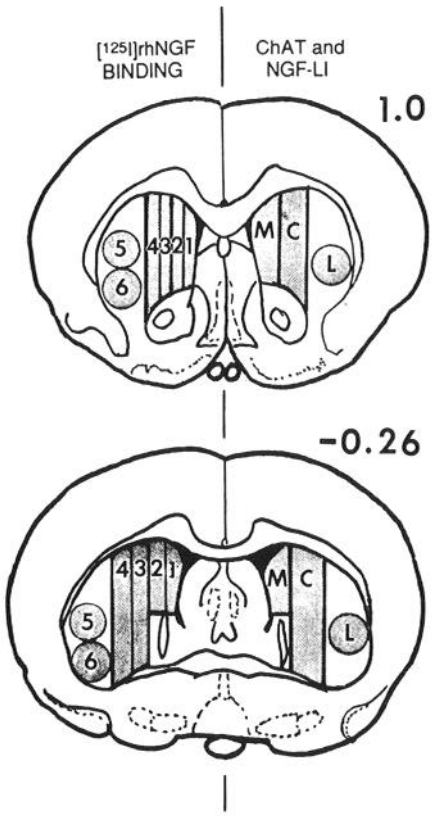


*Figure 1.* Rostral-caudal extent of neostriatal tissue used for the measure of NGF receptor, ChAT, and NGF-LI. Each marker was measured in both hemispheres, but sampling locations for each are represented unilaterally. *Numbers* at the *top right* of each section are the mm anterior to the bregma suture (Paxinos and Watson, 1982). *Left hemisphere,* Distribution of 6 subregions of striatum in which $^{125}$I-rhNGF binding autoradiography was measured bilaterally by image analysis. *Right hemisphere,* Tissues were removed bilaterally from the lateral (*L*), central (*C*), or medial (*M*) striatum for ChAT and NGF-LI measurements.

*$^{125}$I-rhNGF binding assay.* Binding assays were conducted according to the procedure of Richardson et al. (1986), with few modifications (Altar et al., 1991). Each section was prewashed for 3 hr at 22°C in 100 mM phosphate-buffered saline (pH, 7.4) containing 0.5 mM $MgCl_2$, 1 mg/ml cytochrome C, 4 μg/ml leupeptin, 0.5 mM PMSF (BRL, Gaithersburg, MD; first dissolved to 0.1 mg/ml isopropyl alcohol), and then for an additional 3 hr in the same buffer with 110 pM $^{125}$I-rhNGF. This concentration, and concentrations of all radioligands used in this study, is 2–3 times the $K_d$ for $^{125}$I-rhNGF binding to the high-affinity site and thus will label about 90% of displaceable binding sites. Slides containing adjacent brain sections were incubated in the same solutions with the addition of 100 nM rhNGF to define nondisplaceable binding. Thereafter, the sections were washed 3 times for 1 min or for 2 hr in buffer at 22°C. Sections were fixed for 10 min in 4% paraformaldehyde at 22°C, rinsed for 2 sec in water, and dried within 5 min by a stream of room-temperature air.

*$^3$H-PK 11195 binding assay.* $^3$H-PK 11195, a high-affinity antagonist ligand for the mitochondrial benzodiazepine binding site (MBBS), was used to label gliosis in brain sections (Benavides et al., 1987; Altar and Baudry, 1990) from animals injected with quinolinic acid. Sections 12 μm thick were thawed and incubated for 45 min at room temperature in a 50 mM Tris-HCl buffer (pH, 7.4) that included 120 mM NaCl and 3 nM $^3$H-PK 11195 (specific activity, 80 Ci/mmol; DuPont-New England Nuclear; Benavides et al., 1987). Ten micromolar Ro 5-4864

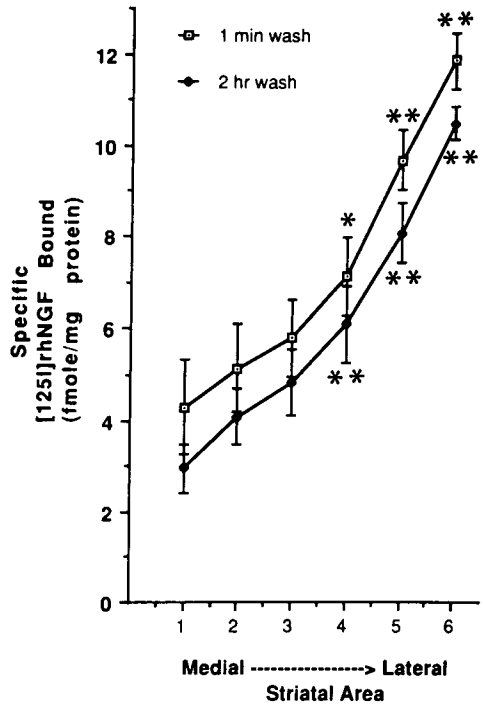


*Figure 2.* Displaceable $^{125}$I-rhNGF bound to the 6 subregions of the neostriatum illustrated in Figure 1 in sections washed in unlabeled buffer for 1 min or 2 hr. Values are means ± SEM for 5 brains. *, $p < 0.05$; **, $p < 0.01$ versus medial striatum (region 1; Dunnett's $t$ test; {6, 25 df}) in the 1-min and 2-hr wash groups.

