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Award Number: DAMD17-98-1-8630

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

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REPORT DATE: October 2000

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2000	Annual (15 Sep 99 - 14 Sep 00)

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)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel 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 second year, critical studies have been performed and completed. In the first 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. In the second experiment, lentiviral delivery of GDNF was performed in MPTP-treated monkey. It was revealed that this therapy prevented the motor deficits and nigrostriatal degeneration normally displayed by parkinsonian animals. These data indicate that lentiviral delivery of GDNF may be an efficacious means by which to prevent the structural and functional consequences of nigrostriatal degeneration seen in in patients with PD.

14. SUBJECT TERMS
Neurotoxin

15. NUMBER OF PAGES
38

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

FOREWORD

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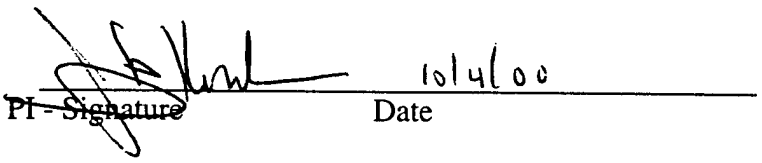
N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.



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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 MPTP-treated 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.

We have just completed a large study which has been accepted as a Research Article in *Science*. This study examines the influence of lenti-GDNF upon the structural and functional consequences of nigrostriatal degeneration in 1) aged monkeys and 2) MPTP treated monkeys. Portion of the aged monkey experiment were reported in the previous Progress Report.

The second experiment examined the effects of lenti-GDNF upon motor behavior and nigrostriatal degeneration in MPTP-treated monkeys. Initially, twenty young adult Rhesus were initially trained 3 days per week until asymptotic performance was achieved on a hand-reach task in which the time to pick up food treats out of recessed wells was measured. Each experimental day, monkeys received 10 trials per hand. Once per week, monkeys were also evaluated on a modified parkinsonian clinical rating scale (CRS). All monkeys then received an injection of 3mg MPTP-HCl into the right carotid artery, initiating a parkinsonian state. One week later, monkeys were evaluated on the CRS. Only monkeys displaying severe hemiparkinsonism with the classic crooked arm posture and dragging leg on the left side continued in the study (n=10). It is our experience that monkeys with this behavioral phenotype display the most severe lesions neuroanatomically and do not display spontaneous recovery behaviorally. Based upon CRS scores, monkeys were matched into two groups of five monkeys which received that day lenti-ßGal or lenti-GDNF treatment. Using MRI guidance, all monkeys received lentivirus injections into the caudate nucleus (n=2), putamen (n=3), and substantia nigra (n=1) on the right side using the same injection parameters as in Experiment 1. One week later, monkeys began retesting on the hand reach task 3 times per week for 3 weeks per month (27). For statistical analyses, the times for an individual week were combined into a single score. During the weeks of hand reach testing, monkeys were also scored once per week on the CRS. Individuals blinded to the experimental treatment performed all behavioral assessments. Three months after lentivirus treatment, monkeys received a FD PET scan, were sacrificed 24-48h later, and histologically processed as before.

Within one week following the lentivirus injections, one monkey from each group died. Necropsies from these animals revealed only the presence of mild multifocal random hepatocellular coagulation necrosis. Due to these deaths, all remaining monkeys underwent detailed necropsies following the planned sacrifices and no significant abnormalities in any organs were seen.

Prior to MPTP treatment, all young adult monkeys scored zero on the CRS. Following MPTP, but prior to lentivirus injection, monkeys in the lenti-GDNF and lenti-ßGal groups averaged 10.4±.07 and 10.6±0.6 respectively on the CRS ($p>0.05$). Following lentivirus treatment, significant differences in CRS scores were seen between the two groups (Kolmogorov-Smirnov test; $p<0.0001$). CRS scores of monkeys receiving lenti-ßGal did not change over the three month post-treatment period. In contrast, CRS scores of monkeys receiving lenti-GDNF significantly diminished during the 3 month post-treatment period. Scores began to decrease in the first month post-lenti-GDNF treatment. However, statistically significant differences between lenti-GDNF and lenti-ßGal were only discerned at post-treatment observations 6,7,8, and 9 (Kolmogorov-Smirnov test; $p<0.04$ for each comparison).

Lenti-GDNF treated animals also improved performance on the operant hand reach task. Under-pre-MPTP conditions, animals in both groups performed this task with similar speed. For the "unaffected" right hand, no differences in motor function were discerned for either group relative to pre-MPTP levels or to each other ($p>0.05$). In contrast, performance with the left hand was significantly improved in lenti-GDNF treated animals relative to controls ($p<0.05$). Following MPTP, all lenti-ßGal treated animals were severely impaired, with monkeys often not performing at all, or requiring more than the maximally-allowed 30 seconds. In contrast, three of the four lenti-GDNF monkeys performed the task with the left hand at near normal levels while one lenti-GDNF-treated monkey was impaired and performed this task in a manner similar to the lenti-ßGal treated animals. Between groups, significant differences in performance were discerned on post-treatment tests 4, 6, 7, 8, and 9. ($p<0.05$ for each comparison).

Just prior to sacrifice, all monkeys underwent FD PET scans. Qualitatively, all lenti-ßGal treated monkeys displayed pronounced FD uptake in the left striatum and a comprehensive loss of FD uptake on the right side. In contrast, two of four lenti-GDNF treated animals displayed robust and symmetrical FD uptake on both sides. The remaining two lenti-GDNF monkeys displayed reduced

FD uptake on the right side, but with Ki values 50-100% greater than lenti-ßGal controls. Quantitatively, no differences in FD uptake were observed between groups within the left striatum ($p>0.05$). In contrast, there was a significant (>300%) increase in FD-uptake in lenti-GDNF treated animals in the right striatum relative to lenti-ßGal treated animals ($p<0.05$). When the right striatum was subdivided, significant increases in FD uptake were only seen within the putamen of lenti-GDNF treated animals ($p<0.05$).

Following sacrifice, a strong GDNF-immunoreactive signal was seen in the caudate nucleus, putamen, and substantia nigra of all lenti-GDNF treated, but none of the lenti-ßGal treated animals. The intensity and distribution of GDNF-immunoreactivity was indistinguishable from what we had previously observed in aged monkeys.

All lenti-ßGal treated monkeys displayed a comprehensive loss of TH- immunoreactivity within the striatum on the side ipsilateral to the MPTP injection. In contrast, all lenti-GDNF treated monkeys displayed enhanced striatal TH-immunoreactivity relative to ßGal controls. However, there was variability in the degree of striatal TH-immunoreactivity in lenti-GDNF treated animals and that variability was associated with the degree of functional recovery seen on the hand reach task. Two lenti-GDNF treated monkeys displayed dense TH- immunoreactivity throughout the rostrocaudal extent of the striatum. In these monkeys, the intensity of the TH-immunoreactivity was greater than that observed on the intact side. These two animals displayed the best functional recovery. A third lenti-GDNF treated monkey also displayed robust functional recovery on the hand reach task. However, the enhanced striatal TH-immunoreactivity in this animal was limited to the post-commissural putamen. The fourth lenti-GDNF treated monkey did not recover on the hand reach task. Although putaminal TH-immunoreactivity in this animal was still greater than controls, the degree of innervation was sparse and restricted to the medial post-commissural putamen.

Lenti-GDNF treatment enhanced the expression of TH-immunoreactive fibers throughout the nigrostriatal pathway. Unlike what was observed in aged monkeys, however, some TH-immunoreactive fibers in the striatum displayed a morphology characteristic of both degenerating and regenerating fibers. Large thickened fibers could be seen coursing in an irregular fashion in these animals. Rostrally, these fibers appeared disorganized at times, with a more normal organization seen more caudally. TH-immunoreactive sprouting was also seen in the globus pallidus substantia innominata and lateral septum. These novel staining patterns were not immunoreactive for dopamine ß hydroxylase confirming the dopaminergic phenotype of this response.

Quantitatively, lenti-ßGal treated monkeys displayed significant decreases in the optical density of TH-immunoreactive fibers within the right caudate nucleus (71.5%; $p<.006$) and putamen (74.3% $p<0.0007$) relative to the intact side. When analyzed as a group, TH optical density in the right caudate nucleus and putamen of lenti-GDNF treated monkeys was significantly greater than that seen in lenti-ßGal treated monkeys ($p<0.001$ for both) and was similar to that seen on the intact side of these animals ($p>0.05$ for both).

