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Award Number: DAMD17-98-1-8630

TITLE: Gene Therapy in a Nonhuman Primate Model of Parkinson's Disease

PRINCIPAL INVESTIGATOR: Jeffrey Kordower, Ph.D.

CONTRACTING ORGANIZATION: Rush-Presbyterian - St. Luke's Medical Center
Chicago, Illinois 60612-3833

REPORT DATE: October 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1999	Annual (15 Sep 98 - 14 Sep 99)

4. TITLE AND SUBTITLE

Gene Therapy in a Nonhuman Primate Model of Parkinson's Disease

5. FUNDING NUMBERS

DAMD17-98-1-8630

6. AUTHOR(S)

Jeffrey Kordower, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Rush-Presbyterian - St. Luke's Medical Center
Chicago, Illinois 60612-3833

e-mail:
jkordowe@rush.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

This grant is studying the ability to deliver the trophic factor glial derived neurotrophic factor (GDNF) via in vivo and ex vivo viral vectors in nonhuman primates. In this first year, two studies have been performed and completed. In the first experiment, lentiviral vectors delivering the marker gene ßgalactosidase was injected into three monkeys to test the ability of this vector to demonstrate sustained expression in the nonhuman primate nigrostriatal system. This study revealed that robust and consistent gene expression could be achieved. In the second experiment, lentiviral delivery of GDNF was attempted in aged nonhuman primates. These injections resulted in a dramatic increase in dopaminergic markers in this model of early Parkinson's disease. These data indicate that lentiviral delivery of GDNF may be an efficacious means by which to upregulate impaired dopaminergic function in patients with PD.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Neurotoxin	26
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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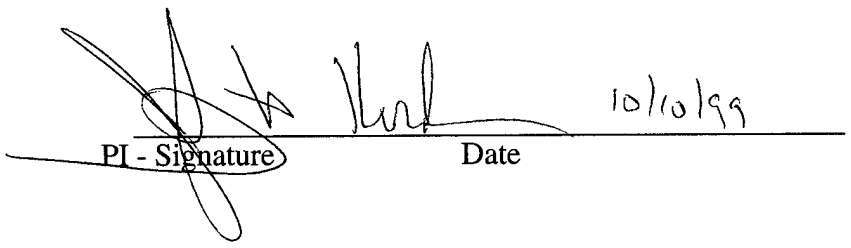

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5) Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder that affects over 1,000,000 Americans. Symptoms include tremor, bradykinesia and rigidity, all of which invariably increase in severity as the disease progresses. Pathologically, there is progressive loss of striatal dopamine and degeneration of dopaminergic neurons within the substantia nigra pars compacta. Palliative symptomatic treatment can be achieved by dopamine (DA) replacement therapy using the dopamine precursor, levodopa. However, "wearing off effects" with disabling dyskinesias complicates symptomatic treatments. As PD progresses, motor and nonmotor symptoms emerge which are not responsive to levodopa. Since treated patients show a life expectancy similar to age-matched controls, patients can survive with crippling symptoms for many years. Thus, new innovative treatment strategies are needed to sustain the quality of life for these individuals. Recently, surgical treatment strategies such as neural transplantation (e.g. 1), pallidotomy (e.g. 2) or deep brain stimulation (e.g. 3) have gained considerable attention for the treatment of PD. However, preventing neuronal degeneration, rather than replacing neurons or disrupting basal ganglia circuitry may be a more parsimonious way of sustaining nigrostriatal and clinical function in patients with PD. The present proposal plans to use a neuroprotection strategy and determine whether delivery of glial derived neurotrophic factor (GDNF) via *in vivo* gene therapy systems can reverse motor deficits and nigrostriatal dysfunction in aged nonhuman primates.

6) Body of Report

The studies being performed are using lentivirus as an in vivo delivery system for GDNF. This delivery system has never been attempted previously in nonhuman primates. Thus we first needed to establish whether successful gene transfer could be achieved in this species using ß-Galatosidase as a marker. The methods and results from this first feasibility study are detailed in the enclosed manuscript.

A second study has just been completed examining the structural and functional consequences of lentiviral delivery of GDNF in aged Rhesus monkeys. Eight aged (24-27 year old) female Rhesus monkeys received injections of lentivirus encoding for ßgalactosidase (lenti-ßGal; n=4) or GDNF (lenti-GDNF; n=4) into the striatum and substantia nigra and were sacrificed 3 months later. All injections were localized to the caudate nucleus, putamen, and supranigral regions. All aged monkeys receiving lenti-GDNF but no monkeys receiving lenti-ßGal, displayed robust GDNF-immunoreactivity within the striatum and substantia nigra. Using standard staining procedures, the GDNF-ir within the striatum was extremely dense and distributed throughout the neuropil. When the primary antibody concentration was diminished 10-fold to decrease this intense neuropil staining, striatal GDNF-ir perikarya were observed. Numerous GDNF-ir perikarya were seen within the substantia nigra of lenti-GDNF injected monkeys.

In lenti-GDNF infused aged monkeys, robust anterograde transport of the trophic factor was observed. Intense GDNF-ir was observed within the globus pallidus and substantia nigra pars reticulata. GDNF-ir fibers emanating from putaminal injection sites were seen coursing medially towards and into the globus pallidus. These staining patterns were clearly distinct from the injection site and exquisitely respected the boundaries of these structures.

All monkeys underwent fluorodopa positron emission tomography (PET) prior to surgery and just prior to sacrifice three months following the lentivirus injections. Enhanced fluorodopa uptake was observed within the caudate nucleus and putamen in lenti-GDNF treated monkeys while no such increases were seen in monkeys receiving lenti-ßGal.

Stereological counts of TH-ir neurons revealed a dramatic increase in TH-ir nigral neurons within the substantia nigra of lenti-GDNF treated aged monkeys. On the left side that did not display GDNF-ir, lenti-GDNF infused animals contained 76,929$\pm$4918 TH-ir neurons which was similar to what was seen in ß Gal infused animals (68,543 $\pm$5519). In contrast, there was an 85% increase in TH-ir

neurons on the right side that displayed robust GDNF-ir. Lenti-ßGal infused monkeys contained 63,738±6094 TH-ir nigral neurons while lenti-GDNF treated monkeys contained 118,170 ± 8631 TH-ir nigral neurons ($p<.001$). A similar pattern of changes was seen for the volume of substantia nigra neurons. TH-ir substantia nigra neurons from Lenti-ßGal and lenti-GDNF treated monkeys were similar in size on the left side where there was no GDNF expression (11147.5±351 μm^3 and 11458.7±379 μm^3 respectively). In contrast, a 35% increase in neuronal volume was seen on the GDNF-rich right side in lenti-GDNF injected aged monkey (lenti-ßGal 10707.5±333 μm^3; lenti-GDNF 16653.7 ±1240μm^3; $p<.001$).

A novel in situ-immunocytochemical technique, combined with computer assisted optical density measurements, was employed to assess the relative differences in THmRNA within nigra neurons in lenti-ßGal and lenti-GDNF treated aged monkeys. The pattern of results was similar to what was observed with TH-ir neuronal number and volume. On the left side, the optical density of THmRNA within nigral neurons was similar between lenti-ßGal and lenti-GDNF treated monkeys (78.28±2.78 and 80.58±2.5 respectively). In contrast, there was a significant increase in the optical density for THmRNA in lenti-GDNF treated monkeys (98.3+1.5) relative to lenti-ßGal treated monkeys (77.2+2.3) on the right side ($p<.01$).

Within the striatum, lentiviral delivery of GDNF increased a number of markers of dopaminergic function with the pattern of changes similar to that seen within the substantia nigra. Optical density measurements were performed to assess the relative intensity of TH-ir staining within the caudate nucleus and putamen. On the left side where there was no GDNF expression, the intensity of TH-ir within the caudate nucleus and putamen were similar in aged monkeys injected with lenti-ßGal or lenti-GDNF. In contrast, significant increases in TH-ir optical density were seen in on the right side of lenti-GDNF infused monkeys relative to lenti-ßGal treated animal. In this regard, there was a 44.1% and a 38.9% increase in TH-ir optical density within caudate nucleus and putamen respectively. At the time of sacrifice, tissue punches were taken throughout the caudate nucleus and

putamen of all monkeys. Measurement of dopamine and homovanillic acid revealed significant increases in the right caudate nucleus and putamen in lenti-GDNF treated aged monkeys relative to lenti-ßGal treated animals.

7) Key Research Accomplishments

Robust lentiviral gene transfer can occur in the nonhuman primate brain.

Robust lentiviral gene transfer can occur in the nonhuman primate brain without cytotoxicity

Long-term gene transfer of GDNF can occur in the aged monkey brain.

Lentiviral transfer of GDNF augments nigrostriatal function in aged monkeys.

8) Reportable Outcomes

Kordower, J.H., Bloch, J., Ma, S.Y., Chu, Y., Palfi, S., Roitberg, B.Z., Emborg, M., Hantraye, P., Déglon, N., and Aebischer, P. Lentiviral gene transfer to the nonhuman primate brain. **Experimental Neurology**, in press.

Emborg, M., Bloch, J., Ma, S.Y., Chu, Y., Palfi, S., Roitberg, B.Z., Stansell, J., Hantraye, P., Déglon, N., and Aebischer, P. Lentiviral gene transfer to the nonhuman primate nigrostriatal system. **Soc. Neurosci. Abstr.** 25: 744, 1999.

9: Conclusions: Lentiviral gene transfer can occur successfully in the nonhuman primate brain without cytotoxicity. Relatively long-term gene transfer can occur in the aged monkeys brain and using this method to deliver the trophic factor GDNF can augment nigrostriatal function in these animals . If the experiments planned in the upcoming years also prove successful, this technology should be tested for its clinical utility in patients with early Parkinson's disease.