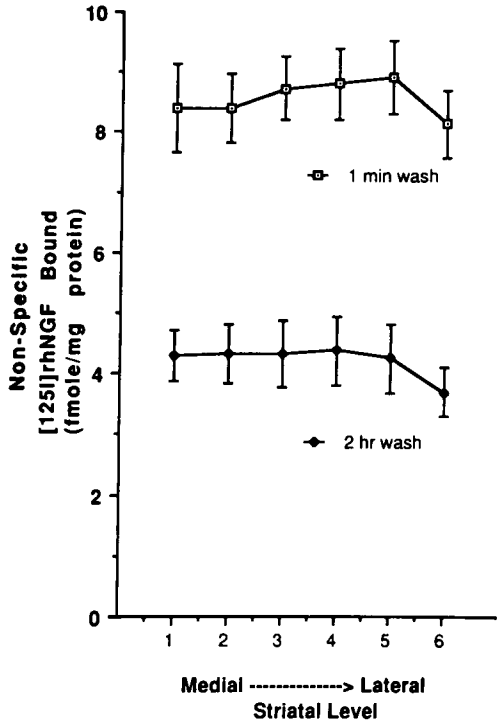


*Figure 3.* Nondisplaceable $^{125}$I-rhNGF bound to 6 subregions of the neostriatum in the presence of 100 nM unlabeled rhNGF. The sections used for this figure were the same as those used to define nondisplaceable binding in Figure 2. No regional differences in nondisplaceable binding were obtained with either wash condition.

(Schoemaker et al., 1982; provided by Hoffman-LaRoche, Nutley, NJ) is a potent and selective MBBS agonist that differs structurally from PK 11195 and was used with adjacent sections to define nondisplaceable binding. Sections were washed for 2 × 1 min at 4°C in the buffer, rinsed 3 × 1 sec in distilled water, and dried within 5 min.

*$^3$H-mazindol autoradiography.* The density of dopamine nerve terminals in the neostriatum was measured with a modification of the $^3$H-mazindol autoradiography procedure of Javitch et al. (1984). Sections were preincubated in binding buffer (50 mM Tris, pH = 7.9 at 4°C, 120 mM NaCl, and 5 mM KCl) for 5 min at 4°C and incubated for 40 min at 4°C in 60 nM $^3$H-mazindol (DuPont-New England Nuclear Research Products, Arlington Heights, VA). Nomifensine (100 μM) was used to define nondisplaceable binding. The sections were washed twice for 1 min in buffer at 4°C, once for 5 sec in water, and dried within 5 min.

The labeled sections and $^3$H- or $^{125}$I-containing radioactivity standards (Amersham, Inc.) were exposed at room temperature for 2 weeks ($^3$H-mazindol), 4 weeks ($^3$H-PK 11195), or 5 d ($^{125}$I-rhNGF) to film sensitive to β-emissions (Hyperfilm, Amersham, Inc.).

*Image analysis.* The autoradiographic films were developed in D-19 (3 min) and fixer (5 min), with an in-between 30-sec wash in stop solution (all reagents from Kodak). The developed films were imaged with an RAS 3000 image analysis system (Amersham, Inc., Arlington Heights, VA) according to the regional pattern illustrated in the left hemispheres of Figure 1. The amounts of binding for each ligand were quantified, and images of total, rhNGF-displaceable, and displaceable binding were produced with computer-assisted image analysis (Altar et al., 1984). The displaceable binding of $^{125}$I-rhNGF was averaged between the 1-min and the 2-hr washes, and these data were averaged for regions 1 and 2 (medial), 3 and 4 (central), and 5 and 6 (lateral) shown in Figure 1 to compare with the regions in which ChAT was measured by dissection.

*NGF-like immunoreactivity (NGF-LI) and ChAT activity.* Tissue samples were homogenized according to the procedure of Korsching and Thoenen (1983) with the addition of 0.5% BSA and 0.1% gelatin to the homogenization buffer. Microtiter plate wells (Maxisorb, Nunc, Kamstrup, Denmark) were coated with 100 μl of protein A-purified rabbit anti-rhNGF (Bennett et al., 1990). The plate bottoms were coated with 10 μg/ml of antibody in coating buffer (0.05 M $Na_2CO_3$, pH 9.6) for 18 hr at 4°C. Excess antibody was removed, and the nondisplaceable binding sites were blocked by the addition of 150 μl per well of PBS containing 5 gm/liter of BSA and thimerosal (blocking buffer), followed by incubation at room temperature for 1–2 hr.

