Biochemical Pharmacology, Vol. 57, pp. 135–142, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(98)00280-9

COMMENTARY

A Commentary on Glial Cell Line-Derived Neurotrophic Factor (GDNF)

FROM A GLIAL SECRETED MOLECULE TO GENE THERAPY

*Martha C. Bohn**

CHILDREN'S MEMORIAL INSTITUTE FOR EDUCATION AND RESEARCH, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL, CHICAGO, IL 60614, U.S.A.

ABSTRACT. Glial cell line-derived neurotrophic factor (GDNF) was identified as a consequence of the hypothesis that glia secrete factors that influence growth and differentiation of specific classes of neurons. Glia are a likely source of additional neurotrophic factors; however, this strategy has not been applied extensively. The discovery of GDNF in 1993 led to an abundance of studies that within only a few years qualified GDNF as a *bona fide* neurotrophic factor. Of particular interest are studies demonstrating the effectiveness of GDNF protein in ameliorating neurodegeneration in animal models of Parkinson's disease and amyotrophic lateral sclerosis (ALS). It remains to be determined whether GDNF will be an effective therapy in humans with these diseases. However, since these diseases are slowly progressive and the CNS relatively inaccessible, the delivery of GDNF as a therapeutic molecule to the CNS in a chronic manner is problematic. Studies addressing this problem are applying viral vector mediated transfer of the *GDNF* gene to the CNS in order to deliver biosynthesized GDNF to a specific location in a chronic manner. Recent studies suggest that these GDNF gene therapy approaches are effective in rat models of Parkinson's disease. These studies are reviewed in the context of what developments will be needed in order to apply GDNF gene therapy to the clinic. BIOCHEM PHARMACOL **57**;2:135–142, 1999. © 1998 Elsevier Science Inc.

KEY WORDS. neurotrophic factor; dopamine; adenovirus; Parkinson's disease; amyotrophic lateral sclerosis; motor neurons; neuronal cell death

GLIAL CELLS AS A SOURCE OF NEUROTROPHIC FACTORS

The discovery of molecules with therapeutic value often occurs by chance rather than by purposeful experimentation. Such is the case for GDNF†, the first member identified of a new family of neurotrophic factors with therapeutic potential for DA and other types of neurons. The contribution of my research group to the discovery of GDNF began over a decade ago when we became intrigued by a study of Schubert and colleagues that at the time received little attention. That study, aimed at identifying cell-specific proteins in the nervous system, used two-dimensional gel electrophoresis to compare the proteins expressed by a variety of neuronal and glial derived clonal cell lines. The surprising result was that the gel patterns of proteins secreted by the cell lines were strikingly different, whereas the patterns of intracellular proteins were nearly identical [1]. This observation suggested the secreted protein fraction of a cell line as an obvious source for identifying cell-specific proteins. In turn, this was an approach likely to uncover novel molecules that have trophic actions on other cell types. However, the lack of methods at that time for identifying low abundance proteins from two-dimensional gels or for identifying cell-specific mRNAs by differential display left this concept begging for the advanced molecular technologies available to us today. Nevertheless, Jürgen Engele, then a postdoc in my laboratory, pursued this concept by applying cumbersome bioassays. Using cultures of embryonic DA neurons, several glial tumor cell lines and one neuronal cell line, PC12 pheochromocytoma [2], were screened for secretion of factors that promoted several growth indices of DA neurons, including neuronal survival, extent of neurite outgrowth, and level of high affinity DA uptake. It was found that the media conditioned by the three glial cell lines, B49, R33, and JSC1, all secreted potent DA activity, while, in contrast, no DA activity was secreted by the PC12 line [3]. Using these bioassays, Lin and colleagues [4] purified a glycoprotein from the B49 cell line with potent DA neurotrophic activity known as GDNF, a novel glycoprotein with homology to the transforming growth factor-β superfamily. GDNF was found to support the survival of DA

* Corresponding author: Martha C. Bohn, Ph.D., Children's Memorial Institute for Education and Research, 2300 Children's Plaza, Box 209, Chicago, IL 60614. Tel. (773) 868-8052; FAX (773) 868-8066; E-mail: m-bohn@nwu.edu