All lenti-ßGal treated monkeys displayed a dramatic loss of TH-immunoreactive neurons within the substantia nigra on the side ipsilateral to the MPTP injection. In contrast, the nigra from all four of the lenti-GDNF treated displayed complete neuroprotection regardless of the degree of functional recovery. In lenti-ßGal treated monkeys, intracarotid injections of MPTP resulted in an 89% decrease in the number, and an 81.6% decrease in the density, of TH-immunoreactive nigral neurons on the side ipsilateral to the toxin injection ($p<0.001$). In contrast, lenti-GDNF treated monkeys displayed 32% more TH-immunoreactive nigral neurons ($p<0.001$) and an 11% increase in TH-immunoreactive neuronal density ($p<0.05$) relative to the intact side. In lenti-ßGal treated animals, MPTP significantly reduced (32%) the volume of residual TH-immunoreactive nigral neurons on the lesion side relative to the intact side ($p<0.001$. In contrast, the volume of TH-immunoreactive neurons in lenti-GDNF treated animals was significantly larger (44.3%) on the lesioned side relative to the intact side

($p<0.001$). Finally, the optical density of TH mRNA was quantified bilaterally in all animals. In lenti-ßGal treated animals, there was a significant decrease (24.0%) in the relative optical density of TH mRNA within residual neurons on the MPTP-lesioned side relative to the intact side ($p<0.03$). In contrast, lenti-GDNF treated animals displayed a significant increase (41.7%) in relative optical density of TH mRNA relative to the intact side or lenti-ßGal treated animals ($p<0.001$).

Sections from all monkeys were stained for CD45, CD3 and CD8-markers to assess the immune response following lentiviral vector injection. These antibodies are markers for activated microglia, T cells, and leukocytes including lymphocytes, monocytes, granulocytes, eosinophils, and thymocytes. Staining for these immune markers was weak, and often absent in these animals. Mild staining for CD45 and CD8 were seen in two animals. Some CD45-ir cells displayed a microglial morphology. Other monkeys displayed virtually no immunoreactivity even in sections containing needle tracts.

Two additional intact young adult Rhesus monkeys received lenti-GDNF injections into the right caudate and putamen and the left substantia nigra using the same injection protocol. These animals were sacrificed 8 months later and were evaluated via immunohistochemistry and ELISA for long term gene expression. Robust GDNF-immunoreactivity was seen in the right caudate, right putamen and left ventral midbrain in both animals. In the right substantia nigra, many GDNF-immunoreactive neurons were seen. This labeling represents retrograde transport of GDNF following injections of lenti-GDNF into the right striatum. Further, dense GDNF-immunoreactive fiber staining, representing anterograde transport of the trophic factor, was seen within the right substantia nigra pars reticulata. Tissue punches taken at the time of sacrifice revealed significant levels of GDNF produced by striatal cells 8 months following lenti-GDNF injections. On the side without a striatal injection, 0.130 ± 0.062 and 0.131 ± 0.060 ng/mg protein of GDNF were seen in the caudate nucleus and putamen, respectively. Significantly higher GDNF levels were observed within the caudate nucleus (2.25 ± 0.312 ng/mg protein; $p<0.001$) and putamen (3.5 ± 0.582 ng/mg protein; $p<0.001$) on the lenti-GDNF injected side.

7) Key Research Accomplishments

Robust lentiviral gene delivery can occur in the MPTP-treated primate brain.

Robust lentiviral gene delivery can occur in the MPTP-treated primate brain without cytotoxicity

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

Lentiviral delivery of GDNF prevents and reverses motor deficits in parkinsonian monkeys.

Lentiviral delivery of GDNF prevents the degeneration of the nigrostriatal system in MPTP-treated monkeys

8) Reportable Outcomes

Kordower, J.H., Bloch, J., Emborg, M., Ma, S.Y., Chu, Y., Palfi, S., Leventhal, L., Roitberg, B.Z, Brown, D., Holden, J., Taylor, M., Carvey, P., Hantraye, P., Déglon, N., and Aebischer, P. Lentiviral-vector mediated GDNF delivery prevents motor deficits and nigrostriatal degeneration in nonhuman primate models of Parkinson's disease. **Science**, in press.

9: Conclusions: Lentiviral gene transfer can reverse the motor deficits seen in MPTP treated monkeys and prevent the degeneration of the nigrostriatal system seen following injection of the parkinsonian toxin. This occurs without cytotoxicity. Long-term transfer of the GDNF gene can occur using this method to deliver the trophic. 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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3. Vitek JL (1997) Stereotaxic surgery and deep brain stimulation for Parkinson's disease. In: Movement Disorders. Neurologic principles and practice. Eds. RL Watts and Koller WC. New York.

11: Appendices

1) Copy of Science paper
2) Letter of acceptance from Science.
3) Copies of Neuroscience and Nectar abstracts

LENTIVIRAL VECTOR-MEDIATED EXPRESSION OF GDNF PREVENTS MOTOR DEFICITS AND NIGROSTRIATAL DEGENERATION IN NONHUMAN PRIMATE MODELS OF PARKINSON'S DISEASE

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Abstract

We tested whether lentiviral vector-mediated delivery of glial cell-line derived neurotrophic factor (lenti-GDNF) could be trophic for degenerating dopaminergic nigrostriatal neurons in two nonhuman primate models of Parkinson's disease (PD). Lentiviral vectors expressing GDNF or the marker gene ß-Galactosidase (lenti-ßGal) were injected into the striatum and substantia nigra of nonlesioned aged (approximately 25 year old) Rhesus monkeys or young adult Rhesus monkeys treated one week prior with 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine (MPTP). Following sacrifice 3 months post-injection, all lenti-GDNF treated monkeys displayed extensive GDNF expression in both structures with evidence for anterograde and retrograde transport of the neurotrophic factor. Lenti-GDNF treated aged monkeys displayed increased numbers of tyrosine hydroxylase (TH) immunoreactive-nigral neurons, nigral neuronal volume, nigral TH mRNA, striatal TH-immunoreactivity, striatal dopamine, striatal homovanillic acid and fluorodopa uptake on PET scans. In adult unilaterally MPTP-treated monkeys, lenti-GDNF treatment reversed motor deficits seen on a parkinsonian rating scale and an operant hand reach task and prevented the MPTP-induced loss of fluorodopa uptake seen on PET scan. In these animals, lenti-GDNF completely prevented the loss and atrophy of TH-ir nigral neurons as well as reductions in TH mRNA in nigral perikarya. In fact, lenti-GDNF significantly enhanced these measures relative to the intact side. Lenti-GDNF also prevented the loss of TH-immunoreactivity in the striatum. In two additional intact rhesus monkeys, robust GDNF expression following lenti-GDNF was seen for 8 months with 2.25±.312 and 3.5±.582 ng/mg protein measured in the caudate and putamen, respectively. These data indicate that chronic administration of GDNF to the nigrostriatal system using a lentiviral vector delivery system can prevent the structural and functional consequences of nigrostriatal degeneration in primate models of PD. This intervention might be a viable therapeutic strategy for PD patients.

Parkinson's disease (PD) is a progressive disorder resulting from degeneration of dopaminergic neurons within the substantia nigra. Surgical therapies aimed at replacing lost dopaminergic neurons or disrupting aberrant basal ganglia circuitry have recently been tested (1). However, these clinical trials have focused on patients with advanced disease and the primary goal of forestalling disease progression in newly diagnosed patients has yet to be realized.