After washing the wells with buffer (PBS containing 0.5 ml/liter of Tween-20 and 0.1 ml/liter of thimerosal), standards (rhNGF) and samples diluted in PBS containing 5 gm/liter of BSA, 0.5 ml/liter of Tween-20, and 0.1 ml/liter of thimerosal (assay diluent) were added to the wells in 100-μl volumes and incubated for 2 hr at room temperature. The wells were washed in buffer, and 100-μl aliquots of horseradish peroxidase-labeled rabbit anti-rhNGF were added to each well. After a 2-hr incubation at room temperature, the plates were washed as above and incubated for 20 min with 100 μl of orthophenylene diamine (Sigma, St. Louis, MO) at 2.2 mmol/liter in PBS (pH, 7.2) with 0.012% (v/v) hydrogen peroxide. The formation of color was stopped with 100 μl/well of 2.25 M sulfuric acid, and the absorbance was read at 490 nm minus 405-nm background. The data were reduced using a 4-parameter curve-fitting program developed at Genentech, Inc., based on an algorithm for least-squares estimation of nonlinear parameters.

The activity of choline acetyltransferase (ChAT) was measured by the Fonnum procedure (Fonnum, 1981) with 10 mM EDTA sodium salt and 0.2% (v/v) Triton X-100 added to the homogenization solution. Each dissected neostriatum was homogenized in 20 vol 0.32 M sucrose. Two- and 4-μl aliquots were used to measure protein content (Lowry) and ChAT activity, respectively. The sensitivity of the ChAT assay was 0.12 nmol ACh formed from the acetylation of choline by ChAT in the presence of $^3$H-acetyl coenzyme A, as determined with ChAT extracted from bovine brain (Sigma).

*Data analysis.* Statistical analysis was accomplished with Student's $t$ test (paired comparisons) or Dunnett's $t$ test (multiple comparisons) following a 1-way analysis of variance.

## Results

### *$^{125}$I-rhNGF binding in rat and rabbit*

Considerable amounts of $^{125}$I-rhNGF were bound throughout the neostriatum of either species. In the rat, the percent of total $^{125}$I-rhNGF binding that was displaced by 100 nM rhNGF after

