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Journal Title: Journal of Neuroscience Nursing
Volume: Volume 22, Number 12
Publisher: Lippincott, Williams & Wilkins | 2002-06-15, Pages 4942-4954
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1523/JNEUROSCI.22-12-04942.2002
Permanent URL: https://pid.emory.edu/ark:/25593/v4n17

Final published version:
http://dx.doi.org/10.1523/JNEUROSCI.22-12-04942.2002

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Accessed December 8, 2023 1:32 AM EST
Lentivirally Delivered Glial Cell Line-Derived Neurotrophic Factor Increases the Number of Striatal Dopaminergic Neurons in Primate Models of Nigrostriatal Degeneration

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The primate striatum contains tyrosine hydroxylase (TH)-immunoreactive (ir) neurons, the numbers of which are augmented after dopamine depletion. Gial cell line-derived neurotrophic factor (GDNF) strongly modulates the viability and phenotypic expression of dopamine ventral mesencephalic neurons. The effect of GDNF on TH-ir neurons intrinsic to the striatum has yet to be investigated. In the present study, stereological counts of TH-ir striatal neurons in aged and parkinsonian nonhuman primates revealed that GDNF delivered via a lentiviral vector (lenti-) further increased the number of these cells. Aged monkeys treated with lenti-GDNF displayed an eightfold increase in TH-ir neurons relative to lenti-β-galactosidase-treated monkeys. Unilateral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment alone in young monkeys resulted in a bilateral eightfold increase in TH-ir striatal cells. This effect was further magnified sevenfold on the side of lenti-GDNF treatment. These cells colocalized with the neuronal marker neuronal-specific nuclear protein. Some of these cells colocalized with GDNF-ir, indicating that an alteration in phenotype may occur by the direct actions of this trophic factor. Thus, GDNF may mediate plasticity in the dopamine-depleted primate brain, which may serve to compensate for cell loss by converting striatal neurons to a dopaminergic phenotype.

Key words: striatum; dopaminergic neurons; Parkinson’s disease; primates; GDNF gene therapy; lentivirus

Received Dec. 4, 2001; revised March 21, 2002; accepted March 28, 2002.

This work was supported by a grant from the Department of Defense, a grant from the Parkinson’s Foundation of the National Capital Area, and the Charles and M. V. Shapiro Foundation. We thank Theodora Kladis for expert histological assistance.

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neuronal populations, because the viability and phenotypic expression of serotonergic or GABAergic neurons are not affected by GDNF.

To date, the role of GDNF on primate TH-ir striatal neurons has not been investigated in vivo. We published a study recently that examined the structural and functional effects of GDNF in primate models of dopamine insufficiency (Kordower et al., 2000). This study provided the opportunity to examine these unique tissues and to evaluate the effects of GDNF on striatal TH-ir neurons. The present study assessed whether gene delivery of this trophic factor could augment the number of dopaminergic striatal neurons in primate models of nigrostriatal degeneration. First, we confirmed that dopamine depletion in MPTP-treated nonhuman primates enhances the number of TH-ir striatal neurons. Then we found that in vivo gene delivery of GDNF using a lentiviral vector increased the number of TH-ir and DAT-ir striatal neurons in aged monkeys. Finally, gene delivery of GDNF dramatically increased the number of TH-ir striatal neurons in MPTP-treated monkeys.