† *Abbreviations:* 6-OHDA, 6-hydroxydopamine; ALS, amyotrophic lateral sclerosis; DA, dopaminergic; GDNF, glial cell line-derived neurotrophic factor; GFRα, receptor family for GDNF; GPI, glycosylphosphatidylinositol; IR, immunoreactive; *LacZ*, gene coding for *Escherichia coli* β-galactosidase (EC 3.2.1.23); MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PC12, pheochromocytoma clonal cell line; and TH, tyrosine hydroxylase (tyrosine 3-monooxygenase, EC 1.14.16.2).

neurons in culture at concentrations in the range of 0.01 to 10 ng/mL and was, therefore, much more potent than previously known DA neurotrophic factors [4].

The rapidity with which GDNF research has progressed over the past 4 years is astounding compared with that of other neurotrophic factors. Within 3 years after its discovery, demonstration of all of the classical criteria defining a neurotrophic factor had been fulfilled. The distribution of GDNF expression in the nervous system as well as in peripheral tissues was mapped [5–9]. Retrograde transport of GDNF in the CNS was demonstrated [10], and neuronal targets for GDNF were identified. In addition to DA neurons, these include motor neurons [11], noradrenergic neurons [12, 13]. Pürkinje cells [14], cholinergic neurons [15], serotonergic neurons [16], sympathetic and ciliary neurons [17], sensory neurons [18], and neurons of the myenteric plexus [19–21]. Of these various target neurons, the protective effects of GDNF on neuronal cell death following experimental lesions *in vivo* have been well documented for DA neurons [22–25], motor neurons [26], cholinergic neurons [15], and noradrenergic neurons [12]. Moreover, the requirement for GDNF in the normal development of myenteric plexus, sympathetic ganglia, trigeminal neurons, petrosal and nodose cranial ganglia, and motor neurons in lumbar spinal cord has been demonstrated in *GDNF* knockout mice [19–21]. Finally, in 1996, several groups simultaneously reported a novel heterodimeric receptor system for GDNF consisting of the newly identified GPI-linked subunit, GFRα-1 and the previously known proto-oncogene, c-*ret* [27–30]. Since the discovery of GDNF is highly significant, it is interesting that the glial cell approach has not been exploited further to identify yet additional neurotrophic or neuritotrophic molecules. Glia from different regions of the developing brain are a likely source of such molecules [31]; however, this source is one that remains relatively untapped. On the other hand, while not employing glial cells, the potential of identifying novel secreted factors using cell lines is accentuated by the recent identification of the second member of the GDNF family, neurturin, from media conditioned by COS cells [32].