Glial cell line-derived neurotrophic factor (GDNF) has potent trophic effects on dopaminergic nigral neurons (2-8) suggesting that this factor could provide neuroprotection in patients with early PD. We have shown that intraventricular administration of GDNF failed to improve clinical function or prevent nigrostriatal degeneration in a patient with PD and this failure resulted from an ineffective delivery method (9). Gene therapy is a powerful means to deliver trophic molecules to the central nervous system in a site-specific manner. Robust transfer of marker and therapeutic genes has recently been demonstrated in the rodent and nonhuman primate brain using a lentiviral vector (10-15). The transgene expression is long-term and non-toxic. Using two different nonhuman primate models of PD, we examined whether lentiviral-mediated delivery of GDNF could reverse the cellular and behavioral changes associated with nigrostriatal degeneration in primates. The first model utilized nonlesioned aged monkeys that display a slow progressive loss of dopamine within the striatum and tyrosine hydroxylase (TH) within the substantia nigra without frank cellular degeneration (16). These aged monkeys demonstrate changes within the nigrostriatal system that model some of the incipient cellular changes seen in early PD (17). In the second model, young adult monkeys received unilateral intracarotid injections of 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine (MPTP) to induce extensive nigrostriatal degeneration resulting in a behavioral syndrome characterized by robust motor deficits.

In the first experiment, eight aged (approximately 25 year old) female Rhesus monkeys received injections of lentiviral vectors encoding ß-galactosidase (lenti-ßGal; n=4) or GDNF (lenti-GDNF; n=4) targeted for the striatum and substantia nigra (18) and were sacrificed 3 months later. Post-

mortem, all GDNF injections were localized to the caudate nucleus, putamen, and supranigral regions (19) as revealed by standard staining procedures (20). All aged monkeys receiving lenti-GDNF displayed robust GDNF-immunoreactivity within the right striatum (Fig. 1A) and substantia nigra (Fig. 1C). In contrast, no monkeys receiving lenti-ßGal displayed specific GDNF-immunoreactivity in the right striatum (Fig. 1B). Rather, these monkeys displayed robust expression of ßGal similar to what we have reported previously (21). In lenti-GDNF-treated animals, GDNF-immunoreactivity within the striatum was extremely dense and distributed throughout the neuropil (Fig. 1). When the primary antibody concentration was decreased 10-fold, the intense striatal neuropil staining was diminished and GDNF-immunoreactive perikarya were easily seen. Numerous GDNF-immunoreactive perikarya were also seen within the substantia nigra of lenti-GDNF injected monkeys. Within the striatum and substantia nigra, Nissl stained sections revealed normal striatal cytoarchitecture without significant cytotoxicity. Macrophages were occasionally observed within the needle tracts. Gliosis was similar across treatment groups and was principally confined to the regions immediately surrounding the needle tracts.

Lenti-GDNF injections resulted in marked anterograde transport of the trophic factor. Intense GDNF-immunoreactivity was observed within fibers of the globus pallidus (Fig. 1D) and substantia nigra pars reticulata (Fig. 1E) following striatal injections. GDNF-containing fibers emanating from putaminal injection sites were seen coursing medially towards and into the globus pallidus (Fig. 1D). These staining patterns were clearly distinct from the injection site and respected the boundaries of the striatal target structures. In contrast, anterograde transport of ßGal was not observed in lenti-ßGal monkeys. This suggests that secreted GDNF, and not the virus per se, was anterogradely transported.

Aged monkeys underwent fluorodopa (FD) positron emission tomography (PET) before surgery and again just prior to sacrifice (22). Prior to treatment, all monkeys displayed symmetrical FD uptake in the caudate and putamen bilaterally (ratio: 1.02 ± 0.02) Fig. 2A and 2B left side).

Similarly, there was symmetrical (4% difference) FD uptake in all lenti-ßGal treated monkeys following lentivirus injections (Fig. 2A right). In contrast, FD uptake was significantly asymmetrical (27%) in lenti-GDNF treated monkeys with greater uptake on the side of the GDNF expression ($p<0.007$; Fig. 2B right). With respect to absolute values, lenti-ßGal animals displayed a trend towards reduced FD uptake post-treatment relative to baseline levels ($p=0.06$). Qualitatively, three of four lenti-GDNF treated monkeys displayed clear increases in FD uptake on the treated side. This increase in Ki value between the groups just failed to reach statistical significance ($p=0.06$).

Within the striatum, lentiviral delivery of GDNF increased a number of markers of dopaminergic function (23). Optical density measurements were performed to assess the relative intensity of TH staining within the caudate nucleus and putamen (Fig. 3A, 3B). On the left side where there was no lenti-GDNF expression, the intensity of TH-immunoreactivity within the caudate nucleus and putamen was similar between groups (Figs. 3A, 3B). In contrast, significant increases in TH-ir optical density were seen in the right striatum of lenti-GDNF infused monkeys (Fig. 3A) relative to lenti-ßGal treated animals (Fig. 3B) or the contralateral side (Fig. 3A). In this regard, there was a 44.1% and a 38.9% increase in TH-ir optical density within the caudate nucleus and putamen respectively (Fig. 4D). At the time of sacrifice, tissue punches were taken throughout the caudate nucleus and putamen of all monkeys. Relative to lenti-ßGal treated animals, measurement of dopamine (DA) and homovanillic acid (HVA) revealed significant increases in the right caudate nucleus (140% DA, $p<0.001$; 207% HVA, $p<0.001$) and putamen (47.2% DA, $p<0.05$; 128% HVA, $p<0.01$) in lenti-GDNF treated aged monkeys (Figs. 4E and 4F).

Lentiviral delivery of GDNF to aged monkeys resulted in an increase in the number of TH-immunoreactive neurons within the substantia nigra. (Fig. 3C, 3D). Regardless of the extent of GDNF- immunoreactivity within the midbrain, the organization of TH-ir neurons was similar in all animals and TH- immunoreactive neurons were not observed in ectopic locations within this locus.

Stereological counts revealed an 85% increase in the number of TH-ir nigral neurons on the side receiving lentivirally delivered GDNF (Fig. 4A) relative to lenti-ßGal treated animals. On the side (left) that did not display GDNF-immunoreactivity, lenti-GDNF treated animals contained 76,929±4918 TH- immunoreactive neurons. This is similar to what was seen in lenti-ßGal infused animals (68,543 ±5519). Whereas lenti-ßGal infused monkeys contained 63,738±6094 TH-immunoreactive nigral neurons in the right side, lenti-GDNF treated monkeys contained 118,170 ± 8631 TH- immunoreactive nigral neurons in this hemisphere ($p<0.001$).

A similar pattern was seen when the volume of TH-ir substantia nigra neurons was quantified (Fig. 4B). TH-immunoreactive neurons from lenti-ßGal and lenti-GDNF treated monkeys were similar in size on the left nigra where there was no GDNF expression (11,147.5±351 μm^3 and 11,458.7±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 monkeys (lenti-ßGal 10,707.5±333 μm^3; lenti-GDNF 16,653.7±1240μm^3; $p<0.001$).

While stereological counts of TH mRNA-containing neurons were not performed, there was an obvious increase in the number of TH mRNA-containing neurons within the right substantia nigra in lenti-GDNF treated monkeys (Fig. 3E) compared to lenti-ßGal containing animals (Fig. 3F). With regard to the relative levels of TH mRNA expression within individual nigral neurons (24), the pattern of results was similar to that observed with TH-ir neuronal number and volume (Fig. 4C). On the left side, the optical density of TH mRNA 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 (21.5%) increase in the optical density for TH mRNA in lenti-GDNF treated monkeys (98.3±1.5) relative to lenti-ßGal treated monkeys (77.2±2.3) on the right side ($p<0.01$).

In Experiment 2, twenty young adult Rhesus were initially trained 3 days per week until asymptotic performance was achieved on a hand-reach task in which the time to pick up food treats out of recessed wells was measured (25,26). Each experimental day, monkeys received 10 trials per hand. Once per week, monkeys were also evaluated on a modified parkinsonian clinical rating scale (CRS). All monkeys then received an injection of 3mg MPTP-HCl into the right carotid artery, initiating a parkinsonian state. One week later, monkeys were evaluated on the CRS. Only monkeys displaying severe hemiparkinsonism with the classic crooked arm posture and dragging leg on the left side continued in the study (n=10). It is our experience that monkeys with this behavioral phenotype display the most severe lesions neuroanatomically and do not display spontaneous recovery behaviorally (25). Based upon CRS scores, monkeys were matched into two groups of five monkeys which received that day lenti-ßGal or lenti-GDNF treatment. Using MRI guidance, all monkeys received lentivirus injections into the caudate nucleus (n=2), putamen (n=3), and substantia nigra (n=1) on the right side using the same injection parameters as in Experiment 1. One week later, monkeys began retesting on the hand reach task 3 times per week for 3 weeks per month (27). For statistical analyses, the times for an individual week were combined into a single score. During the weeks of hand reach testing, monkeys were also scored once per week on the CRS. Individuals blinded to the experimental treatment performed all behavioral assessments. Three months after lentivirus treatment, monkeys received a FD PET scan, were sacrificed 24-48h later, and histologically processed as before.