**MATERIALS AND METHODS**

**Experiment 1: effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on striatal tyrosine hydroxylase-immunoreactive cells.** This experiment was performed to confirm previous findings now using stereological counting procedures to show that MPTP-treated monkeys have increased numbers of TH-ir striatal neurons (Betarbet et al., 1997). Four rhesus monkeys (4–5 years of age) received two unilateral intracarotid injections of MPTP separated by 1 month according to a protocol described previously (Kordower et al., 2000) (Table 1). Briefly, animals were tranquilized with ketamine (10 mg/kg, i.m.) and then maintained on an anesthetic plane with isoflurane (1–2%). The animals were put in the supine position. For each injection, a right-sided incision was made along the median edge of the sternocleidomastoid muscle. The carotid sheath was opened and the common carotid artery, internal jugular vein, and vagus nerves were identified. The common carotid was exposed below the carotid bifurcation. The external carotid artery was then ligated. A 2.5 gauge butterfly needle was inserted into the common carotid artery in a direction retrograde to blood flow; for each injection, 20 ml of saline containing 3 mg of MPTP-HCl was infused at a rate of 1.33 ml/min (15 min). After the infusion was completed, 3 ml of saline was delivered, and then the incision was closed. Two additional untreated rhesus monkeys served as unoperated controls.

**Experiment 2: lentiviral delivery of ganglioside myelin-associated glycoprotein factor to aged monkeys.** This experiment investigated the effects of in vivo GDNF gene delivery on the number of striatal TH-ir cells in aged rhesus monkeys. The details of procedures performed on these monkeys have been reported previously (Kordower et al., 2000). In this experiment, four aged rhesus monkeys (24–27 years of age) received intrastratal injections of lentivirus encoding for GDNF, and three monkeys received control injections of lentivirus encoding for β-galactosidase (β-gal) (Table 1). All animals received six lentivirus injections targeted for the caudate nucleus (n = 2 injections; 5 and 10 μl), putamen (n = 3; 10, 10, and 5 μl), and substantia nigra (n = 1; 5 μl). Coordinates were based on magnetic resonance imaging as described previously (Kordower et al., 2000). Monkeys were killed 3 months after the surgery. The construction of the vectors has been described previously (Kordower et al., 1999, 2000; Deglon et al., 2000). Three months after the lentivirus treatment, all animals were killed.

**Experiment 3: lentiviral delivery of glial cell line-derived neurotrophic factor to monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.** This experiment tested the hypothesis that GDNF delivered via a lentiviral vector would increase the number of TH-ir striatal neurons in MPTP-treated monkeys. Eight monkeys comprised this experiment, and the methodological details of which have been reported previously (Kordower et al., 2000). Briefly, monkeys received a single injection of MPTP (10 mg/kg) into the right carotid artery followed 1 week later by injections of GDNF delivered by a lentiviral vector (lenti- (n = 4) or lent-β-gal (n = 4) into the right caudate nucleus, putamen, and substantia nigra. The injection scheme was identical to that described above for aged monkeys. Three months after the lentivirus treatment, all animals were killed.

**Preparation of tissues.** Rhesus monkeys were killed with an overdose of pentobarbital, perfused transcardially with saline, and postfixed with 4% paraformaldehyde solution in phosphate buffer (0.1 M), pH 7.4, at 4°C. The brains were removed immediately and cut into coronal blocks for postfixation. Tissue blocks were then washed in a series of cold graded sucroses and sectioned (40 μm) in the coronal plane on a freezing microtome.

**Immunohistochemistry.** Sections through the forebrain and midbrain were processed for the visualization of TH, DAT, or GDNF immunoreactivity using modifications of procedures published previously (Kordower et al., 1999, 2000). Briefly, endogenous peroxidase activity was eliminated with a 20 min incubation in 0.1 M sodium periodate. Background staining was then inhibited by a 1 hr incubation in 3% normal serum and 2% bovine serum albumin (BSA), after which the tissue was incubated for 48 hr in the primary antibody. The concentrations of the primary antibodies were as follows: TH (1:20,000; Chemicon, Temecula, CA); DAT (1:500; generously provided by Dr. Alan Levey, Emory University, Atlanta, GA); or GDNF (1:250; Transduction Laboratories, Lexington, KY). Using a labeled antibody procedure, sections were then sequentially incubated in the appropriate biotinylated IgG (1:200; Vector Laboratories, Burlingame, CA) for 1 hr, washed in a Tris-buffered saline solution containing 0.05% Triton X-100, and incubated in “Elite” avidin–biotin complex (ABC) (1:1000; ABC kits; Vector Laboratories) for 75 min. The chromogen solution that completed the reaction consisted of 0.05% diaminobenzidine and 0.005% H2O2. For the GDNF staining, the immunohistochemical reaction product was intensified by adding 2.5% nickel II sulfate to the chromogen solution. Sections were mounted on gelatin-coated slides, dehydrated through graded alcohols (70, 95, and 99%), cleared in xylene, and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA). Additional sections were processed simultaneously in an identical manner except for the deletion of the primary antibody or substitution of the primary antibody with an irrelevant IgG as a negative control. No specific immunoreactivity was seen for any stain under these conditions.