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11: Appendices

1) Galley proofs from Experimental Neurology paper.
2) Copy of Neuroscience abstract

Experimental Neurology **160,** 1–16 (1999)
Article ID exnr.1999.7178, available online at http://www.idealibrary.com on IDEAL®

Lentiviral Gene Transfer to the Nonhuman Primate Brain

Jeffrey H. Kordower,* Jocelyne Bloch,† Shuang Y. Ma,* Yaping Chu,* Stéphane Palfi,* Ben Zion Roitberg,*,‡ Marina Emborg,* Philippe Hantraye,§ Nicole Déglon,† and Patrick Aebischer†

*Departments of Neurological Sciences and Research Center for Brain Repair, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612; ‡Department of Neurosurgery, University of Illinois Medical Center, Chicago, Illinois 60612; §CEA CNRS URA 2210 Service Hospitalier Frederic Joliot, CEA, DSV, DRM, Orsay cedex, France; and †Division of Surgical Research and Gene Therapy Center, Lausanne University Medical School, Switzerland

Received April 16, 1999; accepted June 28, 1999

Lentiviral vectors infect quiescent cells and allow for the delivery of genes to discrete brain regions. The present study assessed whether stable lentiviral gene transduction can be achieved in the monkey nigrostriatal system. Three young adult Rhesus monkeys received injections of a lentiviral vector encoding for the marker gene β galatosidase (βGal). On one side of the brain, each monkey received multiple lentivirus injections into the caudate and putamen. On the opposite side, each animal received a single injection aimed at the substantia nigra. The first two monkeys were sacrificed 1 month postinjection, while the third monkey was sacrificed 3 months postinjection. Robust incorporation of the βGal gene was seen in the striatum of all three monkeys. Stereological counts revealed that 930,218; 1,192,359; and 1,501,217 cells in the striatum were βGal positive in monkeys 1 (n = 2) and 3 (n = 1) months later, respectively. Only the third monkey had an injection placed directly into the substantia nigra and 187,308 βGal-positive cells were identified in this animal. The injections induced only minor perivascular cuffing and there was no apparent inflammatory response resulting from the lentivirus injections. Double label experiments revealed that between 80 and 87% of the βGal-positive cells were neurons. These data indicate that robust transduction of striatal and nigral cells can occur in the nonhuman primate brain for up to 3 months. Studies are now ongoing testing the ability of lentivirus encoding for dopaminergic trophic factors to augment the nigrostriatal system in nonhuman primate models of Parkinson's disease. © 1999 Academic Press

INTRODUCTION

The transfer of genes into postmitotic cells *in vivo* can have far reaching implications for understanding basic functions of specific systems within the central nervous system as well as potentially delivering therapeutic molecules to brain regions vulnerable to neurodegenerative diseases (See 30, for review). Currently, a number of vector delivery systems exist that can be applied toward these goals. In this regard, recombinant and defective herpes simplex virus, as well as adenovirus, display moderate-to-high rates of transduction efficiency. However, these gene transfer technologies may also be toxic and immunogenic. Although less immunogenic, adenoassociated has lower transduction efficiency compared to herpes simplex virus and adenovirus. Recently a new HIV-based vector system has begun to be evaluated. This lentivirus integrates into the genome of nonproliferating brain cells (4, 23, 24, 25, 27). Recent studies have demonstrated stable long-term expression of the reporter gene β galactosidase (βGal) following lentivirus injections into multiple regions across the rodent neuraxis (4, 24, 25). Lentiviral-mediated gene transfer has been demonstrated to have potent biologic properties as injections of lentivirus encoding for the antiapoptotic gene Bcl-xL as well as nerve growth factor prevents the experimental degeneration of cholinergic basal forebrain (5). Furthermore, lentiviral delivery of glial-derived neurotrophic factor (GDNF; 10) prevents the loss of axotomized dopaminergic nigrostriatal neurons.

For *in vivo* gene therapy strategies to be of value to humans, safety and efficacy need to be established in the best animal models available. For Parkinson's disease, the best animal model is the MPTP-treated monkey. However, at present, studies examining the safety and the efficiency of *in vivo* gene transfer procedures in nonhuman primates are limited. Davidson and coworkers (9) initially demonstrated short-term (7-day) gene transfer using adenovirus in Rhesus monkeys. Bohn and coworkers (6) attempted *in vivo* gene delivery to African green monkeys. Successful transduction was highly variable and accompanied by significant immunogenicity and cytotoxicity using adenoviral vectors. Finally Bankiewicz and colleagues (1, 2) have successfully achieved βGal and tyrosine hydroxylase expression in rhesus monkeys for up to 3 months postinjec-

0014-4886/99 $30.00

tion. To date, there are no data evaluating the safety and efficiency of lentiviral gene transfer to the nonhuman primate brain. One of our ultimate goals is to determine whether lentiviral delivery of GDNF can prevent parkinsonism in nonhuman primate models of Parkinson's disease. As a prelude to functional and structural studies using MPTP-treated monkeys, the present report describes the robust lentiviral transfer of the βGal gene to the striatum and substantia nigra of rhesus monkeys.

METHODS

Subjects. Three young adult male (4–6 kg) Rhesus monkeys served as subjects. These animals were housed singly with food and water available *ad libitum.* All experimentation was performed according to NIH guidelines.

Surgery. Coordinates for stereotaxic injection were based upon MRI guidance. For the caudate nucleus, the more rostral injection was targeted to the head of the caudate nucleus at its largest extent. The second caudate injection was targeted 4 mm more caudally at the level of the body of the caudate nucleus. This deposit was at a level just caudal to the decussation of the anterior commissure. The first two putaminal injections were at the same rostrocaudal levels as the two caudate injection. The final putaminal injection was placed 4 mm caudally at the level of the lateral geniculate nucleus. Prior to surgery, monkeys were anesthetized with an intramuscular injection of Ketamine (10 mg/kg) and Xylazine (2 mg/kg). Once in an anesthetic plane, the monkeys were placed in a specially designed MRI compatible stereotaxic unit modeled after a Kopf primate stereotaxic apparatus. The angle of the head was established by measuring the height of the incisor tooth using a standard micromanipulator and a modified electrode holder. Then the monkey was transferred to the 1.5 Tesla MRI unit, overlapping T1 and T2 images, where 4-mm images were obtained using standard procedures. The coordinates for injection sites into the caudate, putamen, and substantia nigra were then ascertained using the MRI's computer software.

On the day of surgery, monkeys were tranquilized with Ketamine (10 mg/kg, im). The monkeys were then intubated, anesthetized with isoflurane (1–3%), and replaced in the same stereotaxic unit. The angle of the head was reestablished to be the same as when the animal received his MRI scan. Under sterile conditions, a sagittal incision was made. On the left side, a craniotomy was made over the striatum. On the right side, a burr hole was made over the substantia nigra. Each monkey then received six lentiviral injections. Five injections were made in the left striatum. Two of these injections were made in the caudate nucleus (5 μl rostral and 10 μl 4 mm caudal) and three were placed in the putamen (10 μl rostral, 10 μl mid, and 5 μl caudal; all injections separated by 4 mm in a rostrocaudal plane). In addition, a single 5-μl injection was placed into the right substantia nigra. The viral particles injected can be deduced from the titer, which is 2×108 TU/ml (transducing unit per milliliter). Thus, each 5-μl injection corresponds to 1×106 TU and each 10-μl injection corresponds to 2×106 TU. All injections were performed manually through a 10-μl Hamilton syringe at a rate of 0.5 μl per minute. The needle was left *in situ* for an additional 3 min to allow the virus to diffuse from the needle tip. The craniotomy and burr hole were filled with Gelfoam. The subcutaneous tissues were closed with 4-0 Coated Vicryl and the skin was closed with 4-0 Ethilon. The monkeys were sacrificed either 1 month ($n = 2$) or 3 months ($n = 1$) postinjection.

Construction of the lentiviral vector. The cDNA coding for the β-galactosidase (LacZ) containing a nuclear localization signal was cloned in the SIN-W-PGK transfer vector. A 400-bp fragment (*Eco*RV–*Pvu*II) of the U3 region of the 3′-LTR was deleted to obtain the self-inactivating vector (SIN; 34). This plasmid was further modified by insertion of the posttranscriptional *cis*-acting regulatory element of the woodchuck hepatitis virus (WHV; a 587-bp fragment: position 1093–1684 of the WHV complete genome: GenBank Accession No. J04514; 35). This element significantly increases transgene expression in a variety of contexts, apparently through a combination of stabilization of nascent RNA transcripts and facilitation of their cytoplasmic export (7, 11, 12). The mouse phosphoglycerate kinase (PGK) 1 promoter was used as internal promoter. The packaging construct and the VSV-G envelope used in this study were the pCMVΔR-8.91 and the pMD.G plasmids described previously (33). High-titer stocks were obtained by ultracentrifugation. The batch of virus was tested for the absence of replication-competent viral vectors (25). The titer of 2×10^8 TU/ml was determined on 293T cells. The cells were plated at a density of 2×10^5 cells per well on six-well tissue culture dishes (Costar). Serial dilutions of the viral stock were added and the number of LacZ-infected cells was analyzed 48 h later.

Histology. All monkeys were tranquilized with Ketamine (10 mg/kg, im), intubated, and anesthetized with Nembutal (25 mg/kg, iv). Monkeys were then sequentially perfused with warm (100 ml) and ice cold (100 ml) saline, followed by fixation with a 4% Zamboni's solution (500 ml). The brains were removed from the calvaria and placed in a 30% sucrose/phosphate-buffered saline (PBS) solution until fully immersed. Frozen (40 μm) thick sections were then cut in the coronal plane on a sliding knife microtome and stored in a cryoprotectant solution.

X-galactosidase (x-Gal) histochemistry. One series of sections from each brain was treated with detergent (2 mM $MgCl_2$, 0.01% sodium deoxycholate, 10% Nonidet P-40 in PBS, pH 7.2) for 10 min and then incubated for 4 h at room temperature in the X-Gal substrate solution, which consisted of 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_63H_2O$, 2 mM $MgCl_2$, and X-Gal (1 mg/ml in PBS, pH 7.2). Sections were mounted on gelatin-coated slides, dehydrated through graded alcohols (50, 70, 95, and 99%), cleared in xylenes, and coverslipped with Permount.

Immunohistochemistry. Sections were also stained for X-Gal immunofluorescence, GFAP, and NeuN using standard procedures. After the cryoprotectant was washed from the sections, incubating the sections in a solution containing phosphate-buffered saline, 3% normal serum, and 2% bovine serum albumen blocked background staining. Sections were then incubated for 48 h at room temperature in the polyclonal βGal (5′–3′; 1:1000), polyclonal GFAP (Dakopatz; 1:2000), monoclonal TH (Chemicon; 1:20,000), or monoclonal NeuN (Chemicon; 1:1000) antibodies. Some sections were processed for fluorescence visualization. Sections processed for fluorescence (βGal or TH) were then sequentially incubated in the biotinylated goat antirabbit or horse antimouse IgG (1:200) for 1 h and streptavidin conjugated to Cy2 (1:1000) for 1 h at room temperature. Sections processed using immunoperoxidase methods (GFAP and NeuN) were incubated in either the biotinylated goat antirabbit (GFAP) or horse antimouse (NeuN) IgG for 1 h following by a 75-min incubation in the Elite ABC substrate (1:500; Vector Labs). The sections were reacted in a chromogen solution containing 0.05% 3′3 diaminobenzidine and 0.005% hydrogen peroxide. All sections were then mounted, dehydrated, and coverslipped with DPX. For each experiment, control sections were processed in an identical manner except the primary antibody solvent or an irrelevant IgG matched for protein concentration were substituted for the primary antibody. While no staining was observed under these conditions, caution is still required since the possibility of the antibody reacting with structurally related proteins cannot be eliminated. The term immunoreactivity in this study refers to "like immunoreactivity."