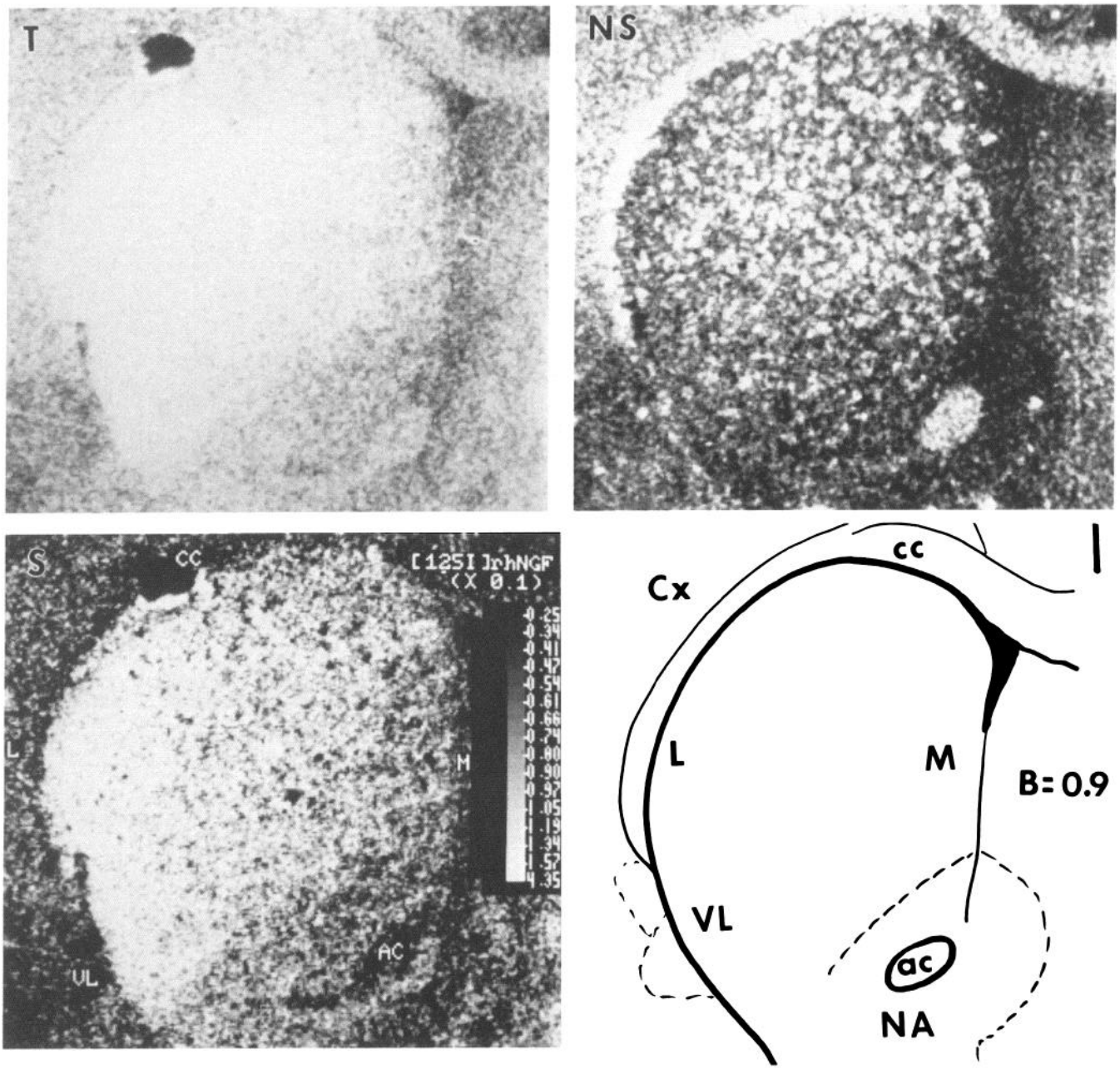


*Figure 4.* Total (*T*), nonspecific (*NS*), and specific (*S*) $^{125}$I-rhNGF binding in the rat neostriatum at a representative coronal section 0.9 mm rostral to bregma (2-hr wash condition). A 110-pM concentration of $^{125}$I-rhNGF was used to define total binding, and a coincubation with 100 nM rhNGF defined nondisplaceable binding. The computer-generated image of displaceable binding was produced by digital subtraction of superimposed total and nondisplaceable binding images. The illustration on the *bottom right* shows prominent anatomical landmarks at this coronal brain level. The gray scale relates image gray value to 0.1 × fmol $^{125}$I-rhNGF bound per mg protein. *ac,* anterior commissure; *B,* mm rostral to the bregma suture; *cc,* corpus callosum; *Cx,* neocortex; *L,* lateral caudate-putamen; *M,* medial caudate-putamen; *NA,* nucleus accumbens; *VL,* ventrolateral caudate-putamen.

1 min or 2 hr of washing in radioligand-free buffer was, respectively, 60 ± 2% and 74 ± 3% in the most lateral caudate-putamen and 32 ± 7% and 40 ± 7% in the most medial caudate-putamen ($p < 0.001$ between lateral and medial caudate-putamen for both wash conditions). These differences were due to variations in displaceable binding (Fig. 2) and not nondisplaceable $^{125}$I-rhNGF binding (Fig. 3), which was uniform throughout the neostriatum (see also Fig. 4). Two nanomolar rhNGF displaced about 80% as much $^{125}$I-rhNGF binding as 100 nM rhNGF. Thus, the 100 nM concentration was routinely used to define displaceable binding, as reported (Richardson et al., 1986; Raivich and Kreutzberg, 1987; Riopelle et al., 1987; Bernd et al., 1988).

Consistent with $^{125}$I-rhNGF binding to a high-affinity site, the amount of displaceable $^{125}$I-rhNGF binding decreased only slightly following 2 hr of wash at 22°C in unlabeled buffer compared with a 1-min wash (Fig. 3). Displaceable binding increased from the medial to the lateral striatum following postincubation washes of 1 min ($F\{5, 24\} = 12.2$; $p < 0.001$) or 2 hr ($F\{5, 24\} = 19.4$; $p < 0.001$; Fig. 3). For example, in the 2-hr group,