**Histological analysis.** In experiment 1, stereological counts of striatal TH-ir neurons were performed bilaterally on MPTP-treated animals and unilaterally in control animals. In experiments 2 and 3, stereological counts of TH-ir, GDNF-ir, or DAT-ir neurons were performed unilaterally on the side of the lenti-GDNF or lenti-β-gal infusions. For all experiments, stereological counts were performed using a computerized optical dissector system. This method allows for an unbiased stereological quantification of a defined portion of a structure independent of its size, shape, orientation, tissue shrinkage, or anatomical level (Emborg et al., 1998). The computerized optical dissector system consists of a computer-assisted image analysis, a microscope, a computer-controlled x, y, z motorized stage, a stereological software program (MicroBrightField Inc., Colchester, VT), and a high-sensitivity video camera. Before each series of measurements, the instrumentation was calibrated. The striatum was outlined under low magnification (1.25×). Five percent of the outlined region was quantified using a dissector counting frame and a systematic random-sampling design. The average section thickness for each section and the antibody penetration throughout the entire tissue section were determined empirically. The total number of striatal neurons was quantified using a high magnification (100×) planapo oil immersion objective with a 0.4 N.A. lens. Under the dissector principle, at least 200 striatal neurons were identified in each case. Once the top of the section was in focus, the z-plane was lowered 1–2 μm. Care was taken to ensure that the top and bottom forbidden planes were not included in the analysis. The total number of TH-ir striatal neurons (N) was calculated using the following formula: \( N = N_v \times V_{striatum} \) where

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Table 1. Experimental groups

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Figure 1. TH-immunostained sections through the striatum of a rhesus monkey receiving a unilateral intracarotid injection of MPTP (A, B) and a normal control (C). A, TH-ir neurons within the striatum on the side ipsilateral to the MPTP infusion. B, TH-ir neurons within the striatum on the side contralateral to the MPTP infusion. C, TH-ir neurons in an untreated rhesus monkey. Scale bar, 30 μm.
Confocal double immunofluorescence of tyrosine hydroxylase with neuronal-specific nuclear protein. Based on tissue availability, doubleimmunofluorescence experiments were performed with tissue from experiment 2 to determine whether the TH-ir striatal cells coexpressed the neuronal marker neuronal-specific nuclear protein (NeuN) or the trophic factor GDNF. Sections were first incubated in a blocking solution (5% normal goat serum, 2% BSA, and 0.3% Triton X-100 in TBS, pH 7.4) for 1 hr to inhibit background staining. Then sections were incubated in primary rabbit polyclonal anti-TH (1:1000; Chemicon) for 24 hr at 4°C. After three washes, sections were incubated in secondary goat anti-rabbit IgG coupled to the fluorescent marker Cy2 (1:200; Jackson ImmunoResearch, West Grove, PA) for 1 hr. After completion of the reaction, sections were washed and background staining was inhibited by incubating the sections in a blocking solution containing 5% normal horse serum, 2% BSA, and 0.3% Triton X-100 in TBS, pH 7.4. Then sections were incubated in mouse monoclonal anti-NeuN (1:1000; Chemicon) for 24 hr at 4°C followed by incubation in goat anti-mouse IgG coupled to the fluorescent marker rhodamine (1:200, Jackson ImmunoResearch) for 1 hr at room temperature.