Within one week following the lentivirus injections, one monkey from each group died. Necropsies from these animals revealed only the presence of mild multifocal random hepatocellular coagulation necrosis. Due to these deaths, all remaining monkeys underwent detailed necropsies following the planned sacrifices and no significant abnormalities in any organs were seen.

Prior to MPTP treatment, all young adult monkeys scored zero on the CRS. Following MPTP, but prior to lentivirus injection, monkeys in the lenti-GDNF and lenti-ßGal groups averaged 10.4±.07

and 10.6±0.6 respectively on the CRS ($p>0.05$). Following lentivirus treatment, significant differences in CRS scores were seen between the two groups (Kolmogorov-Smirnov test; $p<0.0001$; Fig. 5A). CRS scores of monkeys receiving lenti-ßGal did not change over the three month post-treatment period. In contrast, CRS scores of monkeys receiving lenti-GDNF significantly diminished during the 3 month post-treatment period. Scores began to decrease in the first month post-lenti-GDNF treatment. However, statistically significant differences between lenti-GDNF and lenti-ßGal were only discerned at post-treatment observations 6,7,8, and 9 (Kolmogorov-Smirnov test; $p<0.04$ for each comparison).

Lenti-GDNF treated animals also improved performance on the operant hand reach task. Under-pre-MPTP conditions, animals in both groups performed this task with similar speed (Fig. 5B). For the "unaffected" right hand, no differences in motor function were discerned for either group relative to pre-MPTP levels or to each other ($p>0.05$). In contrast, performance with the left hand was significantly improved in lenti-GDNF treated animals relative to controls ($p<0.05$). Following MPTP, all lenti-ßGal treated animals were severely impaired, with monkeys often not performing at all, or requiring more than the maximally-allowed 30 seconds. In contrast, three of the four lenti-GDNF monkeys performed the task with the left hand at near normal levels while one lenti-GDNF-treated monkey was impaired and performed this task in a manner similar to the lenti-ßGal treated animals. Between groups, significant differences in performance were discerned on post-treatment tests 4, 6, 7, 8, and 9. ($p<0.05$ for each comparison).

Just prior to sacrifice, all monkeys underwent FD PET scans. Qualitatively, all lenti-ßGal treated monkeys displayed pronounced FD uptake in the left striatum and a comprehensive loss of FD uptake on the right side (Fig. 2C). In contrast, two of four lenti-GDNF treated animals displayed robust and symmetrical FD uptake on both sides (Fig. 2D). The remaining two lenti-GDNF monkeys displayed reduced FD uptake on the right side, but with Ki values 50-100% greater than lenti-ßGal controls (Fig. 2). Quantitatively, no differences in FD uptake were observed between

groups within the left striatum ($p>0.05$). In contrast, there was a significant (>300%) increase in FD-uptake in lenti-GDNF treated animals in the right striatum relative to lenti-ßGal treated animals ($p<0.05$). When the right striatum was subdivided, significant increases in FD uptake were only seen within the putamen of lenti-GDNF treated animals ($p<0.05$).

Following sacrifice, a strong GDNF-immunoreactive signal was seen in the caudate nucleus, putamen, and substantia nigra of all lenti-GDNF treated, but none of the lenti-ßGal treated animals. The intensity and distribution of GDNF-immunoreactivity was indistinguishable from what we observed in aged monkeys (see Fig. 1).

All lenti-ßGal treated monkeys displayed a comprehensive loss of TH- immunoreactivity within the striatum on the side ipsilateral to the MPTP injection (Fig 6A). In contrast, all lenti-GDNF treated monkeys displayed enhanced striatal TH-immunoreactivity relative to ßGal controls (Fig. 6B). However, there was variability in the degree of striatal TH-immunoreactivity in lenti-GDNF treated animals and that variability was associated with the degree of functional recovery seen on the hand reach task. Two lenti-GDNF treated monkeys displayed dense TH- immunoreactivity throughout the rostrocaudal extent of the striatum (Fig 6B). In these monkeys, the intensity of the TH-immunoreactivity was greater than that observed on the intact side. These two animals displayed the best functional recovery. A third lenti-GDNF treated monkey also displayed robust functional recovery on the hand reach task. However, the enhanced striatal TH-immunoreactivity in this animal was limited to the post-commissural putamen. The fourth lenti-GDNF treated monkey did not recover on the hand reach task. Although putaminal TH-immunoreactivity in this animal was still greater than controls, the degree of innervation was sparse and restricted to the medial post-commissural putamen.

Lenti-GDNF treatment enhanced the expression of TH-immunoreactive fibers throughout the nigrostriatal pathway. Unlike what was observed in aged monkeys, however, some TH-

immunoreactive fibers in the striatum displayed a morphology characteristic of both degenerating and regenerating fibers. Large thickened fibers could be seen coursing in an irregular fashion in these animals. Rostrally, these fibers appeared disorganized at times, with a more normal organization seen more caudally. TH-immunoreactive sprouting was also seen in the globus pallidus (Figs. 6G,6H), substantia innominata (Figs. 6A,6B), and lateral septum. These novel staining patterns were not immunoreactive for dopamine ß hydroxylase confirming the dopaminergic phenotype of this response.

Quantitatively, lenti-ßGal treated monkeys displayed significant decreases in the optical density of TH-immunoreactive fibers within the right caudate nucleus (71.5%; $p<.006$; Fig. 7D) and putamen (74.3% $p<0.0007$; Fig 7D) relative to the intact side. When analyzed as a group, TH optical density in the right caudate nucleus and putamen of lenti-GDNF treated monkeys was significantly greater than that seen in lenti-ßGal treated monkeys ($p<0.001$ for both) and was similar to that seen on the intact side of these animals ($p>0.05$ for both).

All lenti-ßGal treated monkeys displayed a dramatic loss of TH-immunoreactive neurons within the substantia nigra on the side ipsilateral to the MPTP injection (Figs. 7C,7E). In contrast, the nigra from all four of the lenti-GDNF treated displayed complete neuroprotection (Figs. 7D,7F) regardless of the degree of functional recovery. In lenti-ßGal treated monkeys, intracarotid injections of MPTP resulted in an 89% decrease in the number (Fig. 7A), and an 81.6% decrease in the density, of TH-immunoreactive nigral neurons on the side ipsilateral to the toxin injection ($p<0.001$). In contrast, lenti-GDNF treated monkeys displayed 32% more TH-immunoreactive nigral neurons ($p<0.001$) and an 11% increase in TH-immunoreactive neuronal density ($p<0.05$) relative to the intact side. In lenti-ßGal treated animals, MPTP significantly reduced (32%) the volume of residual TH-immunoreactive nigral neurons on the lesion side relative to the intact side ($p<0.001$; Fig. 7B). In contrast, the volume of TH-immunoreactive neurons in lenti-GDNF treated animals was significantly larger (44.3%) on the lesioned side relative to the intact side ($p<0.001$).

Finally, the optical density of TH mRNA was quantified bilaterally in all animals (Fig. 7C). In lenti-ßGal treated animals, there was a significant decrease (24.0%) in the relative optical density of TH mRNA within residual neurons on the MPTP-lesioned side relative to the intact side ($p<0.03$). In contrast, lenti-GDNF treated animals displayed a significant increase (41.7%) in relative optical density of TH mRNA relative to the intact side or lenti-ßGal treated animals ($p<0.001$).

Sections from all monkeys were stained for CD45, CD3 and CD8-markers to assess the immune response following lentiviral vector injection (28). These antibodies are markers for activated microglia, T cells, and leukocytes including lymphocytes, monocytes, granulocytes, eosinophils, and thymocytes. Staining for these immune markers was weak, and often absent in these animals. Mild staining for CD45 and CD8 were seen in two animals. Some CD45-ir cells displayed a microglial morphology. Other monkeys displayed virtually no immunoreactivity even in sections containing needle tracts.