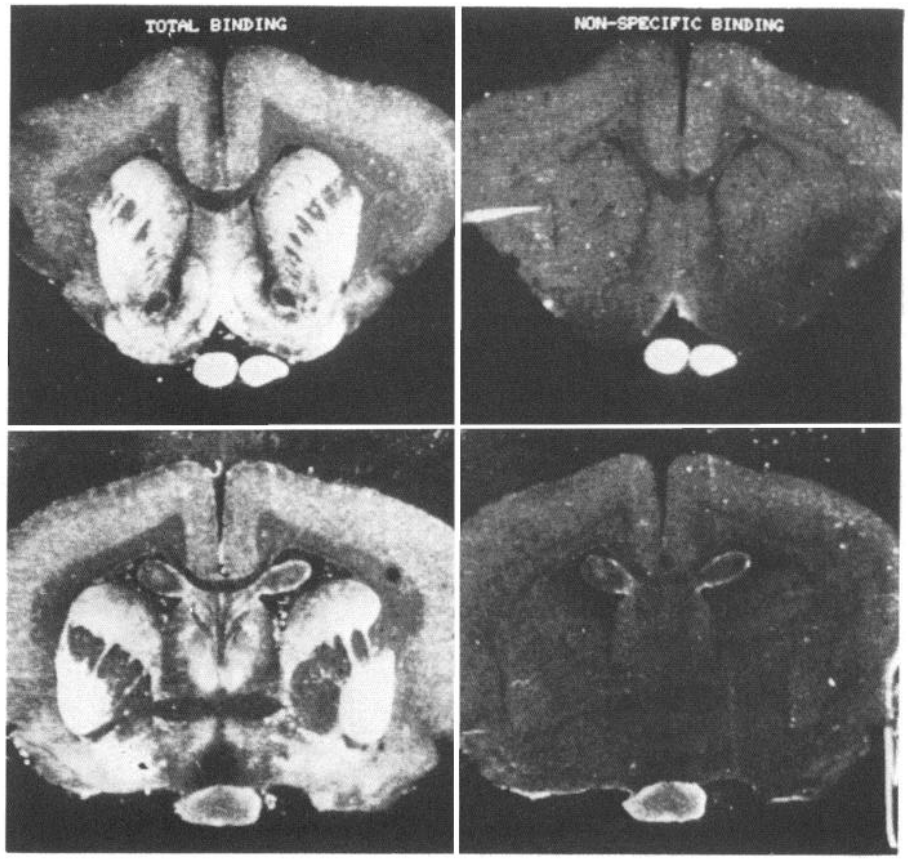


*Figure 5.* Total and nonspecific $^{125}$I-rhNGF binding at 2 levels of the rabbit neostriatum under conditions identical to those used in Figure 4. Note that binding is not displaced by unlabeled rhNGF in the corpus callosum, anterior commissure, and optic nerve (*top*) and optic chiasm (*bottom*).

displaceable $^{125}$I-rhNGF binding in the medial striatum was 2.95 ± 0.5 fmol/mg protein. This increased by about 3-fold to a maximum of 10.5 ± 0.4 fmol/mg protein in the ventrolateral striatum. Digitized autoradiographs of total, nondisplaceable, and displaceable $^{125}$I-rhNGF binding to representative neostriatal sections (2-hr wash) revealed the typical pattern of NGF binding in the rat (Fig. 4) and rabbit (Fig. 5). The gradient in total binding of the rat neostriatum was more readily apparent after subtraction of the uniform nondisplaceable binding image from the total binding image. The image of displaceable $^{125}$I-rhNGF binding most clearly revealed the receptor gradient, with the highest density of NGF receptors present in the lateral and ventrolateral striatum. In the rabbit, the separation of the caudate nucleus from the putamen by the fibers of the internal capsule clearly revealed the greater density of $^{125}$I-rhNGF binding in the putamen and, within the caudate nucleus, the greater density of sites in the ventrolateral than in the dorsomedial portion (Fig. 5). Quantitation showed a 2.5-fold-greater density of displaceable sites in the rabbit putamen than in the caudate (data not shown).

*ChAT activity and NGF-LI in the intact neostriatum*

Increasing amounts of ChAT activity were measured from the medial to lateral portions of the neostriatum (Fig. 6). The 2.4-fold increase in ChAT activity from the medial to lateral neostriatum was highly significant ($F\{2, 8\} = 18.9$; $p < 0.001$), with lateral activities exceeding those found centrally or medially ($p < 0.01$). The average amounts of $^{125}$I-rhNGF binding and ChAT activity in the medial, central, and lateral areas were positively correlated ($r = +0.99$; $p < 0.001$). NGF-LI was also prevalent in the neostriatum but, in contrast to ChAT and $^{125}$I-rhNGF binding, was evenly distributed along the medial-lateral dimension (Fig. 6).

*Effects of striatal quinolinic acid or 6-hydroxydopamine*

Basal levels of displaceable binding of the glial marker, $^{3}$H-PK 11195, were uniformly distributed in the caudate-putamen (Fig. 7) and exceeded by 2-fold the level of background binding defined with Ro 5-4864. Intrastriatal injections of quinolinic acid elevated $^{3}$H-PK 11195 binding by 6–8-fold in the lateral, central, and medial caudate-putamen. In contrast, these lesions lowered displaceable NGF binding by 21%, 33%, and 43% in the lateral, central, and medial caudate-putamen, respectively (Fig. 8). The $^{125}$I-rhNGF binding in the entire cross-sectional area of the striatum and ChAT activity in homogenates of the dissected caudate-putamen were each decreased by 40% following quinolinic acid (Fig. 8).

Injections of 6-hydroxydopamine produced 70% and 77% ($p < 0.01$, Student's $t$ test) depletions of dopamine nerve terminal densities determined with $^{3}$H-mazindol but not displaceable $^{125}$I-rhNGF binding at 1 and 4 weeks after the injection (Table 1).

## Discussion

The present study has revealed that high-affinity binding sites for $^{125}$I-rhNGF in the caudate-putamen of the rat and rabbit are dense and distributed with a 2–3-fold increasing medial-to-lateral gradient. With the present methods, neostriatal binding sites for $^{125}$I-rhNGF were found to be among the most dense in the brain, second only to the interpeduncular nucleus and spinal trigeminal tract (Altar et al., 1991). It is thus surprising that