GDNF ACTIONS ON DA NEURONS

DA factors had long been sought since the early work of the laboratories of Prochiantz and Heller suggested the presence of such factors in specific regions of developing brain [33, 34]. The existence of factors that could rejuvenate DA neurons in the adult brain was also suggested by transplantation studies in which various types of tissue grafts were reported to stimulate the sprouting or regeneration of DA fibers of chemically lesioned DA neurons in the adult mouse, rat, and monkey brain [35]. Despite these tantalizing observations and their significance for developing therapies for Parkinson's disease, it still is not known what mechanisms or endogenous factors are involved in the recovery of damaged DA neurons in the adult brain. GDNF clearly stimulates process growth from immature DA neurons, as demonstrated both in culture [4] and in grafts of fetal mesencephalon grown *in oculo* [6]. But, does GDNF also stimulate sprouting or regeneration of DA neurons in adult brain? Data addressing this issue remain equivocal. An increase in density of fibers immunoreactive (IR) for tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, was observed in mice in which GDNF was injected into the substantia nigra prior to an MPTP lesion or into the striatum at 7 and 16 days after an MPTP lesion [22]. This suggests that GDNF has both protective and restorative effects on DA neurons. In unlesioned rats, GDNF injected into the substantia nigra was reported to stimulate the sprouting of TH-IR fibers towards the injection site and to increase the density of TH-IR in the striatum [36]. In contrast, sprouting toward the injection site and increased TH-IR density following intranigral injection of GDNF were not observed in rats in which the DA neurons were progressively lesioned by striatal injection of 6-OHDA, even though significant effects of GDNF on DA cell survival were observed [37]. However, using a similar progressive lesion model, but delivering biosynthesized GDNF via adenoviral vectors, increased TH-IR fiber staining was observed in both substantia nigra and striatum in one study at 4 weeks [38], but not in another study at 7 weeks [39]. In rats lesioned by 6-OHDA injection into the medial forebrain bundle, a significant increase in TH-IR fibers in the substantia nigra was observed, whereas no increased density of TH-IR was observed in the striatum [40]. Similar increases in TH-IR fibers in the substantia nigra have been reported in MPTP-treated monkeys [25, 41]. In another paradigm in which capsulated GDNF-secreting cells were implanted into the 6-OHDA-lesioned striatum, TH positive fibers were observed growing into the capsules [42]. Since all of these studies have relied on one character of the DA phenotype, i.e. TH-IR, it has remained unclear whether GDNF just increases the expression of TH or stimulates growth of adult DA neurons, or a combination of the two. A recent study addressing this issue used a phenotypic marker other than TH-IR to study the effects of GDNF on sprouting of DA terminals in the striatum. GDNF was found to stimulate axonal sprouting in the partially deafferented striatum as assessed by binding of [^{3}H]*N*-[1-(2-benzo(*b*)thiopenyl)cyclohexyl]piperidine to DA uptake sites [43]. Although this still relies on one marker of the DA phenotype, the result strongly suggests that GDNF stimulates regrowth of DA fibers. On the other hand, an effect of GDNF in improving amphetamine-induced rotational behavior in rats with a unilateral lesion of the striatonigral system has been reported to occur in the absence of an increase in DA levels [44]. This not only emphasizes the presence of other cellular targets for GDNF in the striatum [45], but underscores the importance of looking at the whole picture in evaluating GDNF effects in animal models of Parkinson's disease.

The loss of phenotypic characters as a consequence of neuronal injury in the absence of cell death, although not

reported previously for 6-OHDA and DA neurons, has been reported for other neuronal types. Cholinergic neurons lose staining for choline acetyltransferase and shrink in size following lesions of the fimbria fornix [46]. Another interesting instance is the report that following adrenalectomy, granule neurons in the hippocampus essentially disappear, displaying minimal Nissl staining, and then reappear shortly after injection of dexamethasone [47]. These phenomena underscore the importance of the need for using a marker that is not linked biochemically to the phenotype of the neurons to follow the fate of lesioned neurons, as well as the amazing neurochemical plasticity inherent in even adult neurons. The effects of GDNF in preventing DA neuronal death following MPTP, 6-OHDA, and lesions of the medial forebrain bundle are probably the most remarkable and well-substantiated effects of GDNF in the adult brain. Moreover, three studies have demonstrated the protective effect of GDNF on DA neurons without relying on the TH-IR phenotype [37, 39, 58]. It still remains equivocal whether or not adult DA neurons actually have a capacity for regrowth. This is an essential issue to be addressed since the present trophic factor therapies in development for Parkinson's disease presume that there is the potential for growth in either diseased neurons or their healthy neighbors.