Two additional intact young adult Rhesus monkeys received lenti-GDNF injections into the right caudate and putamen and the left substantia nigra using the same injection protocol (29). These animals were sacrificed 8 months later and were evaluated via immunohistochemistry and ELISA (30) for long term gene expression. Robust GDNF-immunoreactivity was seen in the right caudate, right putamen and left ventral midbrain in both animals. In the right substantia nigra, many GDNF-immunoreactive neurons were seen. This labeling represents retrograde transport of GDNF following injections of lenti-GDNF into the right striatum. Further, dense GDNF-immunoreactive fiber staining, representing anterograde transport of the trophic factor, was seen within the right substantia nigra pars reticulata. Tissue punches taken at the time of sacrifice revealed significant levels of GDNF produced by striatal cells 8 months following lenti-GDNF injections. On the side without a striatal injection, 0.130 ± 0.062 and 0.131 ± 0.060 ng/mg protein of GDNF were seen in the caudate nucleus and putamen, respectively. Significantly higher GDNF levels were observed

within the caudate nucleus (2.25±0.312 ng/mg protein; $p<0.001$) and putamen (3.5±0.582 ng/mg protein; $p<0.001$) on the lenti-GDNF injected side.

Our study demonstrates that delivery of GDNF cDNA into the nigrostriatal system using a lentiviral vector system can potently reverse the structural and functional effects of dopamine insufficiency in nonhuman primate models of aging and early Parkinson's disease. Most critically, lenti-GDNF delivery prevented the motor deficits that normally occur following MPTP administration. In this regard, functional disability was prevented on both a subjective clinical rating scale modeled after the Unified Parkinson's Disease Rating Scale and an objective operant motor test. Consistent expression of GDNF was observed in aged and lesioned monkeys with significant and biologically relevant levels of GDNF observed for up to 8 months post-lentivirus injection. Indeed, the 2.5-3.5ng/mg protein levels produced following lenti-GDNF injections compares very favorably to the 50-152 pg/mg protein of striatal GDNF produced following intrastriatal adenovirus injections in monkeys (31).

This consistent gene expression occurred without significant toxicity to aged monkeys, and minor toxicity in two of the MPTP-treated monkeys, supporting our previous observations (32). Still the death of two monkeys needs to be addressed. Pathological analyses revealed only a mild multifocal random hepatocellular coagulation necrosis in these animals and this was not deemed to be the cause of death. No other young adult or aged monkeys from this or a previous study (32) displayed morbidity or mortality following lentivirus injections. Further, detailed necropsies from the remaining MPTP-treated animals failed to reveal any relevant pathology. While the absolute cause of death remains elusive, we hypothesize that the death of these two monkeys relates to the impact of the surgical procedure one week following the MPTP injections and is unrelated to the lentivirus injection.

In aged monkeys, lentiviral delivery of GDNF augmented host nigrostriatal function as determined by a variety of morphological, physiological, and neurochemical dependent measures. In this regard, lenti-GDNF treated animals displayed marked increases in the size and number of TH-immunoreactive neurons within the substantia nigra, increased the expression of TH mRNA within these neurons, increased the levels of dopamine, dopaminergic metabolites and dopaminergic markers in the striatum, and increased FD-uptake within the striatum as determined by PET scan. Enhanced nigrostriatal dopamine function was consistently associated with the expression of lentivirally delivered GDNF as enhanced nigrostriatal function was only seen on the side with robust gene expression.

We used aged monkeys to model specific cellular changes that occur in aging and the earliest aspects of PD. Phenotypic down regulation of TH gene expression and protein is one of the earliest pathological events within the substantia nigra in PD (33) and analogous changes are seen in aged Rhesus monkeys (34). The number of TH-ir nigral neurons seen in lenti-ßGal injected animals was similar to that previously reported for aged Rhesus monkeys (34). In contrast, lenti-GDNF treated aged monkeys displayed nigral neurons in numbers similar to that seen in young adult animals. The possibility that the lenti-GDNF spurred neurogenesis of dopaminergic nigral neurons cannot be ruled out. However, the delivery of lenti-GDNF to the nigral region resulted in transgene expression throughout the midbrain. Yet, TH-immunoreactive neurons were observed only within established catecholaminergic nuclei and not in ectopic midbrain locations. A more parsimonious explanation is that GDNF upregulated TH-ir in aged nigral neurons that had previously down-regulated TH expression below detectable levels. The enhanced THmRNA expression seen within nigral neurons following lenti-GDNF treatment supports this interpretation.

Lenti-GDNF also prevented the behavioral and neuroanatomical effects of MPTP-induced nigrostriatal degeneration. It is notable that unlike many other neuroprotection paradigms, the lenti-GDNF injections were performed after the parkinsonian state was initiated, thus better

modeling what can be attempted in PD patients. The exact mechanism by which lenti-GDNF exerted its effects requires further elucidation. It is clear that neuroprotection was achieved within the substantia nigra as these neurons do not degenerate within a week of MPTP treatment (35). However, striatal fibers can degenerate during this time and whether the GDNF is preventing degeneration or inducing sprouting of degenerating fibers, still needs to be established. Indeed, there is evidence for both mechanisms as some animals displayed fiber morphology and topography indicative of regeneration.

A critical question is whether preservation of striatal innervation, nigral perikarya, or both is required for functional recovery. Although the number of animals in our study is too small to provide a definitive answer, it is notable that all lenti-GDNF treated monkeys had complete preservation of nigral perikarya. Yet functional recovery on the hand reach task was absent only in the one monkey with sparsest striatal reinnervation. Thus it appears that GDNF-mediated striatal reinnervation is critical for functional recovery in nonhuman primates, a concept supported by recent studies performed in rodents (36,37). The failure to potently protect dopaminergic innervation in the one monkey may be due variability in the speed by which nigrostriatal fibers are lost following MPTP. At the time of the lenti-GDNF injection, dopaminergic fibers in this monkey may have regressed to a level where access to the GDNF was limited and regrowth to the striatum was impossible.

Not only was lenti-GDNF capable of preventing the degeneration of nigrostriatal neurons in MPTP-treated monkeys, it augmented many of the morphological parameters relative to the "intact" side. It is likely that the unilateral 3mg MPTP dose induced a small loss of TH-ir neurons on the contralateral side. Thus the increased numbers of TH-ir neurons may reflect complete neuroprotection on the side of GDNF expression contrasted with a small loss of TH-ir neurons on the non-injected side.

We injected lentivirus into both the striatum and substantia nigra in order to maximize the chance for an effect. For lenti-GDNF therapy to be a practical clinical approach, studies determining the regions of GDNF delivery critical to reverse progressive nigrostriatal degeneration are needed. The importance of related biological events such as anterograde transport of GDNF from injection sites to target regions also needs to be established. Finally, potential adverse events resulting from lenti-GDNF inducing supranormal levels of striatal dopamine needs to be evaluated. Towards this end, vectors with built-in inducible systems that can modulate gene expression in cases of dose limiting side effects need to be developed. Still, the reversal of slowly progressive cellular phenotypic changes seen in aged monkeys, combined with the structural and functional neuroprotection and regeneration seen in MPTP-treated monkeys, indicates that lentiviral delivery of GDNF may provide potent clinical benefits for patients with PD.

Acknowledgments: This research was supported by a grant from the Department of Defense, NS40578, and from the Swiss National Science Foundation and the Swiss National Program in Neurological Diseases. We would like to thank Dr. Timothy Collier for comments on this manuscript, Theodora Kladis for expert histological assistance, Fabienne Pidoux and Maria Rey for the technical assistance in the production of the lentiviral vectors, Kim Gibbons for assistance with PET scans, and Dr. John Sladek Jr. for photographic assistance.

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18. The cDNA coding for a nuclear-localized β-galactosidase (LacZ) and the human GDNF containing a Kozak consensus sequence (a 636 bp fragment: position 1-151 and 1-485 genbank accession numbers L19062 and L19063) were cloned in the SIN-W-PGK transfer vector (Zufferey et al., J. Virol. 1999). The packaging construct used in this study was the pCMV-R-8.92 (destruction of the *Bam*HI restriction site in the coding region of the rev gene from the pCMV-R-8.91 plasmid (Zufferey et al., Nature Biotech. 1997)). To decrease further the risk of recombination and production of replication competent retroviruses, the Rev gene was inserted in the pRSV-Rev plasmid. The viral particles were produced in 293T cells as previously described (J. H. Kordower et al., *Exp. Neurol.* ibid). The titers (3-5x10^8TU/ml) of the concentrated LacZ-expressing viruses (200,000 and 250,000 ng p24/ml in the experiment 1 and 450,000 ng p24/ml in the experiment 2) was determined on 293T cells. The GDNF-expressing viral stocks were normalized for viral particles content using p24 antigen measurement.