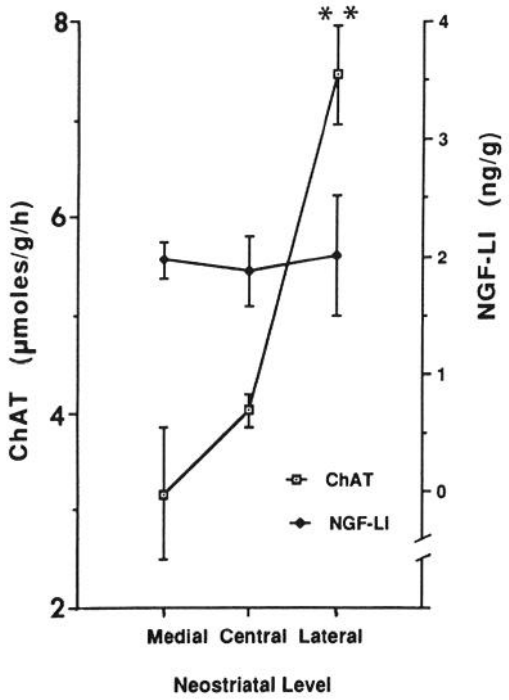


*Figure 6.* ChAT activity (*left ordinate*) and NGF-LI (*right ordinate*) in the 3 neostriatal subregions illustrated in the left half of Figure 1. $n$ = 4–5 determinations, with tissues from 4–5 animals used per determination. **, significance level of $p < 0.01$ (3, 13 df) versus ChAT activity in the medial and central caudate-putamen. *Vertical lines* represent SEM.

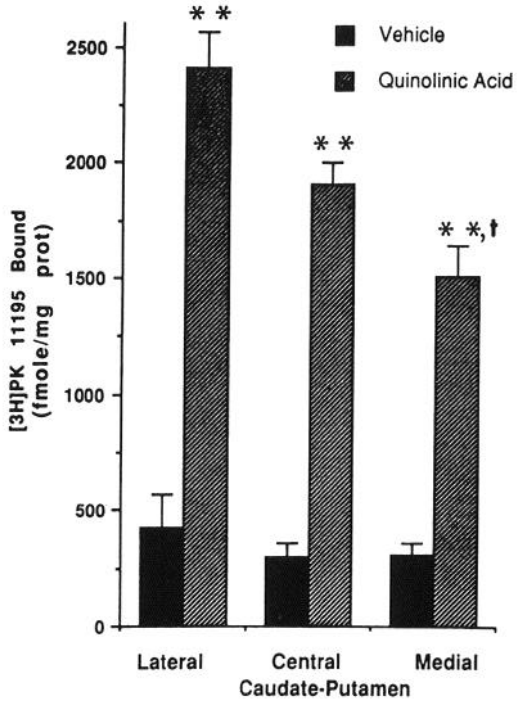


*Figure 7.* Increased density of the peripheral benzodiazepine-binding-site ligand $^3$H-PK 11195 1 week following an intrastriatal injection of 150 nmol quinolinic acid. Values are means ± SEM; $n$ = 5 rats per group. Displaceable binding was elevated 6–8-fold in each caudate-putamen subregion. **, $p < 0.01$; †, $p < 0.05$ versus binding in the lateral caudate-putamen of quinolinic acid-treated rats.

previous studies have been equivocal as to the presence of NGF binding sites in the caudate-putamen of the human or rat. In large part, this has probably been due to the lack of selective high-affinity binding by the monoclonal antibodies and incomplete saturation of high-affinity sites by $^{125}$I-muNGF. The 192-monoclonal antibody (MAb) IgG directed against the mouse NGF receptor labels the low-affinity site in Schwann cells and PC12 cells (Chandler et al., 1984; DiStefano and Johnson, 1988) as well as high-affinity sites in PC12 cells (Chandler et al., 1984). Because the high- and low-affinity NGF receptors are encoded by a single gene, the MAb probably recognizes a common epitope on these interconverting or posttranslationally modified receptors. Even so, NGF receptors either have not been found in the human caudate or putamen with the MAb 82.11 (Hefti and Mash, 1989) or were shown with the MAb 192 to bind only in the ventrolateral monkey putamen (Kordower et al., 1988; Schatteman et al., 1988). NGF-receptor antibodies also fail to label NGF receptors in the rat neostriatum in some studies (Batchelor et al., 1988; Gage et al., 1989) or identify only sparse, scattered receptors (Gibbs et al., 1989; Kiss and Patel, 1989; Woolf et al., 1989). However, in agreement with our findings, NGF receptors have been colocalized with ChAT (Kordower et al., 1988; Kiss and Patel, 1989; Woolf et al., 1989) and are found in lower density in medial striatum (Pioro and Cuello, 1990).

Interpretations of the high-affinity nature of direct NGF ligand-binding studies are less equivocal, because $^{125}$I-rhNGF concentrations, wash, and other conditions have been shown to optimize high-affinity binding and minimize or eliminate low-affinity binding. Nevertheless, prior radioligand studies of the

**Table 1. $^3$H-mazindol and $^{125}$I-rhNGF binding in the caudate-putamen after local infusion of 6-hydroxydopamine**

| | Displaceable ligand bound (fmol/mg protein) | | | | | |
|---|---|---|---|---|---|---|
| | $^3$H-mazindol | | | $^{125}$I-rhNGF | | |
| Time after infusion | Intact | Lesion | % Decrease | Intact | Lesion | % Decrease |
| 1 week | 41 ± 2.6 | 9.2 ± 2.6** | 77 | 8.7 ± 0.5 | 9.8 ± 0.9 | – |
| 4 weeks | 58 ± 2.4 | 18 ± 4.7** | 70 | 10.4 ± 0.7 | 9.8 ± 0.8 | – |

Six male Sprague–Dawley rats received an infusion into the central neostriatum of the vehicle (Intact) or 6-hydroxydopamine (Lesion) as described in Materials and Methods. The density of dopamine nerve terminals ($^3$H-mazindol binding) and NGF binding sites was measured in adjacent sections with saturating concentrations of each radioligand with appropriate unlabeled compounds to define nondisplaceable binding.