Confocal double immunofluorescence of tyrosine hydroxylase with glial cell line-derived neurotrophic factor. To assess double labeling of TH-ir cells with the neurotrophic marker GDNF, sections were immersed in the blocking solution as described above for 1 hr and then incubated in primary mouse monoclonal anti-TH (1:10,000; Incstar, Stillwater, MN) for 24 hr at 4°C. After three washes, sections were incubated in secondary goat anti-mouse IgG coupled to the fluorescent marker Texas Red (1:200; Jackson ImmunoResearch) for 1 hr at room temperature. Then sections were blocked with 5% normal horse serum, 2% BSA, and 0.3% Triton X-100 in TBS, pH 7.4, and incubated in goat polyclonal anti-GDNF (1:200; Chemicon) for 24 hr at 4°C and in biotinylated secondary donkey anti-goat IgG (1:200; Vector Laboratories) coupled to the fluorescent marker Cy3 for 1 hr at room temperature. Statistical analysis. The data were analyzed using a two-way ANOVA followed by a Fisher’s LSD post hoc test when there was a significant overall ANOVA (Statview 4.0; Abacus Concepts, Calabasas, CA).

RESULTS

Experiment 1: effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on striatal tyrosine hydroxylase-immunoreactive cell number

In all animals, numerous TH-ir neurons were distributed within the nonhuman primate caudate nucleus and putamen. In normal control animals, these cells were preferentially distributed around the periphery of the striatum, just interior to surrounding white matter. In MPTP-treated monkeys, TH-ir striatal neurons were also distributed along the periphery of the striatum. However, unlike normal controls, they were abundantly distributed throughout the central portions of the caudate nucleus and putamen as well.

Greater numbers of TH-ir neurons were seen in MPTP-treated monkeys relative to normal controls (Figs. 1A–C, 2). In present study, we performed stereological counts to confirm previous findings (Betarbet et al., 1997) using nonstereological counting procedures. Stereological counts in the present study demonstrated that MPTP-treated monkeys have increased numbers of TH-ir striatal neurons. Statistical analyses revealed significant differences in TH-ir striatal neurons between treatment groups (factorial ANOVA: F(2,7) = 21.2; p < 0.001) (Fig. 2). Monkeys with intracarotid MPTP injections displayed significant increases in the number of TH-ir cells ipsilateral to the MPTP injection (mean ± SD, 1,557,526 ± 137,769) compared with untreated controls (453,528 ± 137,769; p < 0.004). Interestingly although the MPTP treatment was unilateral, the number of TH-irpositive striatal cells was also augmented on the side contralateral to MPTP treatment (1,834,752 ± 129,477). This number of TH-ir striatal neurons was statistically greater than that seen in control animals (453,528 ± 137,769; p < 0.001) (Fig. 2) and similar in magnitude to that seen on the side ipsilateral to MPTP treatment (p = 0.90).

Experiment 2: lentiviral delivery of glial cell line-derived neurotrophic factor in aged monkeys

Lentiviral delivery of GDNF to aged monkeys resulted in a significant increase in striatal TH-ir and DAT-ir neurons compared with β-gal-treated control animals (Figs. 3, 4, 5, 6). The increase in the number of TH-ir striatal neurons was seen at every level of the striatum (Fig. 4B,D,F). In lenti-β-gal-treated animals, TH-ir striatal neurons were scattered in the striatum but again were preferentially located within the lateral aspects of the caudate nucleus and putamen (Fig. 4A,C,E). In contrast, numerous TH-ir striatal neurons were distributed throughout the center core of this structure in lenti-GDNF-treated animals (Fig. 4). Stereological counts through the striatum of aged monkeys receiving lentivirus encoding for GDNF revealed that the number of TH-ir labeled neurons was significantly augmented compared with lenti-β-gal-treated cohorts (factorial ANOVA: F(1,5) = 137.4; p < 0.0001) (Fig. 5A). Lenti-GDNF-treated aged monkeys displayed 2,851,744 ± 179,249 TH-ir cells compared with 350,009 ± 28,105 TH-ir striatal neurons in lenti-β-gal-treated animals. This represents a more than eightfold increase in the number of TH-ir cells (Fig. 5A). Similar significant increases mediated by lenti-GDNF were seen in DAT-ir neurons (F(1,5) = 155.2; p < 0.0001) (Figs. 5C, 6). In lenti-GDNF-treated monkeys, 2,550,508 ± 143,991 DAT-ir cells were seen in the striatum on the side ipsilateral to the lentivirus/MPTP infusion. In contrast, only 379,625 ± 43,649 DAT-ir striatal neurons were seen in Parkinsonian monkeys treated with lenti-β-gal (Fig. 5C). This represents a 6.7-fold increase in the number of TH-ir neurons.