GDNF AS THERAPEUTIC MOLECULE FOR PARKINSON'S DISEASE

The striking survival effect of GDNF on DA neurons in the adult brain is well documented in animals. However, what causes the loss of DA neurons in humans with Parkinson's disease is not known. Therefore, in considering GDNF as a therapy for diseased neurons, it is significant that GDNF protects DA neurons not from just one type of damage, but from agents that cause damage through different mechanisms such as oxidative stress and generation of free radicals. GDNF recombinant protein injected as a bolus or slowly infused through the use of a minipump into the brains of adult rat, mouse, and monkey protects DA neurons against death elicited by several insults, including physical lesioning of the medial forebrain bundle [23], 6-OHDA-induced cell death [24], and toxicity induced by MPTP [22]. Moreover, the protective effects of GDNF are observed when the protein is provided either to the DA cell bodies in the midbrain or to the DA terminals in the striatum. GDNF also protects against the damage following ischemia induced by occlusion of the middle cerebral artery [48]. The intracellular mechanisms through which GDNF protects neurons from dying most likely are mediated through the GPI-linked GFRα-1 and c-ret tyrosine kinase receptor system [27, 49]. Although the downstream mechanisms remain an area of intense investigation, GDNF has been reported to stimulate phosphotidylinositol-3-kinase activity in sympathetic neurons [50], and to block the increase in nitric oxide in the cerebral hemisphere following occlusion of the middle cerebral artery [48]. Models of disease in animals cannot predict with certainty that a neurotrophic factor will overcome the effects of disease in a neuron in humans or even slow the progression of the disease. However, the broad actions of GDNF against several types of damage leading to neuronal death make it hopeful that GDNF will be an effective therapeutic molecule for Parkinson's disease.

On the other hand, the broad actions of GDNF, especially those on other types of neurons, may become troublesome if large amounts of GDNF are repeatedly injected or chronically infused into the brain, as would seem to be needed for such a slowly progressive disease as Parkinson's. In previous clinical studies in which patients received large amounts of brain derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF) or nerve growth factor (NGF), serious side-effects led to the cessation of the trials [51–54]. Intracerebral injections of GDNF have resulted in significant loss of body weight in animals [36, 55, 56]. It remains to be determined whether such weight loss or other side-effects in humans become problematic in clinical trials using repeated injections of GDNF protein in Parkinson's patients. However, since there are, at present, no approved therapies that slow down or prevent the death of DA neurons in Parkinson's patients, success in these trials would be a remarkable step. Evidence that GDNF administration to humans with this devastating disease ameliorates symptoms, even if short-lived, could be considered a success in terms of developing novel therapies for neurodegenerative diseases based on the neurotrophic factors. The reason that even a transient effect could be considered successful is that there are technologies on the horizon that could more successfully provide chronically increased levels of GDNF to support DA neurons in a more specific manner than that of injecting or infusing large amounts of recombinant protein into the brain in a relatively nonspecific manner.

GDNF GENE THERAPY

Several laboratories have been studying viral vectors as a means for delivering GDNF in animal models of Parkinson's disease. Adenoviral vectors are highly efficient in infecting cells in the brain following direct stereotaxic injection (for review, see Ref. 57). In our studies, we injected an adenoviral vector harboring human GDNF into the ventral mesencephalon near the cell bodies of the DA neurons. Then 6-OHDA was injected into the striatum where it was taken up by the DA terminals, causing a slow progressive lesion. To study only those DA neurons that projected to the lesion site in the striatum without relying on the DA phenotype, these were prelabeled with the retrograde tracer fluorogold. In untreated rats and in rats that received control vectors harboring either the cellular marker gene *LacZ* or an inactive deletion mutant of *GDNF*, the 6-OHDA resulted in a loss of about 70% of the fluorogold-labeled DA cell bodies. In rats injected with the GDNF adenovirus, a remarkable protection against