Double labeling immunofluorescence procedure. In order to identify the cell types infected with the lentiviral vector, an indirect immunofluorescence double-label technique was employed to label βGal-positive cells with a neuronal and glial markers. In the striatum, β gal immunofluorescence was codetected with NeuN, a neuronal marker, or GFAP, a glial marker. In the substantia nigra, identical colocalization studies were performed with the addition of experiments colocalizing βGal with tyrosine hydroxylase. For each experiment, background staining was inhibited with a 1-h incubation in a blocking solution (5% normal goat serum, 2% bovine serum albumin [BSA], and 0.3% Triton X-100 in TBS, pH 7.4) at room temperature. Sections were then incubated in primary rabbit polyclonal antibody to β-gal (1:500) overnight at 4°C. After washes, the sections were incubated in the secondary goat anti-rabbit IgG coupled to the fluorescent marker Texas Red (1:200) for 1 h. Following completion of the reaction, the sections were blocked again in a blocking solution (5% normal horse serum, 2% BSA, and 0.3% Triton X-100 in TBS, pH 7.4), and incubated in one of the following the primary antibodies: mouse monoclonal anti-NeuN (Chemicon; 1:500), mouse monoclonal anti-GFAP (Sigma; 1:500), or mouse monoclonal anti-TH (Incstar 1:10000) for 24 h at 4°C. After incubation in the secondary antibody (biotinylated horse anti-mouse IgG 1:200) for 1 h at room temperature, the sections were placed in fluorolink Cy 2-labeled streptavidin (1:1000) for 1 h at room temperature. Following several washes, sections were mounted on gelatin-coated slides, dehydrated through graded alcohols (50, 70, 95, and 99%), cleared in xylenes, and coverslipped with DPX. For control, the first or second primary antibodies were deleted. All fluorescence images were analyzed with the Olympus Confocal Fluoroview microscope equipped with argon and krypton lasers.

Stereological analysis β-Gal-positive neurons. The total number of lentivirus-positive neurons within the caudate nucleus, putamen, and substantia nigra was determined using the optical fractionator procedure (13, 17, 22, 29). The optical fractionator, a design-based stereological method for estimating total number of structures, is optically disected using a high magnification objective with a high numerical aperture (1, 4) in a known fraction of a defined reference space without affected by tissue shrinkage (13, 17, 22, 29). Briefly, the optical fractionator system consisted of a computer-assisted image analysis system including an Olympus BX-60 microscope hard-coupled to a Prior H128 computer-controlled x-y-z motorized stage, a high-sensitivity Hitachi 3CCD video camera system (Hitachi, Japan), and a Macintosh 8500 computer. All analyses were performed using NeuroZoom software, custom-designed morphology and stereology software developed in collaboration between Mount Sinai School of Medicine and the Scripps Research Institute (31) by an observer blinded to the survival time of each animal. Prior to each series of measurements, the instrument was calibrated. The region of lentivirus-positive neurons in the caudate, putamen, or substantia nigra was outlined at low magnification (1.25× objective) and at least 15% of the outlined region was measured with a systematic random design of disector counting frames (9890 μm^2) using a 100× planapo oil immersion objective with a 1.4 numerical aperture. The average thickness of the sections was empirically mea-

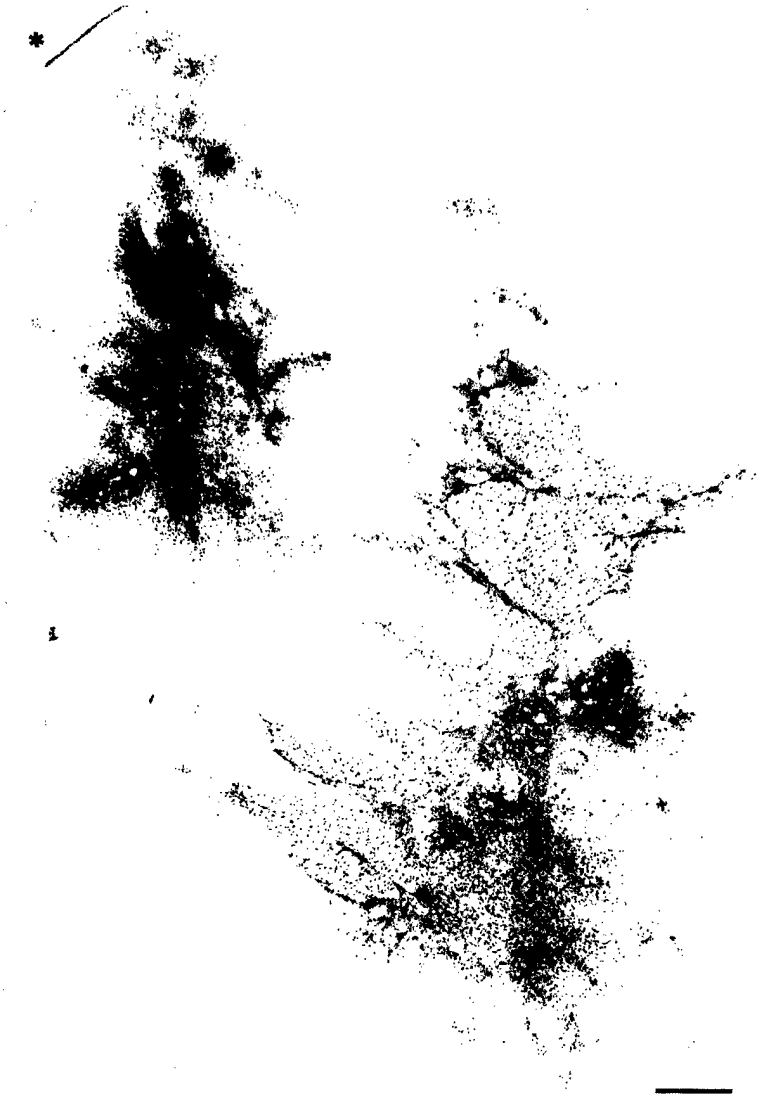

FIG. 1. Low power photomicrograph through the caudate nucleus of a monkey illustrating the area of βGal transduced (X-Gal histochemically stained) cells from two lentivirus injections. For orientation, the asterisk denotes the lateral ventricle. Scale bar represents 1000 μm.

sured at 30 μm but neurons were only counted within a 20-μm height of tissue, with guard heights of 5 μm at the top and 5 μm at the bottom of each section. Between 40 and 58 sections through the striatum of each monkey were evaluated. Thirteen sections through the substantia nigra were analyzed in the one monkey receiving an accurate lentivirus injection. The total number of lentivirus positive neurons (N) within the caudate nucleus, putamen, and substantia nigra was estimated using the following formula $N = Q\ t/h\ 1/\text{asf}\ 1/\text{ssf}$, where Q is the total disector number of lentivirus-positive neurons actually counted by optical scanning using uniform, systematic, and random design procedures in each disector for all measurements. The height (h) of the disector is known relative to the thickness of the section (t). The areal sampling fraction (asf) is the percentage (15%) of the section-sampling fraction (ssf, the area containing lentivirus positive neurons; 13, 17, 22, 29).

The percentage of βGal-positive cells that double labeled for the neuronal marker NeuN and the astrocytic marker GFAP were determined using stereological principals. Random but systematically chosen microscopic fields through areas of positive labeling from a full series of sections were evaluated using an Olympus inverted confocal microscope and Fluoroview software. In separate experiments, βGal-immunofluorescent cells were colocalized with either NeuN or GFAP. Within each field, the number of cells stained singly for βGal (Texas red), NeuN, or GFAP (Cy2: green fluorophore) or

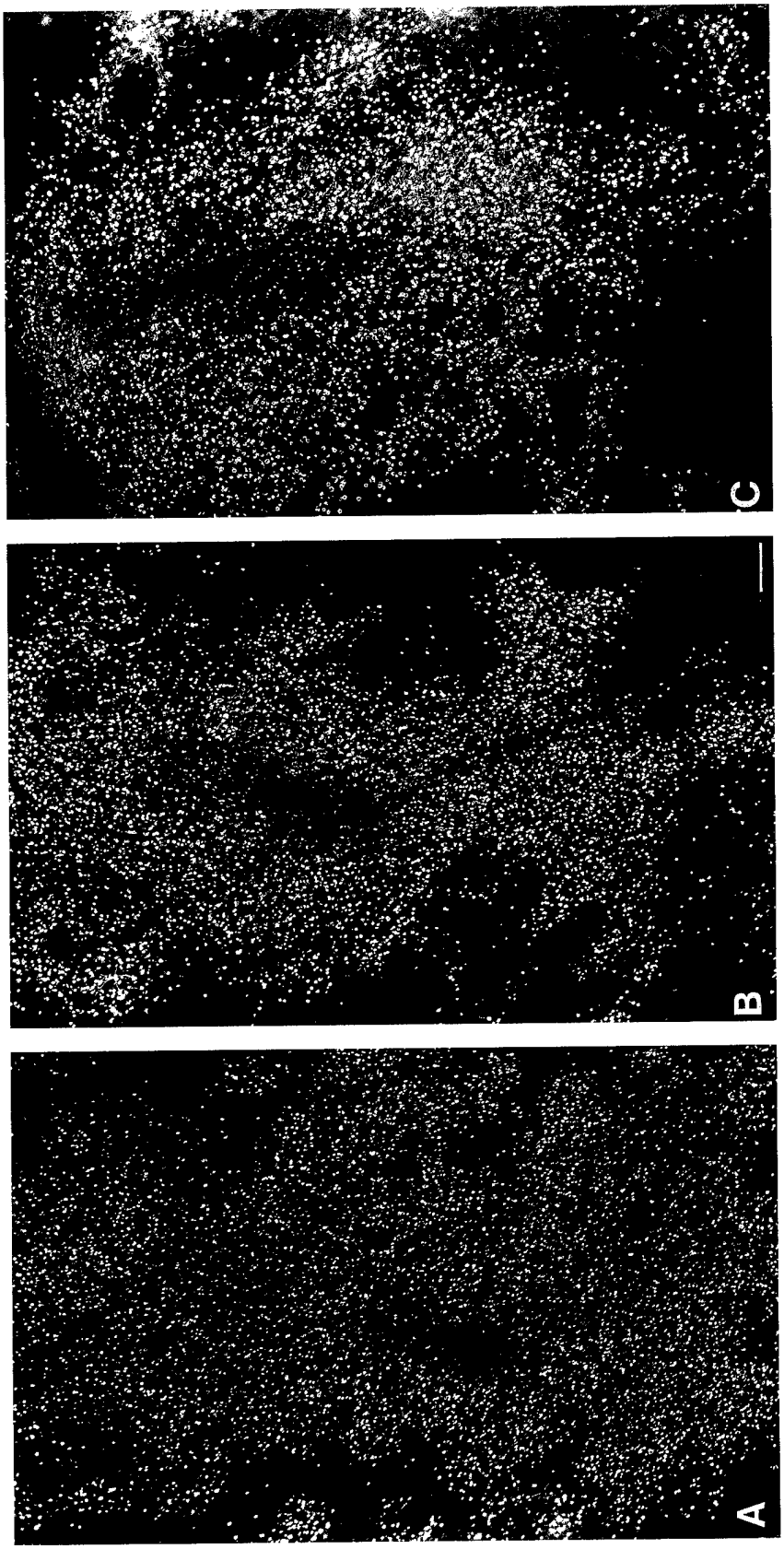

FIG. 2. Low power fluorescent photomicrographs of sections through the caudate nucleus of Rhesus monkeys (A) RH6055, (B) RH6058, and (C) RH6075 immunostained for βGAL. Monkeys RH6055 and RH6058 were sacrificed 1 month postinjection while RH6075 was sacrificed 3 months postinjection. Note the robust transfer of this marker gene in all three animals. Scale bar in B represents 165 μm in all panels.