GDNF-ir neurons were observed in aged monkeys treated with lenti-GDNF (Fig. 7A) but not in aged monkeys treated with lenti-β-gal (Fig. 7B). Stereological counts of GDNF-ir neurons were also performed (Fig. 5B). No GDNF-ir striatal neurons were seen in lenti-β-gal-treated animals, indicating that the injection schema used did not induce an upregulation of endogenous GDNF to within detectable levels. In contrast, 825,836 ± 13,466 GDNF-ir neurons were seen within the striatum of lenti-GDNF-
treated animals. Morphologically, the location of GDNF-ir cells varied depending on the site of lentiviral vector injections. In the penumbra of the injection site, GDNF-ir-positive cells appeared to be of similar size and displayed a similar morphology to that normally displayed by medium-sized spiny neurons. Additional tissues were not available to perform colocalization experiments to confirm that these cells are indeed medium spiny neurons. Robust extracellular GDNF staining was also observed in the penumbra of the injection site. In contrast, ovoid GDNF-ir-positive cells were distributed primarily throughout the periphery of the injection area (Fig. 7A). Similarly, ovoid TH-ir- and DAT-ir-positive cells were located in the same regions of the striatum as the ovoid GDNF-ir cells.

Correlations were performed to examine whether a significant association existed between GDNF-induced alterations of TH-ir, GDNF-ir, and DAT-ir striatal cells. Significant correlations were observed between the various cell types: TH-ir/GDNF-ir \((r = 0.98; p < 0.0001)\), TH-ir/DAT-ir \((r = 0.98; p < 0.0001)\), and GDNF-ir/DAT-ir \((r = 0.98; p < 0.0001)\) (Fig. 5).

**Immunofluorescence**

Double-immunofluorescence experiments were performed to determine whether the TH-ir striatal cells coexpressed the neuronal marker NeuN or the trophic factor GDNF. Laser confocal microscopic images through the striatum of lenti-GDNF-treated monkeys confirmed that virtually all of the TH-ir cells colocalized with the neuronal marker NeuN (Fig. 8). These double-labeled cells were medium-sized and located in the penumbra of the
lenti-GDNF injection sites. Laser confocal microscopic images throughout the striatum of lenti-GDNF-treated monkeys indicated that numerous medium-sized neurons were immunopositive for GDNF and that the degree of colocalization between TH and GDNF was regionally specific. Few GDNF-ir cells located within the core of the injection site were immunoreactive for TH (Fig. 9). In contrast, many TH-ir-positive cells coexpressed GDNF-ir in regions distal to the transfection site. These double-labeled cells displayed an ovoid shape. Again, no GDNF-ir immunofluorescence was observed in β-gal-treated animals.

**Experiment 3: lentiviral delivery of glial cell line-derived neurotrophic factor in monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine**

In monkeys rendered parkinsonian with MPTP, lenti-GDNF augmented the number of TH-ir striatal neurons by more than sevenfold relative to lenti-β-gal-treated animals (Fig. 10). In lenti-β-gal-treated animals, stereological counts of TH-ir neurons revealed 121,380 ± 19,600 positive cells on the treated side. In contrast, the striata of lenti-GDNF-treated animals contained 786,387 ± 406,682 TH-ir-positive neurons. This difference in the number of TH-ir striatal neurons between lenti-β-gal- and lenti-GDNF-treated animals was statistically significant ($t_{(1,6)} = 3.267; p < 0.017$).