19. All experimentation was performed in accord with NIH guidelines and institutional animal care approval. Level II Biosafety procedures were employed. Using MRI guidance, each monkey received six stereotaxic injections of lenti-ßGal or lenti-GDNF bilaterally into the caudate nucleus, putamen and substantia nigra. Injections were made into the head of the caudate nucleus (10μl), body of the caudate nucleus (5μl), anterior putamen (10μl), commissural putamen (10μl), post-commissural putamen (5μl), and substantia nigra (5μl). Injections were made through a 10μl Hamilton syringe connected to a pump at a rate of 0.5μl/min. During the injection, the needle was raised 1-2mm to better disperse the lentivirus through the intended target. The needle was left in place for an additional 3 min to allow the injectate to diffuse from the needle tip. The left side was injected 6 weeks prior to the right. During the first surgical session, there was a technical failure with the virus aggregating in the needle preventing its injection into the brain. This was confirmed upon post-mortem examination using GDNF-immunohistochemistry and ßGal histochemistry. Thus, the left side served as an additional control for the right side.

20. GDNF immunohistochemistry was performed with a commercially available antibody (R and D systems; 1:1000) using the ABC method and nickel intensification. Deletion or substitution for the primary antibody served as controls. Under control conditions, no staining was observed.

21. J. H. Kordower et al., *Exp. Neurol.* ibid.

22. All procedures followed an overnight fast. Following sedation with Ketamine (10-15 mg/kg) the animal was intubated and femoral angiocatheters placed for tracer injection and blood sampling. Anesthesia was then maintained by

1-2% isofluorane for the remainder of the procedure. Carbidopa (2-3 mg/kg IV) was administered 30 min prior to the FD study. The animal was placed in a stereotaxic head holder constructed of materials compatible with PET scanning, and a transmission scan acquired for correction of the emission data for attenuation. FD (185MBq) was administered over 30 s and a 90 min 3D dynamic emission scan started. The scan included 22 frames with durations increasing from 1 min initially to 5 min at the end. The bed was moved cyclically by the interplane distance between each pair of 5 min scans to give a net coronal sampling interval of 2.125 mm. Regions of interest (ROI) were placed on the caudate nucleus, putamen and occipital cortex in individual morphometric MR images co-registered with the FD image data. Cortical time courses were used as input functions to generate functional maps of the uptake rate constant Ki by the modified graphical method (C. S. Patlak and R. G. Blasberg, *J. Cereb. Blood Flow Metabol.* **5**, 584 (1985)). Striatal ROI were transferred to the functional maps and the Ki values were evaluated as the ROI means for each structure.

23. All monkeys were perfused with saline. The brain removed, immersed in ice cold saline for10 min and slabbed on an monkey brain slicer. Slabs through the head of the caudate and putamen were punched bilaterally with a 1mm brain punch. These punches were processed for HPLC (Kordower et al., Cell Transplantation 4: 155-171, 1995). The tissue slabs were immersed in Zamboni's fixative. Stereological counts and volumes of TH-ir neurons were performed using NeuroZoom software employing the optical disector method for cell counting and the nucleator method for measuring neuronal volume. (M. Emborg et al., J. Comp. Neurol. Ibid).

24. The TH riboprobe was prepared as previous described (Kordower et al., Ann. Neurol. ibid). The probe was conjugated to 2mM biotin-14-CTP (Gibco.), 1μg PvuI-linearized pBS-TH3'. 5mM DTT, 50 units RNAsin, 4units T3RNA polymerase, 0.5mM CTP, and 0.25mM of ATP, GTP, and UTP. Tissue was processed for immunohistochemistry via the ABC method using this probe as the primary antibody. Optical density measurements were performed using NIH Image.

25. J. H. Kordower; Cell Transplantation, ibid.

26.M. Emborg et al., J. Comp. Neurol. Ibid

27. Testing was performed during weeks 2-4 for month one, and weeks 1-3 for months two and three. Monkeys were not tested for the first week in month 1 to allow them time to recover from surgery. Testing was not performed for the final week of months 2 and 3 to allow for routine veterinary care (month 2) and transportation to the University of Wisconsin for PET scans (month 3).

28. Web site for Fig

29. Web site for Fig.

30. Brain punches were homogenized in 150:l buffer I (0.1M Tris buffered saline, pH 8.1, containing 1 mM EDTA, 1% aprotinin, 10 µg/ml leupeptin, 14 µg/ml pepstatin, 4 mM PMSF) for 30 seconds in the ice slurry. Equal amount buffer II (0.1M Tris buffered saline, pH 8.1, containing 1 mM EDTA, 1% aprotinin, 10 g/ml leupeptin, 14 µg/ml pepstatin, 4 mM PMSF, and 0.5% NP-40) was then added. The tubes were shakened for 2 hours in the cold room and spun down to remove tissue debris. The supernatant was collected for ELISA and protein measurements. The ELISA reaction was completed in Dynatech 96-well plate according to the ELISA manufacturer's instructions (Promega GDNF E_{max} ImmunoAssay Systems Kit G3520, Madison, WI). The optical densities were recorded in ELISA plate reader (Dynatech Plate Reader at 450 nm wave length). Some lysates were diluted to ensure all the optical densities were within the standard curve. The concentrations or GDNF were calculated against 6-point standard curve and then were adjusted to pg GDNF/mg total protein. The total protein in each tissue lysate was measured using Bio-Rad protein assay kit (Bio-Rad, CA).

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Figure Legends:

Figure 1: (A) Dense GDNF-ir within the head of the caudate nucleus and putamen in a lenti-GDNF treated aged monkey. (B) In contrast, no GDNF-ir was observed in these regions in a lenti-ßGal treated animal. (C) Dense GDNF-ir was observed within the midbrain of a lenti-GDNF treated animal. (D) GDNF-ir within the forebrain of a lenti-GDNF treated monkey. The staining within the putamen (pt) is from an injection site. The staining within both segments of the globus pallidus (GPe and GPi) is the result of anterograde transport. (E) Anterogradely transported GDNF was also seen in the substantia nigra pars reticulata. Note that the holes in the tissue sections were made post-mortem for HPLC analysis. Asterisk in E represents a lenti-GDNF injection site (CP = cerebral peduncle). Scale bar in E represents 1600μm for panels A, B, D; 1150 μm for panels C and 800μm for panel E.

Figure 2: PET scan data the evaluating the influence of lenti-GDNF upon FD uptake in (A and B) intact aged monkeys and (C and D) young adult MPTP-treated monkeys. (A) There was no change in FD uptake from baseline to 3 months post-lentivirus injection in Lenti-ßGal treated aged monkeys (B) In contrast, Lenti-GDNF injections manifested increased FD uptake on the side of GDNF expression relative to pre-operative levels in aged monkeys. Ki values (min^{-1}) for the striatum are as follows: (left side) lenti-ßGal preop 0.0068±.0001; lenti-ßGal post-op 0.0062±0.0002; (right side) lenti-ßGal preop 0.0068±0.0002 lenti-ßGal post-op 0.0065±0.0001. (left side) lenti-GDNF preop 0.0072±0.0005; lenti-GDNF post-op 0.0068±0.0003; (right side) lenti-GDNF preop 0.0076±0.0004; lenti-GDNF post-op 0.0081±0.0003.

(C) Following MPTP lesions, a comprehensive loss of FD uptake was seen within the right striatum of lenti-ßGal treated young adult monkeys. (D) In contrast, FD uptake was enhanced in lenti-GDNF treated monkeys. Ki values (min^{-1}) for the striatum are as follows: Lenti-ßGal left: 0.0091±0.0004; Lenti-ßGal right: 0.0017±0.0005; lenti-GDNF left: 0.0084±0.0004; lenti-GDNF right: 0.0056±0.0018.