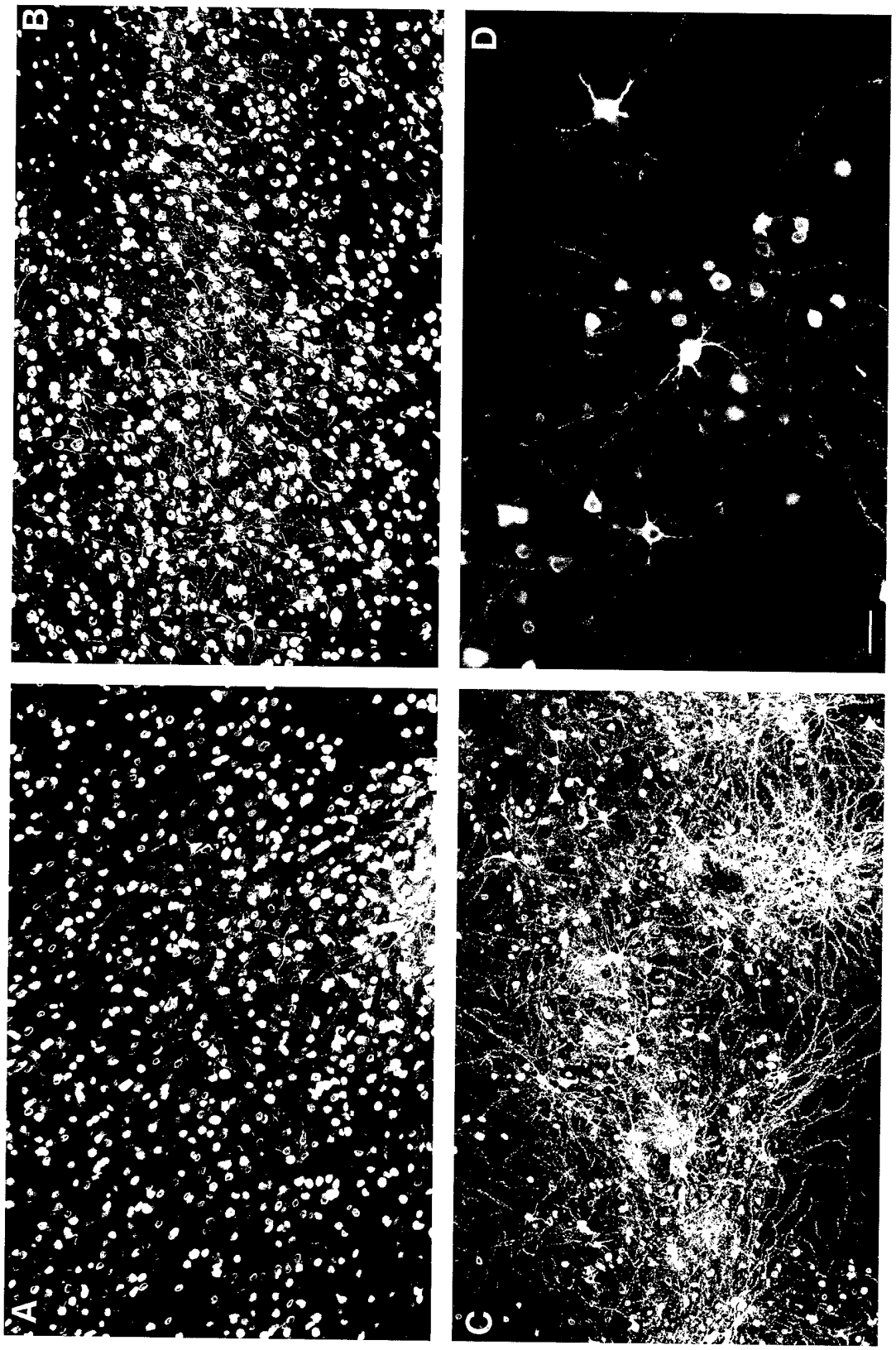

FIG. 3. Medium power fluorescent photomicrographs through the caudate nucleus of Rhesus monkeys (A) RH6055, (B) RH6058, and (C and D) RH6075 immunostained for βGAL. (A and B) Note that most labeled cells in monkeys sacrificed 1 month postinjection displayed mainly nuclear staining although some fiber staining can be observed. (C and D) In contrast, extensive networks of βGal-immunoreactive processes can be seen in the monkey sacrificed 3 months postinjection. Scale bar in D represents 70 μm for A–C, 35 μm for D.

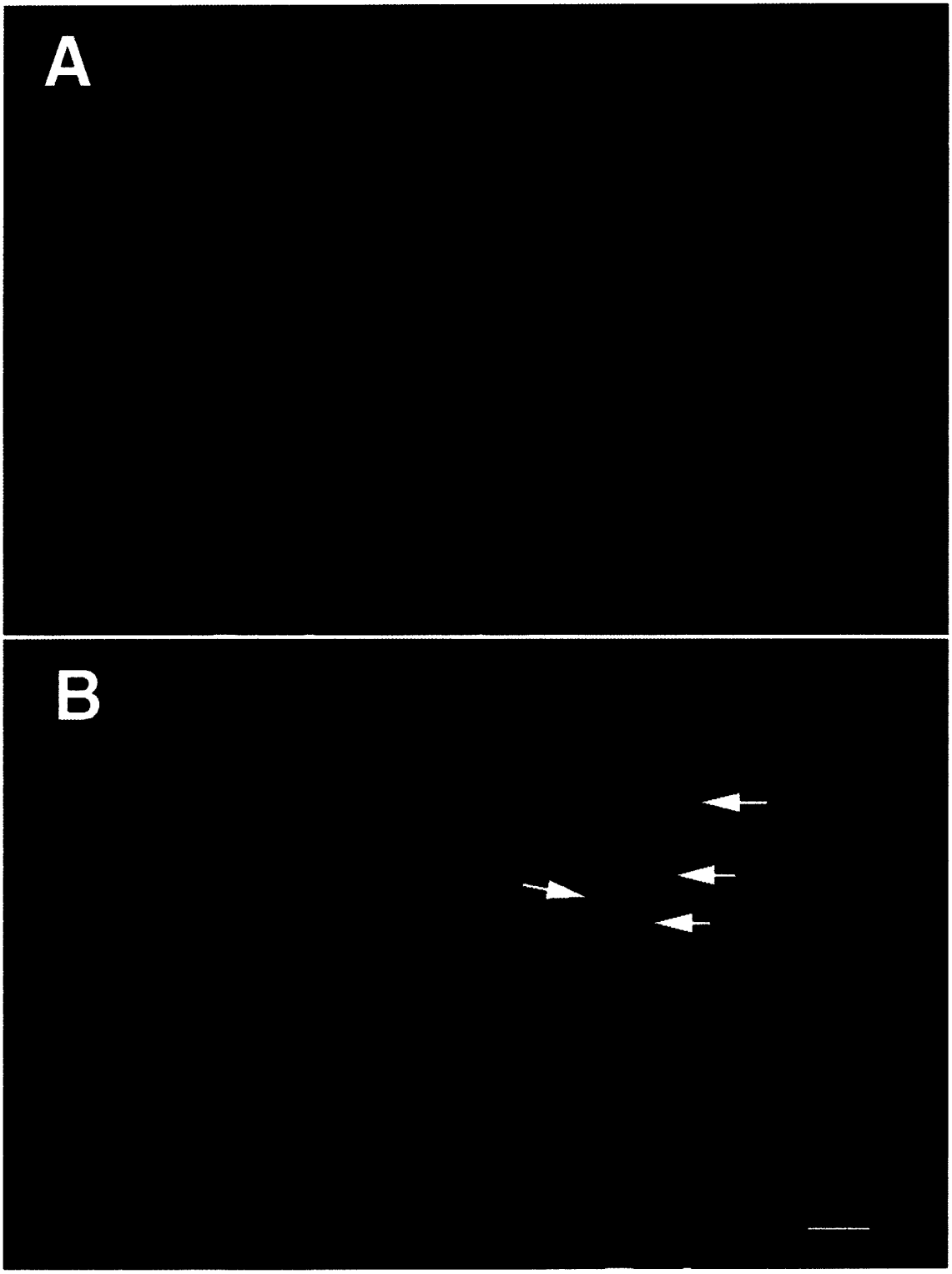

FIG. 4. (A) Low and (B) high power photomicrographs of laser-confocal microscopic images of βGal immunostained cells with labeling of this transgene seen in perikarya, processes and spinelike structures. Scale bar in A, 35 μm, 8 μm for B.

were double labeled for βGal and either NeuN or GFAP (yellow in the merged image) were quantified.

RESULTS

Lentiviral injections into striatum. All monkeys tolerated the surgery and lentivirus injections without noticeable complications. Both βGal histochemistry and immunofluorescence revealed robust gene expression within the striatum of all three monkeys (Figs. 1 and 2). Numerous βGal-positive cells were scattered throughout both the caudate nucleus and putamen on the side of the injection (Figs. 1 and 2). Sections incubated in a solution lacking the lacZ substrate did not display positive staining. Additionally, βGal labeling was never observed in the uninjected contralateral striatum. βGal-positive cells were identified up to 1.8 and 1.9 cm from the needle tract in the caudate nucleus and putamen, respectively. In both monkeys humanely killed at 1 month postinjection, the βGal reaction product was localized principally to striatal perikarya (Fig. 3A), although reaction product could be observed within a number of striatal processes as well (Fig. 3B). In the monkey sacrificed 3 months postinjection, a greater number of striatal cells displayed extensive labeling of processes (Figs. 3C and 3D). In some cells, spine-like structures appear to be labeled (Figs. 4A and 4B). The presence of βGal within nonnuclear cellular compartments was observed to a similar degree in sections processed for x-Gal histochemistry and βGal-immunohistochemistry. In contrast to labeled neurons in the striatum, there were no βGal-positive cells within the substantia nigra on the side ipsilateral to the striatal injection, indicating that the lentivirus was not retrogradely transported.

Stereological counts of βGal-positive cells in the striatum were performed on sections stained for x-Gal histochemistry (Table 1). Robust and consistent gene transfer was observed. For the two animals sacrificed 1 month postinjection, 930,218 and 1,192,359 βGal-positive cells were counted. For the monkey sacrificed 3 months postinjection, 1,501,217 βGal-positive cells were observed.

Confocal microscopy and double immunofluorescence was employed to assess the cell types expressing βGal.

TABLE 1

Stereological Counts of βGal Positive Cells

Case No.	Survival time	Caudate nucleus	Putamen	Striatum total	Substantia nigra
RH6055	1 month	764,149	166,069	930,218	N/A
RH6058	1 month	593,142	599,217	1,192,359	N/A
RH6075	3 months	567,453	933,764	1,501,217	187,305

Note. N/A, not available.

In all monkeys, both NeuN-immunoreactive neurons and GFAP-immunoreactive astrocytes colocalized the marker gene βGal. Qualitatively, it appeared that βGal-immunofluorescent cells were colocalized principally in cells that were labeled by the neuronal marker NeuN (Fig. 5), while fewer βGal-immunofluorescent cells colocalized with the astrocytic marker GFAP (Fig. 6). Quantitative analyses in all three monkeys supported these qualitative assessments. Stereological assessments of double label-immunostained sections revealed that the range of βGal/NeuN double-labeled neurons was between 80.53 and 87.54% (Table 2). In support of these data, βGal/GFAP double label experiments revealed a range of βGal/GFAP double-labeled astrocytes in the range of 14.79–20.73% (Table 2).