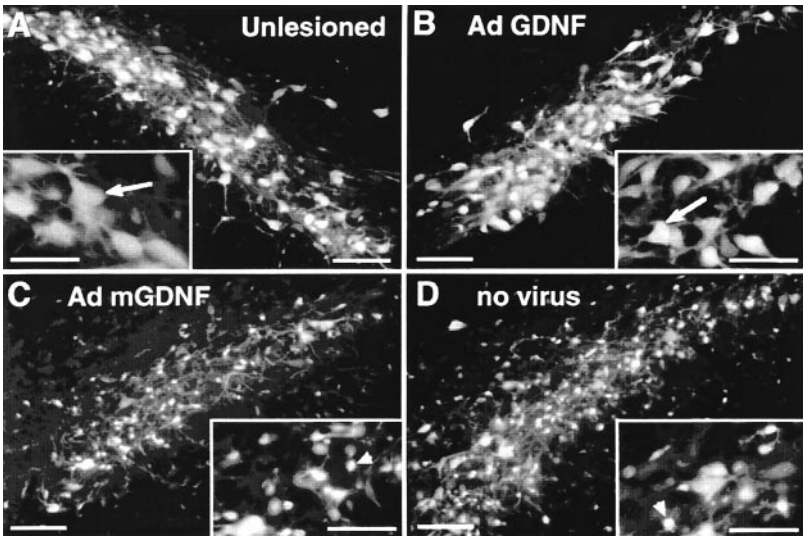

FIG. 1. Effect of *in vivo* gene therapy with adenovirus (Ad) GDNF on degeneration of DA neurons. Six weeks following intrastriatal 6-OHDA, many large, fluorogold (FG) positive cells (DA neurons, arrows) were observed in the substantia nigra on the unlesioned side (A) and on the lesioned side of a rat treated with Ad GDNF (B). In contrast, fewer large FG positive cells, but many small, secondarily labeled FG positive cells (microglia and other non-neuronal cells, arrowheads) were noted in rats treated with Ad mGDNF (C) or untreated (D). Scale bars in panels A–D are 100 μm and 50 μm for the insets. Reprinted with permission from Ref. 58 (Choi-Lundberg DL, Lin Q, Chang Y-N, Chiang YL, Hay CM, Mohajeri H, Davidson BL and Bohn MC, Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science* 275: 838–841, 1997). Copyright 1997 American Association for the Advancement of Science.*

6-OHDA-induced cell death was observed. An average of only 20% loss of fluorogold-labeled DA neurons was observed at 2 months, and, in several rats, the degree of protection was greater than 90% (Fig. 1) [58]. In a subsequent study, we observed a similar protective effect when the GDNF adenoviral vector was injected into the striatum near the terminals of the DA neurons [39]. Moreover, the GDNF adenoviral vector prevented the onset of DA-dependent behaviors that occurred in the control rats as a consequence of the unilateral 6-OHDA lesion, including amphetamine-induced rotation and ipsilateral-biased paw use [39]. Bilang-Bleuel and colleagues [38] also observed that striatal injection of a GDNF adenoviral vector increased the number of TH-IR neurons after a 6-OHDA lesion and improved performance on the amphetamine-induced rotation test compared with rats injected with control vector. These studies establish the principle that increasing the levels of biosynthesized GDNF in the vicinity of the DA neurons protects these neurons from degeneration in rodent models of Parkinson's disease. Moreover, following injection of a GDNF adenoviral vector, nanogram levels of GDNF transgene protein persist for up to 2 months (the longest time yet studied) in the vector injection site [58], suggesting that it is not necessary to inject large amounts of GDNF into the brain to elicit neuroprotective effects as long as the GDNF is being continually biosynthesized in the appropriate site. Interestingly, this site can be near either the DA cell bodies or their terminals, the latter site being more relevant since, in humans, the striatum is more surgically accessible than the midbrain.

These initial reports of GDNF gene therapy being effective in rat models of Parkinson's disease bring us to the brink of a new era in therapeutics for not only Parkinson's disease, but also for other neurodegenerative diseases and injuries to the nervous system, in general (see Refs. 59 and 60 for reviews). What further progress is needed to apply this idea to humans? An obvious starting point to address this question is to take the *in vivo* gene therapy approach into a non-human primate model of Parkinson's disease. The monkey lesioned with MPTP is unquestionably the animal model that most closely mimics neurodegenerative disease in humans [61]. Moreover, MPTP causes Parkinson's disease in humans [62]. A demonstration that GDNF gene therapy can either protect against the effects of MPTP or, more importantly, reverse the effects of a partial MPTP lesion in the non-human primate is pivotal to taking this approach into the clinic. While straightforward in concept, the actual application of vectors to primate brain is more complex than application to rodent brain. To date, there are no published data demonstrating long-term expression in primate brain from any viral vector. For adenoviral vectors, expression up to 1 month has been reported [63, 64], whereas for adenoassociated viral vectors, up to 3 months has been reported [65]. However, no studies have yet systematically compared different classes of vectors injected at varying titers and kinetics over an extended time course in the monkey brain. This type of comparison