Figure 3: (A) TH-ir stained section through the anterior commissure illustrating the increase in TH-ir within the right caudate nucleus and putamen following lenti-GDNF delivery to aged monkeys. (B) Symmetrical and less intense TH-ir staining in a monkey injected with lenti-ßGal. (C) There were greater numbers and larger TH-ir neurons within the substantia nigra of a lenti-GDNF treated animal relative to (D) a lenti-ßGal treated monkey. (E) Lenti-GDNF treated aged monkeys displayed increased THmRNA relative to (F) lenti-ßGal treated monkeys in the SN. Scale bar in F represents 4500µm for panels 250µm for panels C and D and 100µm for panels E and F.

Figure 4: Plots of quantitative data illustrating enhanced nigrostriatal function in lenti-GDNF-treated aged monkeys. Solid bars denote lenti-ßGal treated monkeys while the hatched bars indicate lenti-GDNF treated monkeys. GDNF expression was limited to the right striatum and nigra. ** and *** denote $p<0.01$ and 0.001 respectively.

Figure 5: Following MPTP-treatment, lenti-GDNF injected monkeys displayed functional improvement on (a) the clinical rating scale and (B) the hand reach task. All tests were performed 3 weeks per month (see ref. 15). On the clinical rating scale, monkeys were matched into groups based upon the post-MPTP score. For the hand reach task, each symbol represents the mean of three sessions /per week. Monkeys were not tested on this task during the week between MPTP and lentivirus injection. * denotes $p<0.05$ relative to lenti-ßGal.

Figure 6: (A and B) Low power dark-field photomicrographs through the right striatum of TH-immunostained sections of MPTP-treated monkeys treated with (A) lenti-ßGal or (B) lenti-GDNF. (A) There was a comprehensive loss of TH-ir in the caudate and putamen of lenti-ßGal treated animal. In contrast, near normal level of TH-ir is seen in lenti-GDNF treated animals. Low (C and D) and medium (E and F) power photomicrographs of TH-immunostained section through the substantia nigra of lenti-ßGal (C and E) and lenti-GDNF (D and F) treated animals. Note the loss of TH-ir neurons in the lenti-ßGal treated animals on the side of the MPTP-injection. TH-ir sprouting fibers, as well as a

supranormal number of TH-ir nigral perikarya are seen in lenti-GDNF treated animals on the side of the MPTP injection. (G and H) Brightfield low power photomicrographs of a TH-immunostained section from a lenti-GDNF treated monkey. (G) Note the normal TH-ir fiber density through the globus pallidus on the intact, non-lenti-GDNF treated, side. (H) In contrast, an enhanced network of TH-ir fibers is seen on the MPTP/lenti-GDNF treated side. Scale bar in G represents the following magnifications. A,B,C,D=3500μm; E,F,G,H=1150μm.

Figure 7: Quantitation of lenti-GDNF's trophic effects upon nigral neuronal number, volume, THmRNA and striatal TH-ir in MPTP-treated monkeys. ** and *** denote significant decreases ($p<0.01$ and $p<0.001$) respectively relative to intact side; ttt denotes significant increases relative to the intact side.

LENTIVIRAL DELIVERY OF GDNF IN MPTP-TREATED MONKEYS: NEUROANATOMICAL STUDIES

J. McBride[1*], M.E. Emborg[1], J. Bloch[3], S. Ma[1], Y. Chu[1], L. Leventhal[1], S. Palfi[1], J.Stansell[1], P. Carvey[1], P. Hantraye[5], N. Deglon[3], J. Holden[6], D. Brown[6], M.Taylor[6], P. Aebischer[3] and J. H. Kordower[1]. [1]Dep. Neurol. Sci. and Res. Ctr. for Brain Repair, [2]Dept Pharmacol. Rush Univ., Chicago IL 60612; [3]Div. Surg. Res. and Gene Therapy Ctr, Lausanne Univ. Med. Sch., Switzerland; [5]CEA CNRS URA 2210 Serv. Hosp. F.Joliot, CEA, DSV, DRM, Orsay cedex, France; [6]Dep. Radiop. Univ. WI, Madison,WI.

The present study investigated the neuroanatomical effects of lentiviral delivery of GDNF (lenti-GDNF) to the nigrostriatal system of MPTP-treated monkeys. Eight Rhesus monkeys (5-8yrs) received a unilateral intracarotid infusion of MPTP. One week later, monkeys received MRI-guided stereotaxic injections of lenti-GDNF (n=4) or ß-galactosidase (lenti-ßGal, n=4) into the caudate nucleus, putamen and substantia nigra (SN) and were sacrificed 3 months later. Robust GDNF-ir or ßGal-ir was observed in all monkeys, however, only lenti-GDNF treated monkeys exhibited anterograde transport of the transgene. Following MPTP treatment, the lenti-ßGal group displayed significant decreases in the number (89%), density (81%) and volume (32%) of TH-ir SN neurons on the MPTP treated side relative to the intact side. Additionally, relative levels of THmRNA within residual SN perikarya were decreased (24%) in lenti-ßGal monkeys. In contrast, lenti-GDNF treated monkeys displayed complete neuroprotection. Remarkably, relative to the intact side, lenti-GDNF treated monkeys exhibited significant increases in TH-ir SN cell number (32%), density (11%) and volume (44.3%) as well as a significant increase in THmRNA (41.7%). Lenti-GDNF also prevented the loss of TH-ir in the striatum. These data indicate that lentiviral delivery of GDNF prevents the structural consequences of MPTP lesion and may be a suitable treatment for Parkinson's Disease.

Supported by: U.S.A. Department of Defense and Swiss National Science.

LENTIVIRAL DELIVERY OF GDNF IN MPTP-TREATED YOUNG RHESUS MONKEYS. M.E. Emborg[1*], J. Bloch[3], S. Ma[1], Y. Chu[1], L. Leventhal[1], S. Palfi[1,5], J.Stansell[1], P. Carvey[1,2], P. Hantraye[5], N. Déglon[3], J. Holden[6], D. Brown[6], M.Taylor[6], P. Aebischer[3] and J. H. Kordower[1] [1] Dep. Neurol. Sci., and Res. Ctr. for Brain Repair, [2]Dept Pharmacol. Rush Univ., Chicago IL 60612; [3]Div. Surg. Res. and Gene Therapy Ctr, Lausanne Univ. Med. Sch., Switzerland; [4]Dep. Neurosurg. Univ. IL Med. Ctr., Chicago IL 60612; [5]CEA CNRS URA 2210 Serv. Hosp. F.Joliot, CEA, DSV, DRM, Orsay cedex, France; [6]Dep. Radiop. Univ. WI, Madison,WI.

The present report examined whether lentiviral delivery of glial derived neurotrophic factor (GDNF) can prevent/reverse the behavioral and anatomical consequences of MPTP-induced nigrostriatal degeneration in nonhuman primates. Eight young Rhesus monkeys (male, 5-8yrs., 5.5-8.5 kg) received a unilateral intracarotid infusion of MPTP-HCL (3 mg). One week after infusion the animals were assessed for behavioral impairments and received MRI-guided-stereotaxic injections of lentivirus encoding for GDNF (lenti-GDNF, n=4) or ß-galactosidase (lenti-ßGal, n=4) (infusion rate: 0.5 µl/min). Each animal was injected into the caudate (n=2; 5 µl and 10 µl), putamen (n=3; 10 µl, 10 µl and 5 µl) and substantia nigra (n=1; 5 µl). Monkeys were assessed by a blind observer for performance on a hand reach task, general activity, and clinical dysfunction based upon a clinical rating scale before and 3 months post injection. Lenti-GDNF animals compared with lenti-ßGal presented: a) significant better performance in the hand reach task and clinical score; b) significant increase in F-DOPA uptake in PET scan; c) increased striatal TH-ir optical density; d) increased number of TH-ir nigral neurons; e) increased TH-ir nigral neuronal volume. These results indicate that site specific delivery of GDNF in nonhuman primates by lentiviral vectors can reverse MPTP-induced changes in nigrostriatal function and may be a suitable therapy for treatment of PD. Support: U.S.A. Department of Defense and Swiss National Science

Keywords: Virus, Parkinson's, substantia nigra, regeneration

Theme J: Disorders of the Nervous system and Aging; 131: Degenerative disease: Parkinson's
Theme A: Development and regeneration; 22: Regeneration

LENTIVIRAL DELIVERY OF GDNF IN NONHUMAN PRIMATE MODELS OF PARKINSON'S DISEASE J.H. Kordower, M.E. Emborg[1*], J. Bloch[3], S. Ma[1], Y. Chu[1], L. Leventhal[1], S. Palfi[1,5], J. McBride[1], J.Stansell[1], P. Carvey[1,2], P. Hantraye[5], N. Déglon[3], J. Holden[6], D. Brown[6], M.Taylor[6], and P. Aebischer[3] Dep. Neurol. Sci., and Res. Ctr. for Brain Repair, [2]Dept Pharmacol. Rush Univ., Chicago IL 60612; [3]Div. Surg. Res. and Gene Therapy Ctr, Lausanne Univ. Med. Sch., Switzerland; [4]Dep. Neurosurg. Univ. IL Med. Ctr., Chicago IL 60612; [5]CEA CNRS URA 2210 Serv. Hosp. F.Joliot, CEA, DSV, DRM, Orsay cedex, France; [6]Dep. Radiop. Univ. WI, Madison,WI.