**, $p < 0.01$ versus intact neostriatum, paired $t$ test.

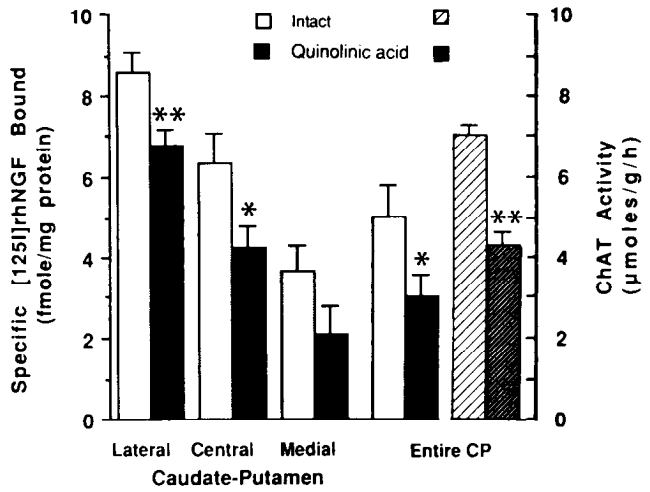


*Figure 8.* Similar decreases in displaceable $^{125}$I-rhNGF binding (*left ordinate*) and ChAT activity (*right ordinate*) in the lateral, medial, or entire caudate-putamen 1 week following an intrastriatal injection of 150 nmol quinolinic acid. *, $p < 0.05$; **, $p < 0.02$ versus hemisphere injected with vehicle in other rats; paired $t$ test. *Vertical lines* represent SEM.

high-affinity NGF receptor with $^{125}$I-labeled muNGF (Richardson et al., 1986; Raivich and Kreutzberg, 1987; Riopelle et al., 1987; Mobley et al., 1989) have found no binding or low and uniform binding throughout the rat caudate-putamen.

The present use of dry-film autoradiography, iodination of rhNGF to very high specific activity, quantitative image analysis and enhancement that can easily detect a 2-fold difference in receptor number, and the exclusion of postbinding organic solvent washes and liquid film emulsions are all likely to have contributed to the more sensitive detection of high-affinity binding than obtained with emulsion autoradiography. Indeed, the techniques used here also afford visualization of high-affinity NGF sites in neocortical and hippocampal areas (Altar et al., 1991) that are undetected with $^{125}$I-muNGF emulsion autoradiography (Richardson et al., 1986; Raivich and Kreutzberg, 1987). The sparse and uniform neostriatal pattern observed with $^{125}$I-muNGF and the lack of binding in cholinergic terminal areas like the cortex and hippocampus indicate that a loss of label from neuropil may occur with the emulsion technique. In support of this conclusion, Burke and Karanas (1990) showed that the processes of ChAT-positive neurons are greater in the lateral than in the medial rat caudate-putamen, while ChAT-positive cell bodies are evenly distributed in this region. Thus, the medial-lateral gradient of $^{125}$I-rhNGF binding sites may result from the presence of these high-affinity sites on cholinergic neuropil and cell bodies.

The medial-lateral gradient in specific binding sites for rhNGF was not due to medial-lateral variations in nonspecific binding (Fig. 3) or to an artifact due to a medial-lateral variation of NGF protein. Nonspecific binding and NGF-LI were each uniformly concentrated throughout the medial-lateral extent of the caudate putamen. Also, tissue section washes for up to 24 hr, which dislodge virtually all bound $^{125}$I-rhNGF from its specific binding site (Altar et al., 1991), do not alter the gradient produced by subsequent 3-hr incubations with $^{125}$I-rhNGF (M. Dugich-Djordjevic and C. A. Altar, unpublished observations). The medial-lateral gradient can instead be explained by the organization of intrinsic NGF-receptor-containing neostriatal elements in a medial-lateral manner. The distribution of cholinergic markers, including high-affinity $^{3}$H-choline uptake (Rea and Simon, 1981) and the density of the ACh transporter ligand $^{3}$H-vesamicol (Marien et al., 1987) are organized in a quantitatively similar 2–3-fold medial-lateral gradient. Also, $^{125}$I-rhNGF binding was positively correlated with the distribution of the cholinergic neuron marker, ChAT, and each was decreased to an equal extent by intrastriatal quinolinic acid. These findings make it most plausible that the high-affinity NGF sites observed throughout the striatum are on cholinergic neurons, and not only in the ventrolateral caudate-putamen (Riopelle et al., 1987). The absence of NGF binding sites on dopamine nerve terminals was shown by the failure of $^{125}$I-rhNGF binding to be altered by 70–77% losses of these terminals following 6-hydroxydopamine. The absence of high-affinity NGF binding sites on dopaminergic nerve terminals is consistent with the lack of a trophic role of NGF for mesencephalic dopamine neurons (Hefti et al., 1989). These findings also suggest that dopamine depletions in the striatum did not elicit an up- or downregulation of NGF receptor number, because the near-saturating concentrations of $^{125}$I-rhNGF used would reveal a change in rhNGF-binding-site number.