is essential to identify an optimal vector and method of delivery for non-human primate, as well as human, brain. Studies that monitor host cellular and humoral responses to different classes of vectors are also essential. It is likely that stable gene delivery can be obtained in primate brain, although this may require the development of new generation viral vectors, or advances in non-virally-mediated gene delivery. It is necessary to develop vectors, whether viral or synthetic, that not only circumvent host cellular and humoral responses, but that also can be regulated by drug administration and be targeted to specific cell types in the brain. Such advances can be made by minimizing the expression of viral proteins and incorporating cellular promoters into vectors along with regulatory elements.

Even if means for optimal vector delivery with persistent transgene expression in the absence of host immune responses can be achieved in the non-human primate brain and these means are applied to demonstrate protective effects in the MPTP monkey, one lingering issue will remain that can be answered only though clinical trials. A healthy neuron damaged with 6-OHDA or MPTP in an animal brain may possess a different capacity for plasticity than a diseased neuron in aged human brain.

GDNF AS THERAPEUTIC MOLECULE FOR MOTOR NEURON DISEASE

A commentary on the potential of GDNF gene therapy for neurodegeneration would not be complete without also considering the potential of GDNF gene therapy to motor neuron disease, and perhaps also to spinal cord injury. The potency of GDNF on DA neurons also extends to other types of neurons, such as motor neurons [11]. In rodents whose motor neurons die as a consequence of genetic mutations or nerve lesions, administration of GDNF protein, injection of adenoviral vectors harboring a *GDNF* gene, or implementation of encapsulated cells secreting GDNF have demonstrated protective effects [66–70]. Since the motor neuron system is much more easily accessible than the striatonigral DA system and diseases such as ALS are rapidly progressive, insidious diseases, the clinical progress of GDNF gene therapy for ALS, especially the *ex vivo* approach, is likely to progress more rapidly than that for Parkinson's disease.

FUTURE PROSPECTS

A decade ago, the prospect that genes might be delivered to the human brain or spinal cord in such a way as to specifically affect a disease process in a particular nerve cell type would have been viewed as science fiction. Today this prospect is not yet a reality, but is certainly within the realm of possibility. The excitement that gene therapy, in general, has engendered has, in part, been its own downfall. Thus far, no clinical trials have met with clear success, although some trials have certainly produced encouraging results (for review, see Ref. 71). The rush to apply gene therapy clinically based on its incredible potential is understandable; however, as fully discussed in the NIH report on gene therapy published in 1993, a solid foundation of basic research on vectors and host responses is lacking [72]. Although many clinical trials are ongoing, the emphasis of research in this area is to develop better, safer vectors. The development of vectors for applications to the nervous system is particularly intriguing in this respect. Vector design should consider the cellular complexity of the nervous system. The incorporation of cellular promoters and enhancers that target transgene expression to specific cell types may be advantageous in offering more specific effects, more stable transgene expression, and different types of host cellular responses. For example, in the case of the striatonigral system, vectors could be designed to target a neurotrophic factor such as GDNF to the DA neurons themselves to elicit an autocrine effect, to adjacent astrocytes to elicit a paracrine effect, or to target neurons in the striatum to elicit an effect mediated by release, uptake, and retrograde transport of the factor. Other promoters that are inducible or repressible though peripheral administration of antibiotics or hormones should also be considered. Although non-virally-mediated DNA transfer to the nervous system is quite inefficient at present, it is likely that novel molecules will be developed to facilitate this process and that, eventually, these will supplant the use of viral vectors. Moreover, new molecular candidates that might have gene therapy applications to neurodegenerative diseases are being discovered daily. For example, genes involved in apoptosis, stimulation or inhibition of axonal growth and regeneration, and neurotrophic factor intracellular signaling mechanisms are interesting candidates for offering protective or restorative effects to diseased or damaged neurons. Finally, a delivery of a concoction of genes whose products act on diverse cellular pathways is likely to be applied to such a complex process as neurodegeneration.