Nissl and NeuN-immunostained sections were employed to evaluate potential cytotoxicity of the lentiviral injection. Nissl-stained sections revealed a minimal inflammatory response in the striatum (Fig. 7). This response was similar in animals sacrificed at one month to the monkey sacrificed at three months postinjection. In the areas directly adjacent to the needle tract, there was minor perivascular cuffing around some blood vessels (Fig. 7B). The maximum distance from a needle tract to a cuffed vessel was 240 and 960 μm in the monkeys sacrificed at 1 month postlentivirus injection and 240 μm in the monkey sacrificed 3 months postinjection. This phenomenon was not observed in vessels slightly more distal to the injection site (Fig. 7A). We assessed between 45–52 Nissl-stained sections through the striatum in these three animals. The total number of "cuffed" vessels observed in these sections was 191 and 215, for the monkeys sacrificed at 1 month postlentivirus injection, and 22, for the monkey sacrificed at 3 months postinjection. Both Nissl (Figs. 7A and 7C) and NeuN (Fig. 7D) immunoreacted sections revealed normal striatal cytoarchitecture bilaterally without any obvious loss of striatal neurons or extensive reactive gliosis on the side of the injection.

Lentiviral injections into the substantia nigra. Lentiviral injections aimed for the substantia nigra in the two monkeys sacrificed 1 month postinjection were localized to the underlying crux cerebri. Only scattered βGal-positive cells were observed within the white matter tracts of these animals (data not shown). The lentivirus injection for the monkey sacrificed 3 months postsurgery was accurately placed in the ventral mesencephalon. In this animal, numerous cells scattered throughout the nigra proper and within the supranigral region exhibited extensive βGal labeling (Fig. 8A). βGal labeling in this region was principally nuclear, although the reaction product was also seen within the cytoplasm and proximal dendrites of a few cells (Fig. 8B). Labeled cells were observed up to 1.8 cm from the needle tract. Quantification of βGal-positive cells was only performed within the nigra for the monkey with

TABLE 2

Relative Levels of Lentiviral Gene Transfer to Neurons and Glia in the Nonhuman Primate Striatum

Case No.	NeuN/βGal (%)	βGal only (%)
RH6055	84.04	15.96
RH6058	87.54	12.45
RH6075	80.53	19.46
Case No.	**GFAP/βGal (%)**	**βGal only (%)**
RH6055	14.79	85.21
RH6058	14.67	85.33
RH6075	20.73	79.27

an accurate injection placement. For this animal, 187,305 βGal-positive cells were quantified (Table 1). Double label experiments revealed that most βGal-positive cells within the nigra colocalized with NeuN and few colocalized GFAP. Some of the βGal-positive cells colocalized with TH.

The lentivirus injection did not cause significant neurotoxicity within the ventral midbrain (Fig. 9). TH-immunostained sections revealed robust staining of dopaminergic cells with an apparently normal collection of TH-ir neurons on the side of the injection (Fig. 9A). Nissl-stained sections revealed healthy appearing neurons with normal morphological features throughout the substantia nigra, even in regions directly adjacent to the needle tract (Figs. 9B and 9C). Significant perivascular cuffing was not observed within the substantia nigra. In this monkey, only 2 "cuffed" vessels were seen in the 12 Nissl-stained sections that were analyzed. These were seen approximately 240 μm from the needle tract.

DISCUSSION

The present experiment was a feasibility study examining whether extensive lentiviral gene transfer could occur in the monkey brain without apparent cytotoxicity. The present data revealed the lentiviral construct results in a robust transfer of the marker gene βgalactosidase to the nonhuman primate nervous system. A number of important features of the present data should be emphasized. Between 930,218–1,501,217 cells within the striatum were βGal positive. This level of transduction is greater than any previously reported test of *in vivo* gene delivery in the central nervous system. Most (between 80 and 87%) of these transfected cells were neurons. This percentage of gene transduction into neurons is similar to what has been reported in rodents as 88.7% of cells successfully transfected with lenti-βGal in rats are neurons (4). It needs to be noted, however, that this feasibility study was performed in a small number of monkeys. The consistent results obtained across the three monkeys lead us to complete the present feasibility study and initiate functional studies, preventing the addition of more monkeys into this experiment. Thus although the monkey sacrificed at 3 months displayed the most robust gene transfer, one cannot, at present, draw meaningful conclusions regarding the sustained expression of the lentiviral delivered transgene in the monkey brain.

In the substantia nigra, 187,305 βGal-positive cells were identified in the monkey sacrificed 3 months postinjection. While this number is less than what was seen in the striatum, it needs to be noted that the nigral injection was in a 5-μl volume, while the striatal injections were made in a 40-μl volume. Indeed, extrapolating for volumetric differences, the number of infected cells in the midbrain of this animal is remarkably similar to the number of βGal-positive cells identified in the striatum. Only this monkey had an accurate placement of lentivirus into the ventral midbrain with the other two having injections placed into the underlying crux cerebri resulting in poor gene delivery. These latter injections illustrate the point that even with MRI guidance, misplaced delivery into white matter bundles can severely compromise the rate of gene transfection.

A critical aspect of the present data is the consistent level of gene transfer across the three monkeys. Most other studies in nonhuman primates using *in vivo* gene transfer approaches have found high variability in transgene expression. Although the numbers of monkeys employed in this feasibility study was relatively small by design, it is still notable that the monkey displaying the largest number of transfected cells was the one that had the longest postinjection survival. The caveats associated with small sample sizes are noted above. Further experiments will be needed to support the notion that long-term gene expression may be achievable using the lentiviral vector system. The use of self-inactivating lentiviral vectors containing post-transcriptional regulatory elements such as the one from the woodchuck hepatitis virus may be responsible for the sustained gene expression. The consistent level of gene transfer across the three subjects employed in the present study strengthens the concept that this gene delivery method might be advantageous for delivering therapeutic genes of interest to the human brain.

An interesting aspect of the present data is the presence of βGal-immunoreactivity within the cytoplasm and processes of some transfected cells despite the fact that the gene is a marker gene for the nucleus of the cell. The fact that similar results were observed with X-gal histochemistry and βGal-immunofluorescence indicates that this finding is not artifactual. Indeed, others have seen βGal within nonnuclear compartments under similar *in vivo* gene delivery conditions (6; Bohn, personal communication) and this may reflect the diffusion of the βGal to other cellular compart-

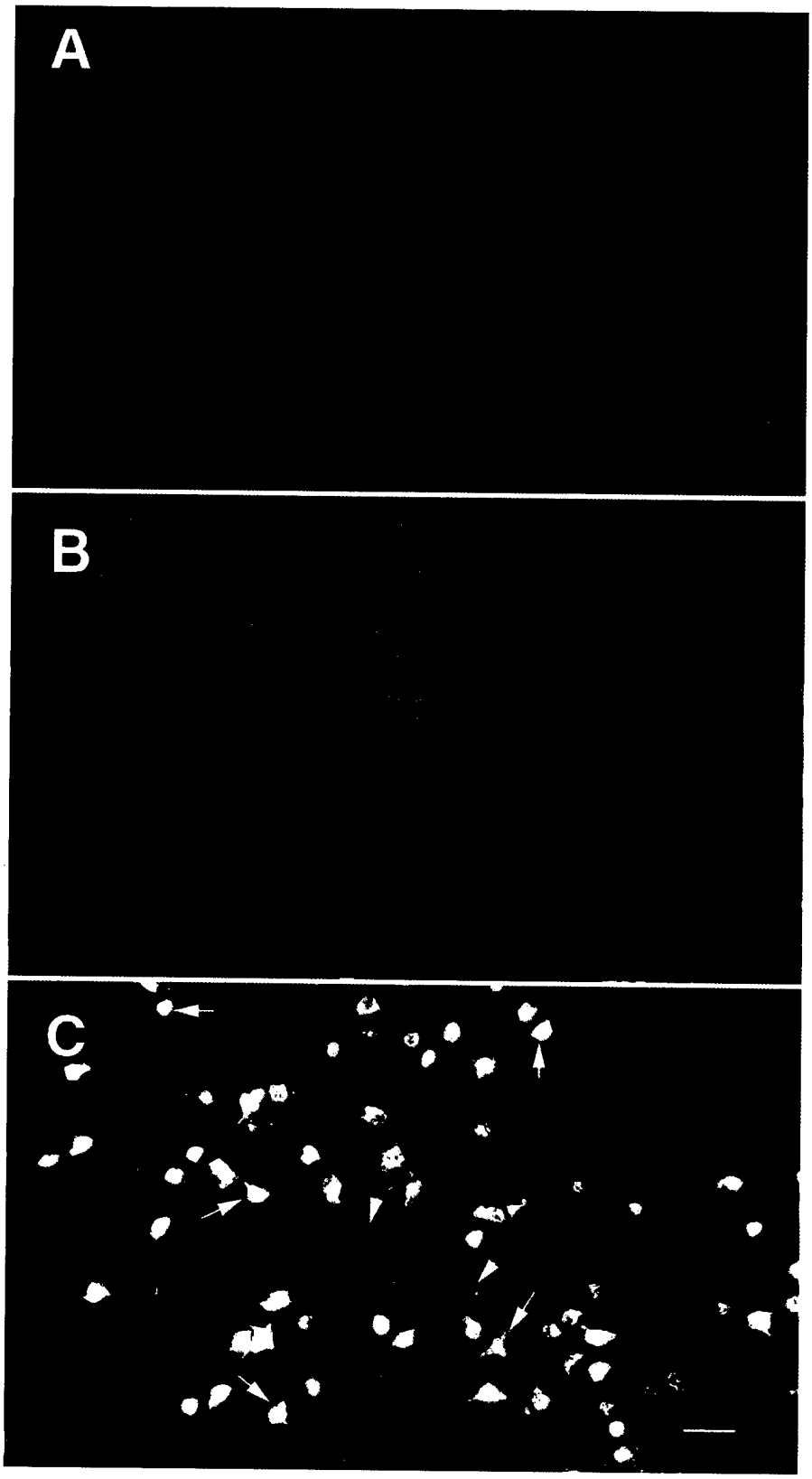

FIG. 5. Laser confocal microscopic images through a series of focal planes through the caudate nucleus stained for (A) β Gal, (B) NeuN, and (C) the composite image. Note the yellow appearing cells in C (arrows), denoting those cells that coexpress βGal and NeuN, indicating that the lentivirus has infected these neurons. In contrast, cells with only red reaction product (arrowheads) represent gene transfer into nonneuronal cells. Scale bar in C represents 35 μm in all panels.