**DISCUSSION**

The present series of experiments demonstrated that the number of TH-ir striatal cells in nonhuman primates increases in response to dopaminergic depletion; this effect is enhanced by the lentiviral delivery of GDNF. Double-labeling studies in
lentivirus-treated animals revealed that these TH-ir cells coexpressed NeuN-ir, indicating that cells expressing this dopaminergic marker were neurons. In addition, TH-ir neurons coexpressed GDNF-ir, indicating that the induction of the TH phenotype may be occurring via an autotrophic mechanism. It should be noted that the increase in DAT-ir cells after GDNF treatment was similar to that observed for TH-ir and that the numbers of TH-ir and DAT-ir cells were strongly correlated. These data suggest that the effects of dopamine depletion and GDNF administration provide concomitant influences on the genes responsible for TH and DAT expression.

A previous study found increases in TH-ir striatal neurons after the unilateral or bilateral nigrostriatal lesions engendered by MPTP in nonhuman primates (Betarbet et al., 1997). This study
quantified TH-ir neurons in four anatomically matched sections and did not have tissue prepared in a manner that was compatible with a stereological analysis. Stereological counts of TH-ir neurons in the present study confirmed the increase in neuronal number after MPTP-induced dopaminergic denervation. Indeed, in the study by Betarbet et al. (1997), bilateral increases in TH-ir striatal neurons were observed after unilateral MPTP injections. Stereological analyses in the present study confirmed this surprising effect as well. The fact that an increase in TH-ir striatal neurons was observed on the “intact side” strongly indicates that the increases seen on the side with lesions were authentic and not attributable to the fact that they were easier to identify and count after removal of the TH-ir striatal neuropil. However, it cannot be ruled out that the increase in TH-ir striatal neurons seen on the intact side may have resulted from a small loss of nigrostriatal neurons that occurred as a result of MPTP crossing over to the contralateral side after the unilateral intracarotid injection of MPTP. In this regard, a small loss of TH-ir has been reported previously in monkeys on the side contralateral to the MPTP injection (Bankiewicz et al., 1999). If MPTP crossover mediated the increase in TH-ir neurons on the intact side, this would suggest that just a small loss of striatal dopamine is capable of eliciting this plasticity response.

It should be noted that the number of TH-ir striatal neurons in the control MPTP/lenti-β-gal-treated animals in experiment 3 was less than what was observed in experiment 1, in which monkeys were treated with MPTP alone. There are a number of factors that could have contributed to this difference in neuronal

Figure 6. Photomicrographs of DAT-ir staining through the striatum of aged monkeys receiving lenti-GDNF (A) or lenti-β-gal (B). Note that large and numerous DAT-ir cells were observed in animals receiving lenti-GDNF compared with those receiving lenti-β-gal. Scale bar, 30 μm.
number. In experiment 1, the monkeys were exposed to multiple MPTP injections and were killed 5 months after surgery, whereas the monkeys in experiment 3 received a single MPTP injection and were killed 3 months postoperatively. Thus, repetitive injections combined with a longer postoperative time course could be responsible for this discrepancy. In addition, technical issues could have played a role in this difference. In each of the three experiments, tissue from control and experimental animals was histochemically processed together. However, tissues from the different experiments were processed independently, using different lots of antibody and different reagents. The intensity of staining was much greater in general through all dopaminergic regions in the tissues processed in experiments 1 and 2 compared with experiment 3; this likely contributed to the differences in absolute numbers. The critical comparisons to be concerned with in this study are the differences between conditions within an experiment rather than the absolute number of neurons responding to dopamine depletion and lenti-GDNF treatment.