Glial cell-derived neurotrophic factor (GDNF) potently supports the viability and phenotypic expression of dopaminergic nigral neurons. The consistent success of GDNF in animal models of Parkinson's disease (PD) has led to a clinical trial that was subsequently abandoned due to minimal efficacy, significant side effects, and post-mortem evidence that this intraventricular GDNF fails to penetrate the brain parenchyma sufficiently to access vulnerable nigrostriatal neurons. We have performed a series of studies in which the GDNF gene was delivered directly to the nigrostriatal system of young, aged and MPTP-treated monkeys using a lentiviral vector system. Robust and consistent gene expression for up to 8 monkeys was seen in all animals. Quantitation revealed the delivery of 2.5-3.5 μg/mg protein of GDNF. Anterograde and retrograde transport of secreted GDNF was noted. Injections of lenti-GDNF to aged monkeys increased the number, size, and THmRNA expression within nigral neurons and dopamine and TH-immunoreactivity within the striatum. Enhanced fluordopa uptake was also seen on PET scan. Injections of lenti-GDNF to MPTP-treated monkeys reversed the motor deficits seen on a clinical rating scale and an operant hand-reach task. The loss of fluorodopa uptake seen on PET was prevented by lenti-GDNF. The loss and shrinkage of nigral neurons, as well as the loss of nigral THmRNA and striatal dopamine was also prevented by lenti-GDNF. Minimal toxicity was observed following lentivirus injections. These data support the concept that lentiviral delivery of GDNF may be Support: Department of Defense and Swiss National Science.

Keywords: Virus, Parkinson's, substantia nigra, regeneration

Theme J: Disorders of the Nervous system and Aging; 131: Degenerative disease: Parkinson's
Theme A: Development and regeneration; 22: Regeneration

THE GLOBAL WEEKLY OF RESEARCH
Science

17 August 2000

Dr. Jeffrey H. Kordower
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Ref: 1047824

Dear Dr. Kordower:

We are glad to accept your paper entitled, "Lentivector-mediated Expression of GDNF prevents Motor Deficits and Nigrostriatal Degeneration in Nonhuman Primate Models of Parkinsons's Disease."

You will be called should editorial changes be required to improve clarity, to save space, or to conform to SCIENCE practice. Please send us the direct-dial telephone number of the author who will be responsible for checking the galley proofs.

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Orla Smith, Ph.D.
Senior Editor

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Published by the American Association for the Advancement of Science

MCMR-UWZ-C (MCMR-RMI-S/8 Sep 00) 1st End
SUBJECT: Annual Report Dated Oct 00
Period Covered: 15 Sep 99 - 14 Sep 00
Award Number: DAMD17-98-1-8630
Principal Investigator: Jeffrey H. Kordower, Ph.D.

Commanding General, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-PLC, 504 Scott Street, Fort Detrick, MD 21702-5012

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The background objectives, methodology and results of technical efforts having scientific merit have been considered in the scientific review of the subject report. The report is:

__✓__ Scientifically acceptable in accordance to the contract Statement of Work.

____ Minor corrections should be made as notated in the reviewer's evaluation.

____ Forward report to the Defense Technical Information Center.

_____ Not scientifically acceptable in accordance to the contract Statement of Work. Report is to be returned to the Principal Investigator for rewrite incorporating the enclosed reviewer's recommendations.

(COR's Signature) 5 DEC 00 (Date)

2 Encls
1. wd
Added 1 encl
2. Reviewer's Evaluation

DAMD# 17-98-1-8630

ANNUAL/FINAL REPORT REVIEW
USAMRMC NEUROTOXIN EXPOSURE TREATMENT RESEARCH PROGRAM

Compliance/Product Analyses
Date of Review by the American Institute of Biological Sciences: November 10, 2000

AIBS #:	**970494**
Grant/Contract/MIPR No:	**17-98-1-8630**
Principal Investigator:	**Jeffrey Kordower**
Institution:	**Rush-Presbyterian-St. Luke's Medical Center**
Report Title:	**Gene Therapy in a Nonhuman Primate Model of Parkinson's Disease**
Report Type:	**Annual Report**
Reporting Period:	**9/15/99 - 9/14/00**

BRIEF CAPSULE SUMMARY OF THE REPORT RESULTS

The PI plans to provide a gene-based therapeutic intervention for parkinsonian syndromes using lentiviral delivery of glial-derived neurotrophic factor (GDNF). Previously, the PI demonstrated that lentiviruses were reasonable vectors for gene transfer to the central nervous system (CNS). The PI observed long-term gene transfer with neither toxicity nor augmentation of nigrostriatal function following trophic factor expression. In the Annual Report, the PI demonstrates that lentivirus gene delivery occurs in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primate brain without subsequent excitotoxicity. Also, the PI reports that GDNF gene delivery can occur in the primate brain and prevents and reverses motor deficits in MPTP-treated monkeys. The PI demonstrates marked improvement in the expression of markers for dopaminergic neurons in the brains of aged nonhuman primates following delivery of GDNF. Lastly, the PI demonstrated that lentiviral delivery of GDNF prevents degeneration of the nigrostriatal system in response to MPTP treatment.

This body of work was submitted and accepted for publication in *Science*. These data provide compelling evidence for the utility of lentiviral delivery of GDNF in amelioration of parkinsonian syndromes.

CONTRACTUAL ISSUES

The work is in compliance with the Statement of Work, is on schedule, has been reported in the peer-reviewed literature, or accepted for publication in the near future. The PI originally proposed to use genetically modified encapsulated cells, but now is using genetically engineered lentivirus. This switch is scientifically sound and has yielded positive results.

TECHNICAL ISSUES

The science is outstanding, and the data are presented as required. There are no obstacles requiring attention or resolution. The proposal was strong, and the PI and investigative team are skilled at these techniques. There are no impediments to the completion of this study.

FORMAT

The report conforms to the USAMRMC requirements.

KEY ACCOMPLISHMENTS

These PI provides one of the first glimpses at the promise of gene therapy. The finding that nigrostriatal degeneration can be significantly diminished by viral delivery of GDNF in a higher animal model is one of the most exciting advances in neuroscience and neurology. The findings are more important considering specific behavioral measures of function that correlate well with the neuropathologic outcome. Furthermore, these studies provide compelling evidence that long-term gene delivery strategies are plausible, and under the conditions of the study reversibility of the neuroprotective effect is not a concern. This work has wider implications in the treatment of neurotoxin-induced neuropathies. Viral delivery of specific neurotrophins, which are notoriously difficult to formulate and deliver using conventional pharmaceutical strategies, may become standard practice in the treatment of chemically- induced or biologically-induced CNS damage.

SUMMARY RECOMMENDATIONS, DISCREPANCIES, AND/OR TECHNICAL ASSISTANCE

No major concerns exist. The PI and investigative team are qualified to perform and evaluate the results of the remaining studies. However, the PI should carefully determine the cause for possible hepatic degeneration due to viral toxicity.

RECOMMENDATION to USAMRMC: accept the report or request revision by PI.

Accept ___XX___ Request Revision _____