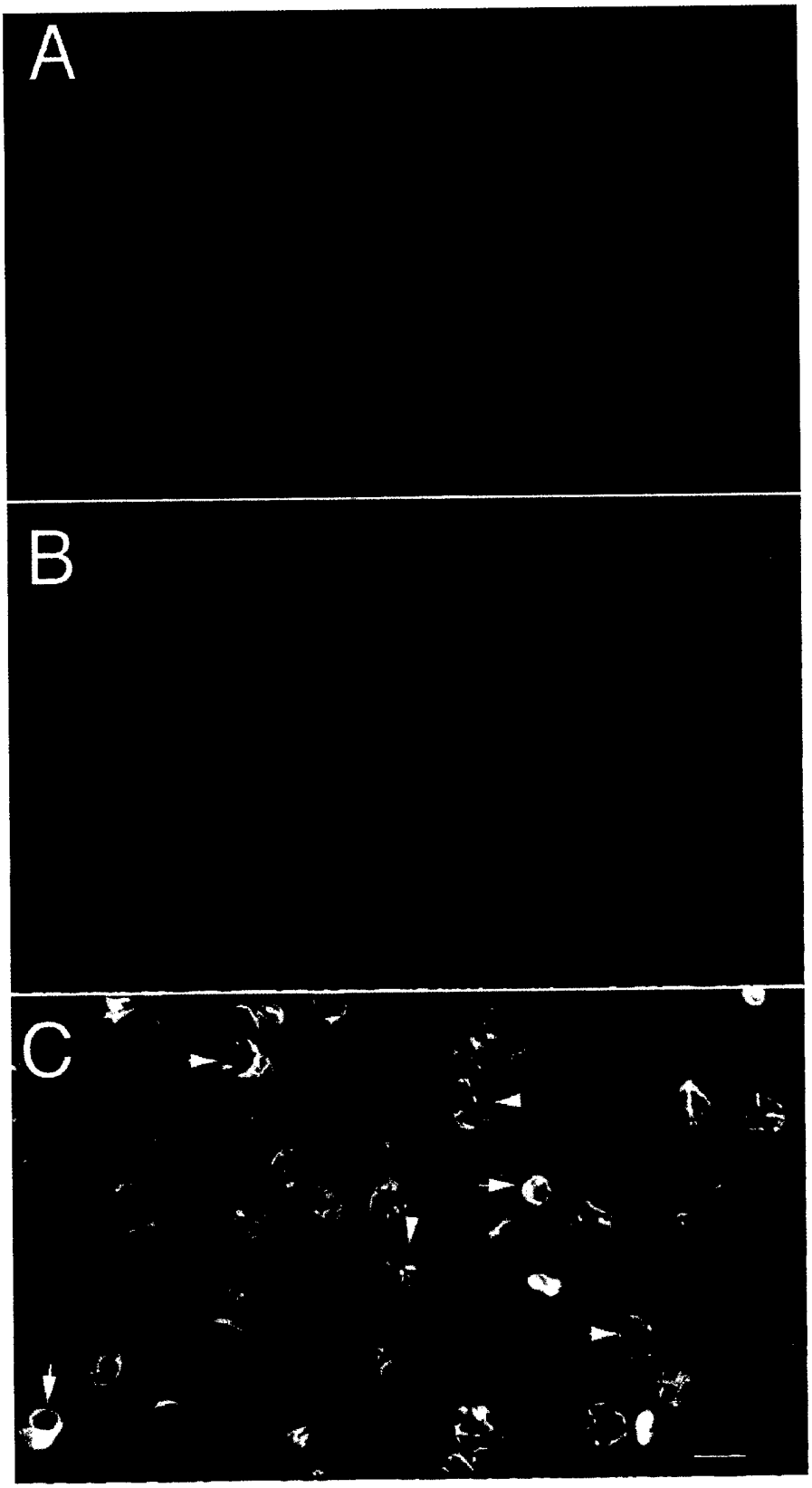

FIG. 6. Laser confocal microscopic images of a field through the caudate nucleus stained for (A) β Gal, (B) GFAP, and (C) the composite image. Note in C the few yellow-appearing cells, denoting that the βGal only infected a few astrocytes arrows. In contrast, cells with only red reaction product represent gene transfer into nonastrocytes (arrowheads). Scale bar in C represents 50 μm in all panels.

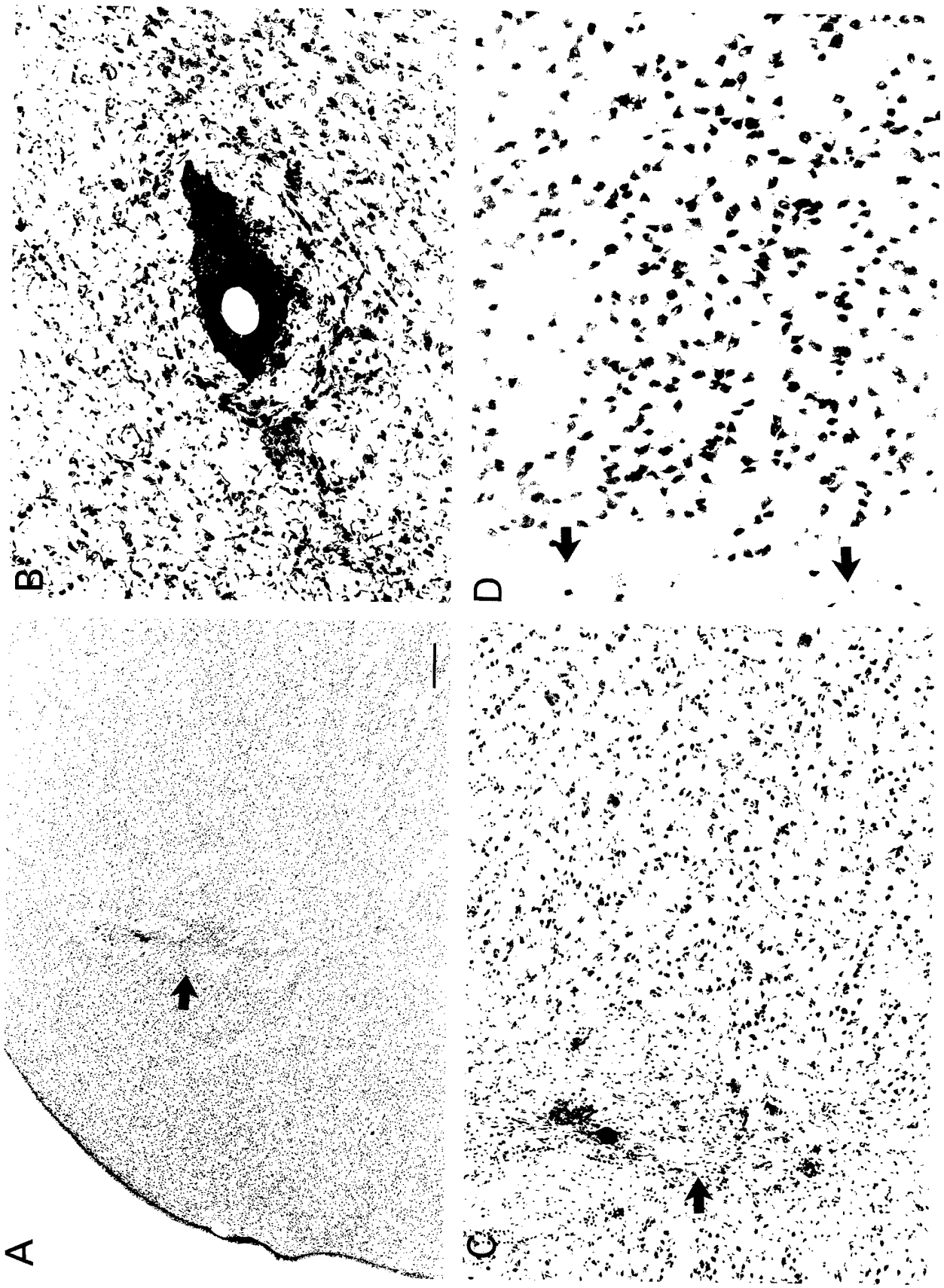

FIG. 7. (A,B,C) Nissl and (D) NeuN-immunostained sections through the caudate nucleus. Note the normal appearing cytoarchitecture of the striatum proximal to the injection site (arrows) in the Nissl (A,C) and (D) NeuN-immunostained sections, indicating that the lentivirus injections were not cytotoxic to the striatum. (B) In some regions proximal to an injection site, there was evidence of perivascular cuffing. Scale bar in D represents the following magnifications: A, 500 μm; B and C, 165 μm; D, 65 μm.

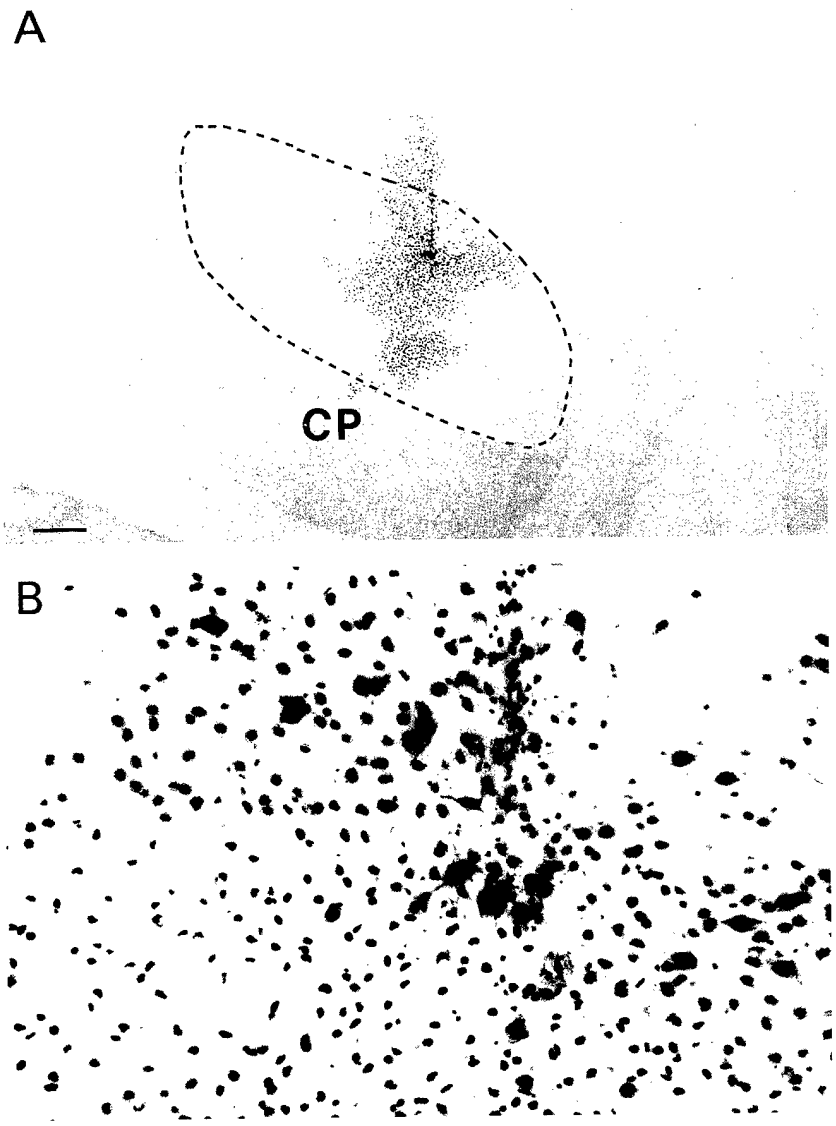

FIG. 8. (A) Low and (B) high power photomicrographs through the midbrain of RH6075, illustrating the breath of lentiviral gene transfer 3 months following an intranigral injection. Sections were stained histologically for βGal. CP, cerebral peduncle. Dashed lines delimit the perimeter of the substantia nigra. Scale bar in A represents the following magnifications: A, 500 μm; B, 65 μm.

ments over time. The fact that βGal was most extensively seen within neurites in the monkey sacrificed 3 months postinjection supports this hypothesis.