The molecular mechanism underlying this plasticity response remains to be elucidated. Endogenous trophic factor release by glia in response to MPTP treatment has been reported as one potential stimulator of the normally quiescent TH gene (Francis et al., 1995) and may account for the increase in TH-ir cells. Regardless of whether this mechanism is in effect, the present data suggest that the increase of TH-ir cells is a compensatory response to striatal dopamine depletion. However, the fact that nonhuman primates become symptomatic so quickly after intra-carotid MPTP administration suggests that this response is insufficient to sustain functional effects in these animals.

In this study, lenti-GDNF dramatically increased the number

Figure 7. Photomicrographs of GDNF-ir in the striatum of aged monkeys receiving lenti-GDNF (A) or lenti-β-gal (B). Note that numerous GDNF-ir cells were observed in animals receiving lenti-GDNF, but no GDNF-ir-positive elements were seen in animals receiving lenti-β-gal. Scale bar, 30 μm.
of TH-ir striatal neurons in both aged monkeys and monkeys rendered parkinsonian via MPTP. Aged rhesus monkeys undergo phenotypic losses of nigrostriatal dopaminergic markers, as evidenced by the fact that 50% of nigral neurons fail to express TH and 30% fail to express DAT (Emborg et al., 1998). Intracarotid injection of MPTP induces a more robust and frank degeneration of nigrostriatal neurons, in which often up to 90% of nigrostriatal neurons are lost (Bankiewicz et al., 1999). It is interesting that

Figure 8. Laser confocal microscopic images through a series of focal planes through the caudate nucleus of an aged monkey treated with lenti-GDNF and stained for TH (A) and NeuN (B). C, The merged image. The yellow cells in C signify that cells coexpress both TH and NeuN (arrow). The arrows depict the same cell in A and B as well as the merged image in C. Scale bar, 15 μm.
lenti-GDNF treatment was equipotent in increasing the number of TH-ir striatal neurons in MPTP-treated animals, in which the lesion was more severe relative to aged monkeys; in aged monkeys the lesion is more modest and does not produce frank neuronal degeneration. This suggests that the underlying status of the host system does not influence the ability of lenti-GDNF to modify the expression of TH-ir striatal neurons.

The mechanism by which GDNF increases the number of...
TH-ir neurons is not yet known. In the nigrostriatal dopaminergic system, GDNF has been shown to signal through a multireceptor complex composed of a novel glycosylphosphatidylinositol-anchored GDNF receptor and the receptor tyrosine kinase product of the c-ret proto-oncogene (Durbec et al., 1996; Trupp et al., 1996). The expression of this receptor complex is maximal during early postnatal development but it is at very low levels in the adult striatum (Trupp et al., 1997). As stated previously, the vast majority of TH-ir cells coexpressed GDNF-ir, suggesting that this trophic factor functioned in an autotrophic manner. Interestingly, there appears to be specificity in this response, because not all cells that were successfully transfected with the lentivirus expressed TH-ir. Unfortunately, sufficient tissue sections were not available to analyze the expression of ret and GDNF receptor α after GDNF delivery and to address some of these issues of mechanisms. Such studies are fertile ground for further investigation.

Some potential mechanisms that mediate the augmentation of striatal TH-ir neurons have been proposed. One hypothesis is that existing GABAergic striatal interneurons are converted to a TH-ir phenotype (Betarbet et al., 1997). This theory is based on a morphology analysis and colocalization of TH-ir with GAD-ir in the MPTP primate model of PD. In the present experiment, double labeling of TH-ir and GAD-ir were inconclusive (data not shown). One other potential mechanism may involve neural stem cells that migrate and differentiate to TH-ir-positive neurons that result from dopamine depletion and/or trophic factor release (Svendsen and Smith, 1999). This later hypothesis has important results from dopamine depletion and/or trophic factor release (Svendsen and Smith, 1999). In this role, GDNF may mediate plasticity in the dopamine-depleted primate brain and may serve to compensate for cell loss by converting striatal neurons to a dopaminergic phenotype. Additional studies are needed to determine whether these TH-ir striatal cells are extant striatal neurons or are generated from differentiated progenitor cells, and whether TH-ir cells are capable of producing dopamine and exerting functional effects.

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