Excitotoxic lesions of the striatum with quinolinic acid promoted gliosis in this region. The increase in glial cells, but not mitochondrial markers such as cytochrome oxidase, are labeled by peripheral-type benzodiazepine-binding-site ligands, including $^{3}$H-PK 11195 (Schoemaker et al., 1982; Dubois et al., 1988). The 6–8-fold increases in glial binding sites for $^{3}$H-PK 11195 and 40% decrease in $^{125}$I-rhNGF binding show that NGF receptors labeled here are probably not on glial cells. Glia are believed to contain the low-affinity form of the NGF receptor (Springer, 1988). Glia are also believed to produce NGF, and gliosis following intrastriatal infusions of quinolinic acid produces a large increase in NGF-LI (C. Bakhit, unpublished observations). Thus, the loss of high-affinity NGF binding sites is probably not due to a downregulation of NGF receptors secondary to decreases in NGF. Instead, these high-affinity sites are probably lost because they are on cholinergic interneurons destroyed by quinolinic acid (Schwarcz et al., 1983). The loss of NGF sites following quinolinic acid predicts that decreases in NGF receptor levels will be found in the caudate nucleus and putamen of patients with Huntington's chorea (Nagai et al., 1983), Alzheimer's disease (Oyangai et al., 1989), or progressive supranuclear palsy (Oyangai et al., 1989), because neostriatal cholinergic interneurons are lost in these disorders.

In contrast to the medial-to-lateral gradient for NGF binding sites, NGF-LI in the striatum was of equal concentration along this dimension. The equal density of NGF-LI through the medial-to-lateral extent of the neostriatum indicates that the source(s) of NGF-LI in the intact neostriatum may be cells that receive a cholinergic input and whose neostriatal distribution is also uniform. One neuronal candidate for these criteria is the striatal GABAergic interneurons. Their ability to produce, store, and release NGF is, however, unknown. Glial cells are also uniformly distributed throughout the intact striatum (see Fig. 7; Benavides et al., 1987) and are other likely sources for NGF.

Because NGF-LI is uniformly distributed in the neostriatum, the heterogeneous content of NGF receptors in the caudate-putamen may dictate preferential effects for NGF in the lateral and ventrolateral portions of this structure. An analogous situation exists for striatal $D_2$ dopamine receptors. They are also

2–3-fold more concentrated in the lateral caudate-putamen (Altar et al., 1984), yet dopamine is uniformly distributed in this structure (Ternaux et al., 1977). Exogenously applied dopamine receptor antagonists increase dopamine metabolism and release to a greater extent in the lateral than in the medial rat striatum (Childs and Gale, 1986; Yamamoto and Pehek, 1990). The presence of regional gradients in NGF receptors in the brain, including the nucleus accumbens (Altar et al., 1991) and striatum, may predict regions where responses to NGF may be the greatest, such as following rhNGF delivery to adult rats via intraventricular (Hagg et al., 1989) or intrastriatal (Gage et al., 1989; Armanini et al., 1990) routes. Exogenously delivered rhNGF may be sequestered in neostriatal subregions in proportion to their receptor density and may prevent or retard the loss of striatal cholinergic neurons in normal aging (McGeer et al., 1971; Strong et al., 1982), Huntington's chorea, or progressive supranuclear palsy (Steele et al., 1964; Nagai et al., 1983; Oyangai et al., 1989).

In conclusion, the increasing medial-to-lateral gradient of NGF binding in the intact caudate-putamen, the parallel response of NGF binding with cholinergic neurons but not dopamine neurons or glia, and the uniform distribution of NGF-LI in this region are consistent with a trophic role for NGF in the neostriatum (Mobley et al., 1985, 1989; Aloe, 1987; Hagg et al., 1989). These binding sites for NGF are likely to play a role in the maintenance of striatal cholinergic neurons throughout the neostriatum and not just for sparsely populated ventrolateral sites, as suggested by prior NGF receptor studies. This conclusion is supported by the observation that the intraventricular delivery of antibodies to muNGF, which enter the striatum (Schweitzer, 1989), lowers ChAT levels in this region if the antibodies are neutralizing against NGF (Vantini et al., 1989). Indeed, NGF augments ChAT activity in the medial caudate-putamen not only in injured or intact neostriatal cholinergic neurons of neonatal rats (Mobley et al., 1985, 1989; Aloe, 1987), but also in intact (Hagg et al., 1989) or injured adult rats (Gage et al., 1989; Armanini et al., 1990).

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