Other groups have evaluated other *in vivo* gene delivery systems in the primate brain. Davidson and coworkers found βGal-positive cells in the Rhesus monkey following adenovirus delivery 1 week following injection (9). Using an adenovirus harboring the herpes simplex viral thymidine kinase. Goodman *et al.* (15) used PCR to localize the vector DNA for up to 6 weeks following delivery. Bohn and colleagues performed the most detailed study of adenoviral expression in the nonhuman primate (6). These experiments resulted in inferior and more inconsistent gene transfer. Using multiple titers and volumes of adenovirus, they found thousands of βGal positive cells in two of three African Green monkeys sacrificed 1 week following injection. Significant variability in βGal gene expression was observed in these monkeys between 1 and 3 months postinjection. As many as 292,448 positive βGal-positive cells were identified in one monkey 1 month following adenoviral injection. However, in the monkey that survived for 3 months postinjection, only 156 and 24 βGal-positive cells were identified at the two injection sites, respectively.

Concomitant with excellent and sustained gene expression in the present study was the apparent absence of a significant cytotoxic or immune response to the

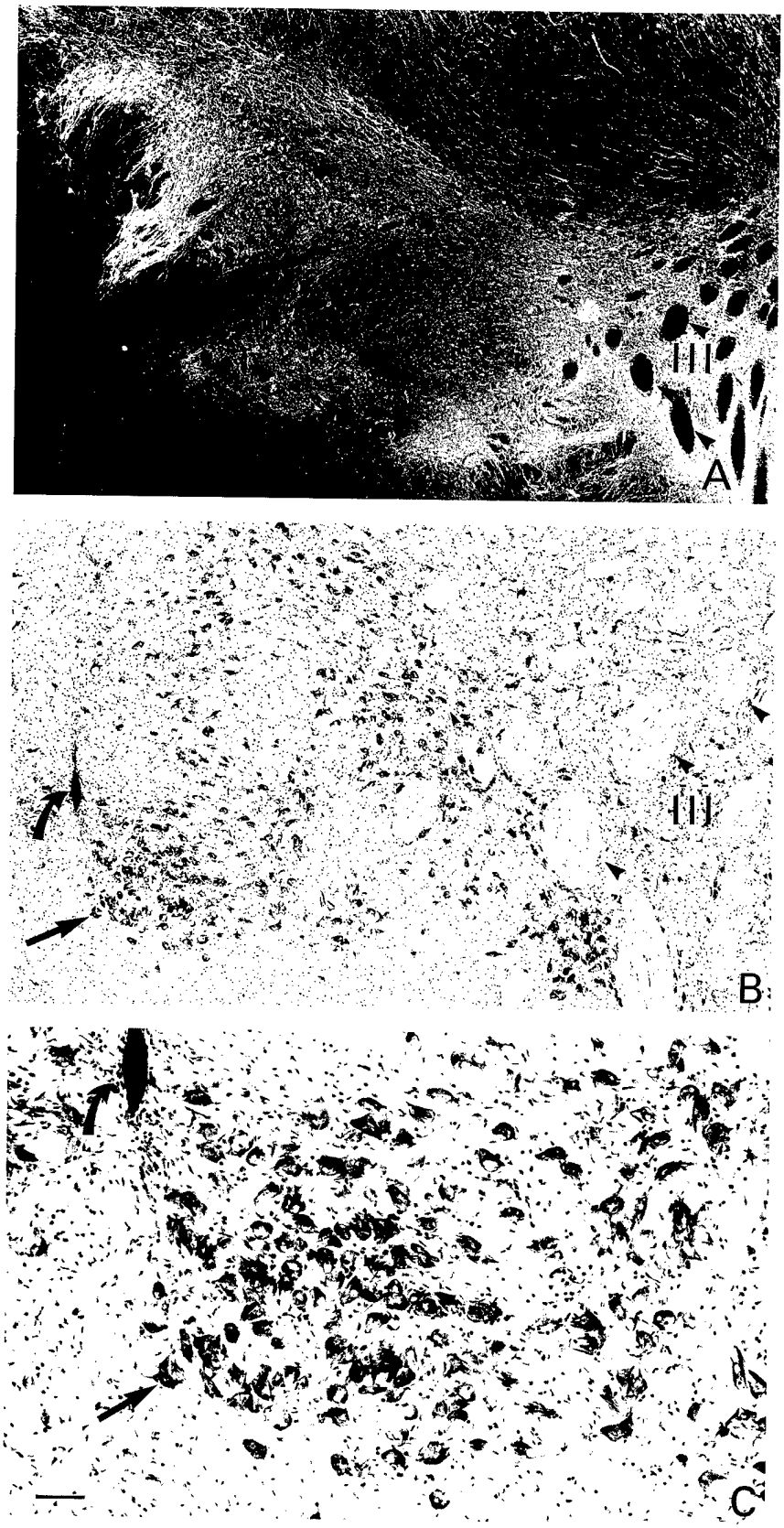

FIG. 9. (A) TH-immunofluorescence through the substantia nigra of a monkey receiving a lentiviral-βGal injection. Note the normal cytoarchitectonic appearance of this region. (B) Low and (C) high power photomicrographs of Nissl-stained sections through the nigral needle tract (curved arrows) illustrate the presence of normal appearing nigral cells adjacent to the lentivirus injection. Scale bar in C represents the following magnifications: A, 325 μm; B, 165 μm; C, 65 μm; and D, 500 μm.

lentivirus injection. Both Nissl- and NeuN-stained sections revealed that the lentiviral injections did not induce notable cytotoxicity in either the striatum or the substantia nigra. Further, only minor perivascular cuffing was observed within the striatum of these animals and the perivascular cuffing that was observed was principally limited to the regions of the injection tract. In contrast, no perivascular cuffing was observed in striatal or midbrain regions slightly distal to the injection site. This lack of toxicity is dissimilar to what has been seen in monkeys previously using other *in vivo* delivery systems. In this regard, adenoviral injections into the monkey striatum results in extensive perivascular cuffing and a mild to moderate immune response in monkeys with reasonable βGal expression and an intense immune response in monkeys with unsuccessful transduction (6). This group concluded that the degree of immune response engendered by adenoviral limits transgene expression. The robust and consistent data gene delivery seen in the present study, in the absence of obvious neurotoxicity and immunogenicity, support the use of lentiviral gene transfer methods in the primate nervous system.

With the feasibility of using lentiviral gene transfer methods in nonhuman primates now established, its use for the delivery of therapeutic genes can be investigated. In rodents, biologically relevant lentiviral gene delivery has been demonstrated in two systems; the cholinergic basal forebrain systems and the dopaminergic nigrostriatal system. In this regards, Blömer and colleagues (5) demonstrated that lentiviral delivery of nerve growth factor can prevent the degeneration of cholinergic neurons within the septodiagonal band region that normally would occur following transection of the fimbria–fornix transection. This model system models, in some respects mimics, the degeneration of cholinergic basal forebrain neurons seen in Alzheimer's disease and has been applied previously to nonhuman primates (14, 20, 28). Furthermore, aged monkeys display cognitive deficits that are reversed with pharmacological augmentation of the cholinergic basal forebrain system (for review, see 3). Determining whether age-related cognitive deficits displayed by nonhuman primates can be reversed by lentiviral delivery of trophic factors would seem to be an important and rationale approach toward determining whether this approach can impact neurological problems.

Perhaps a more straightforward application of the lentiviral gene delivery system involves studies the delivery of the glial derived neurotrophic factor (GDNF) in animal models of Parkinson's disease. Using the same gene transfer system employed in the present study, Déglon and colleagues (10) recently demonstrated that dopaminergic nigral neurons destined to die following axotomy could be rescued by supranigral lentiviral delivery of GDNF. For many reasons, the best animal model of Parkinson's disease is the MPTP-treated monkey. The present study demonstrates that robust and consistent lentiviral gene delivery can be achieved in the nigra and striatum in nonhuman primates. Animal studies have consistently demonstrated that GDNF can provide functional and structural protection and regeneration to dopaminergic nigrostriatal neurons (for reviews, see 17 and 21). However, a recent case report indicated the lack of anatomical and clinical changes following infusions of the GDNF protein into the lateral ventricle of a patient with PD (19, 26). We argued in that study that the lack of any effect of GDNF in the PD patient was principally due to the method and location of GDNF delivery and not due to the absence of GDNF potency. We believe that site-specific delivery of GDNF will be necessary to exploit the trophic effects of this potent molecule in patients and that site-specific delivery of lentiviral gene vectors might be an optimal delivery vehicle. Studies testing this hypothesis, in addition to examining potential peripheral nervous system toxicity, in nonhuman primate models of PD are underway. Should they be successful, then careful consideration for the use of this technology in a clinical trial for PD patients may be warranted.

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LENTIVIRAL VECTOR GENE TRANSFER TO THE NONHUMAN PRIMATE NIGROSTRIATAL SYSTEM. M.E. Emborg[1*], J. Block[2], S.Y. Ma[1], Y.-P. Chu[1], S. Palfi[3], R. Roitberg[1,2], J.Stanzell[1], P. Hantraye[4], N. Deglon[3], P. Aebischer[3] and J.H. Kordower[1]. [1]Res. Ctr. for Brain Repair and Dept. Neurol. Sci., Rush Univ., Chicago, IL60612; [2]Div. Surg. Res. and Gene Ther. Ctr., Lausanne Univ., Switzerland; [3]Dept. Neurosurg., Univ. IL Med. Ctr, Chicago, IL60612; [4]CEA CNRS URA2210 Serv. Hosp. F.Joliot, CEA DSV, DRM Orsay cedex, France.

Viral vectors are a means by which genes can be transferred to the CNS. The present report studied whether stable lentiviral gene can be achieved in the monkey nigrostriatal system. Three Rhesus monkeys (male, 8.1-10 kg, 7-8 yrs.) received injections of lentivirus encoding for the marker gene β-galactosidase (β-Gal). Each animal received lentivirus injections into the caudate (n=2; 5µl and 10µl) and putamen (n=3; 10µl, 10µl and 5µl) in one side, and into the substantia nigra (SN; n=1; 5µl) on the other side. Two monkeys were sacrificed 1 month post-injection while the third monkey was sacrificed 3 months post-injection. Robust incorporation of the β-Gal gene was seen in the striatum of all 3 monkeys. Stereological counts revealed that 930,218; 1,192,359; and 1,501,217 cells in the striatum were β-Gal positive in each monkey, respectively. Only the 3rd monkey had an injection directly placed into the SN and 187,308 β-Gal positive cells were identified. The injections induced only minor perivascular cuffing and no cytotoxic response was observed. Double label experiments revealed that predominantly neurons (80-87%) were transfected. These data indicate that robust transfection of striatal and nigral cells can occur in the nonhuman primate brain for up to three months. Studies are now ongoing testing the ability of lentivirus encoding for dopaminergic trophic factors to augment the nigrostriatal system in nonhuman primate models of Parkinson's disease. (Support: Department of Defense and Swiss National Science)

294.2

CONVECTION-ENHANCED GENE TRANFSFER OF ADENO-ASSOCIATED VIRAL VECTOR IN PARKINSONIAN MONKEYS. Malgorzata Kohutnicka[1], John Bringas[2*], Phil Pivirotto[2], Janet Cunningham[2], William Jagust[2,4], Jamie Eberling[2,4] and Krys S. Bankiewicz[1,2]. [1]Molecular Therapeutic Section, LMNN, NINDS, NIH, Bethesda, MD, [2]Lawrence Berkeley National Laboratory, UC Berkeley, Berkeley CA, [3]Avigen Inc., Alameda, CA, [4]Department of Neurology, UC Davis, Davis CA.

In-vivo delivery of viral vectors results in limited gene transfer to surrounding injection site. For gene therapy to work in the CNS, safe and efficient methods for delivery of viral vectors within target regions must be applied. We used a convection-enhanced delivery (CED) method with an adeno-associated virus (AAV)-based vector in an attempt to maximize the delivery of AAV in four behaviorally stable, unilaterally MPTP-treated monkeys. AAV Lac-Z (n=2) or AAV l-aromatic amino acid decarboxylase (AADC) (n=2) vectors were administered on the MPTP-treated side. A solution (180µl) containing $5x10^{11}$ of AAV/AADC or $2x10^{11}$ of AAV/LacZ was infused into the basal ganglia at 6 sites. No adverse clinical or histopathological effects were observed in any of the animals following AAV CED. AAV/AADC-treated monkeys showed robust cellular AADC-IR staining in over 85% of the striatum and 100% of the globus pallidus . Stereological counts of AAV-infected cells revealed over $20x10^6$ infected cells with a cell density of $2x10e^6$cells/mm^3. Based on double immunostaining and confocal microscopy 1 of 4 neurons, but not glia cells, were found to express AADC in the striatum. These data demonstrates that CED of AAV can result in robust, efficient and safe gene transfer in the striatum of monkeys. Supported by US DOE Contract BG98039.

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RESTORATION OF DOPAMINERGIC ACTIVITY IN PARKINSONIAN MONKEYS BY ADENO-ASSOCIATED VECTOR-MEDIATED GENE TRANFSFER OF AADC. Krys Bankiewicz[1,2*], Judy Harvey-White[1], John Bringas[2], Phil Pivirotto[2], Malgorzata Kohutnicka[1], Janet Cunningham[2], William Jagust[2,3] and Jamie L. Eberling[2,3]. [1]Molecular Therapeutic Section, LMNN, NINDS, NIH, Bethesda, MD; [2]Lawrence Berkeley National Laboratory, UC Berkeley, Berkeley CA 94720; [3]Department of Neurology, UC Davis, Davis CA; [4]Avigen Inc., Alameda, CA.

Parkinson's disease is a common neurodegenerative disease affecting the dopaminergic (DA) system. Both tyrosine hydroxylase (TH) and l-aromatic amino acid decarboxylase (AADC) enzymes are responsible for DA synthesis and are affected by idiopathic and MPTP-induced parkinsonism. We used an adeno-associated virus (AAV)-based vector with AADC cDNA in an attempt to restore DA levels in four behaviorally stable, unilaterally MPTP-treated monkeys. AAV Lac-Z (n=2) or AAV AADC (n=2) vectors were administered on the MPTP-treated side using a convection-enhanced (CED) bulk flow method. Positron emission tomography (PET) using the AADC tracer 6-[18F]fluoro-L-m-tyrosine (FMT) was performed in a blind fashion 1 month prior to and 6 weeks following AAV infusion. One week after the second PET scan, 200/20mg of L-DOPA/carbidopa (Sinemet) was administered orally and animals were sacrificed 40-50 minutes later. Biochemical analysis (tissue punch analysis by HPLC to determine levels of L-DOPA, DA, DOPAC and HVA) and in-vitro measures of AADC activity were performed. Cortical and striatal brain punches contained high levels of L-DOPA in all four monkeys, but only the AAV AADC treated monkeys showed restoration of DA, DOPAC and HVA in the striatum on the MPTP-treated side. In the areas that showed AADC-expression, L-DOPA levels were reduced and DA, DOPAC and HVA levels were increased suggesting rapid metabolic conversion of L-DOPA to DA and its metabolites. AADC activity was restored to normal levels on the MPTP-treated striatum by gene transfer. FMT was converted and stored in the transfected cells as well. These data demonstrates that use of the pro-drug, L-DOPA, following AAV-AADC treatment can restore DA levels in MPTP-treated monkeys. Using this approach, brain levels of DA can be regulated by controlling the levels of the peripherally administered L-DOPA. These results may have direct application for the treatment of idiopathic PD. Supported by US DOE Contract BG98039

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ASTROCYTES RETROVIRALLY TRANSDUCED WITH A GLIAL FIBRILLAR ACIDIC PROTEIN-TYROSINE HYDROXYLASE TRANSGENE ELICI BEHAVIORAL RECOVERY IN EXPERIMENTAL PARKINSONISM N. Cortez F. Trejo,[2] P. Vergara,[1] and J. Segovia[1*]. [1]Dept. Fisiologia, Biofisica y Neurociencia CINVESTAV-IPN; [2]Programa Multidisciplinario de Biomedicina Molecula CINVESTAV-IPN, México, 07300 D.F.

Astrocytes engineered to express a tyrosine hydroxylase (TH) transgene are promising platform for treatment of Parkinson's disease (PD). Among the attribut that recommend astrocytes for the delivery of therapeutic molecules to the CNS v find, that they are normal residents of the CNS, have a long life span, and a capable of releasing different substances. We had previously demonstrated that bo the implantation of C6 glioma cells engineered to express a TH cDNA driven by promoter of the human glial fibrillary acidic protein (GFAP), *gfa2*, or the dire transfer of the transgene into the brain, induce behavioral recovery in a rodent mod of PD. Here we report the construction of a retroviral vector to transduce primary astrocytes to express the *gfa2-TH* transgene. Transduced astrocytes we stereotactically implanted into the dopamine-denervated striata. Lesioned rats w tested for their turning behavior in response to apomorphine. Rats that received *gfa2-TH* transduced astrocytes showed a significant reduction in turning behavi whereas rats that received astrocytes expressing the *lacZ* gene did not change th turning behavior. We obtained striata from implanted rats and observed that in that showed behavioral recovery there was expression of the *gfa2-TH* transgene; t was demonstrated by transgene mRNA expression, determined by RT-PCR, and protein expression, assessed both by immunohistochemistry and western b analysis. These results further recommend astrocytes as a platform for PD g therapy. Supported by a CONACYT (México) grant 27118N (J.S.)

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REGENERATIVE AND PROTECTIVE EFFECTS OF ENCAPSULATED GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF)-PRODUCING CELLS IMPLANTED INTO THE RAT STRIATUM. H. Yoshida*, I. Date, T. Shingo, K. Fujiwara, K. Kobayashi and T. Ohmoto, Dept. of Neurological Surgery, Okayama Univ. Med. Sch., Okayama, Japan 700-8558.

Intrastriatal implantation of encapsulated cells genetically modified to produce glial cell line-derived neurotrophic factor (GDNF), which possesses neurotrophic effect on dopaminergic (DA) neurons, is an useful technique for the treatment of animal models of Parkinson's disease. In this study, we describe the regenerative and protective effects of encapsulated GDNF-producing cells on DA neurons.

We introduced human GDNF gene into the baby hamster kidney (BHK) cells and established GDNF-producing cells (BHK-GDNF). Encapsulated BHK-GDNF or parent BHK (BHK-Control) cells were implanted into the striatum of female Sprague-Dawley rats 2 weeks before or 1 week after intrastriatal injection of 6-hydroxydopamine (6-OHDA). Drug-induced rotation was recorded during the experimental period. Then the animals were sacrificed, and tyrosine hydroxylase immunostaining of the striatum and the substantia nigra was performed.

Histological and chemical examination revealed good survival of encapsulated GDNF-producing cells and constant GDNF production from the capsule. Those animals implanted BHK-GDNF before or after the injection of 6-OHDA showed larger number of surviving DA neurons and less drug-induced rotations than the control animals. The results suggest that the implantation of encapsulated GDNF-producing cells has regenerative and protective effects on DA neurons, and this technique is expected to be applied for the treatment of Parkinson's disease.

294.6

CASPASE INHIBITION AND EX VIVO BCL-2 TRANSDUCTION FOR AUGMENTATION OF MESENCEPHALIC DOPAMINE NEURON SURVIVAL IN VITRO AND FOLLOWING TRANSPLANTATION C.E. Sortwell*[1], B.F. Daley[1], M.R. Pitzer[1], M.W. Halterman[2], H.J. Federoff[2] and T.J. Collier[1]. Dept. of Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612; [2]Dept. of Neurology and Div. of Molecular Medicine and Gene Therapy, Univ. of Rochester, Rochester, NY 14642.

Strategies to inhibit apoptotic cell death of mesencephalic dopamine (DA) neuron have proven effective in enhancing grafted DA neuron survival. Recently, exposur of mesencephalic cell suspensions to a caspase inhibitor immediately prior t grafting was demonstrated to increase grafted DA neuron survival over 3-fo (Schierle et al., 1999). We have identified that the time course of apoptotic ce death of grafted mesencephalic cell suspensions occurs primarily over the first we following transplantation, with greatest cell loss occurring immediately followin implantation. In order to provide protection from apoptosis for the duration of th critical early period, we have developed a dual approach of utilizing caspa inhibition and transduction with the herpes simplex virus (HSV) amplicon vect encoding bcl-2 in a cell reaggregate system. Mesencephalic cells were reaggregat in a rotating incubator in the presence of serum, striatal conditioned media and th caspase inhibitor Ac-YVAD-cmk. After four days in vitro (DIV 4) reaggregat were exposed to either HSVbcl-2, HSVlac (MOI = 0.5) or no vector for 24 hours. Four days post infection, aggregates were either grafted into the unilaterally D denervated striatum of F344 rats or analyzed histochemically. At DIV 3 (4 da post-infection), cell reaggregates exhibited uniform infection throughout, determined by X-gal histochemistry, and possessed numerous tyrosine hydroxylas positive (TH+) neurons. Mesencephalic reaggregates maintained in culture unt DIV 12 similarly possessed high numbers of TH+ cells, with transduc reaggregates displaying X-gal positive or bcl-2 positive cells, although significant fewer in number than observed at DIV 8. Behavioral and survival effects of caspa inhibition and bcl-2 transduction on grafted reaggregates are the subject of ongoi studies with results to be presented. Supported by NIH grants AG00844 (CE AG10851 (TJC) and the Schweppe Foundation.