The author thanks Dr. D. Eugene Redmond, Jr., for his comments on the manuscript. Preparation of this commentary was supported by the Medical Research Institute Council of Children's Memorial Hospital and the National Institutes of Health (NS31957).

References

1. Schubert D, Brass B and Duman J-P, Protein complexity of central nervous system cell lines. *J Neurosci* **6:** 2829–2836, 1986.
2. Greene LA and Tischler AS, Establishment of a noradrenergic clonal cell line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc Natl Acad Sci USA* **73:** 2424–2428, 1973.
3. Engele J, Schubert D and Bohn MC, Conditioned media derived from glial cell lines promote survival and differentiation of dopaminergic neurons *in vitro*: Role of mesencephalic glia. *J Neurosci Res* **30:** 359–371, 1991.
4. Lin L-FH, Doherty DH, Lile JD, Bektesh S and Collins F, GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* **260:** 1130–1132, 1993.

5. Stratford-Perricaudet LD, Makeh I, Perricaudet M and Briand P, Widespread long-term gene transfer to mouse skeletal muscles and heart. *J Clin Invest* **90:** 626–630, 1992.
6. Strömberg I, Björklund L, Johansson M, Tomac A, Collins F, Olson L, Hoffer B and Humpel C, Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons *in vivo*. *Exp Neurol* **124:** 401–412, 1993.
7. Schaar DG, Sieber B-A, Dreyfus CF and Black IB, Regional and cell-specific expression of GDNF in rat brain. *Exp Neurol* **124:** 368–371, 1993.
8. Trupp M, Ryden M, Jörnvall H, Funakoshi H, Timmusk T, Arenas E and Ibanez CF, Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. *J Cell Biol* **130:** 137–148, 1995.
9. Choi-Lundberg DL and Bohn MC, Ontogeny and distribution of glial cell line-derived neurotrophic factor (GDNF) mRNA in rat. *Brain Res Dev Brain Res* **85:** 80–88, 1995.
10. Tomac A, Widenflak J, Lin L-F, Kohno T, Ebendal R, Hoffer BJ and Olson L, Retrograde axonal transport of glial cell line-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. *Proc Natl Acad Sci USA* **92:** 8274–8278, 1995.
11. Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C, Armanini M, Simmons L, Moffet B, Vandlen RA, Koliatsos VE and Rosenthal A, GDNF: A potent survival factor for motoneurons present in perhiperhal nerve and muscle. *Science* **266:** 1062–1064, 1994.
12. Arenas E, Trupp M, Akerud P and Ibanez CF, GDNF prevents degeneration and promotes the phenotype of brain noradrenergic neurons *in vivo*. *Neuron* **15:** 1465–1473, 1995.
13. Maxwell GD, Reid K, Elefanty A, Bartlett PF and Murphy M, Glial cell line-derived neurotrophic factor promotes the development of adrenergic neurons in mouse neural crest cultures. *Proc Natl Acad Sci USA* **93:** 13274–13279, 1996.
14. Mount HTJ, Dean DO, Alberch J, Dreyfus CF and Black IB, Glial cell line-derived neurotrophic factor promotes the survival and morphologic differentiation of Purkinje cells. *Proc Natl Acad Sci USA* **92:** 9092–9096, 1995.
15. Williams LR, Inouye G, Cummins V and Pelleymounter MA, Glial cell line-derived neurotrophic factor sustains axotomized basal forebrain cholinergic neurons *in vivo*: Dose–response comparison to nerve growth factor brain-derived neurotrophic factor. *J Pharmacol Exp Ther* **277:** 1140–1151, 1996.
16. Beck KD, Irwin I, Valverde J, Brennan TJ, Langston JW and Hefti F, GDNF induces a dystonia-like state in neonatal rats and stimulates dopamine and serotonin synthesis. *Neuron* **16:** 665–673, 1996.
17. Ebendal T, Tomac A, Hoffer BJ and Olson L, Glial cell line-derived neurotrophic factor stimulates fiber formation and survival in cultured neurons from peripheral autonomic ganglia. *J Neurosci Res* **40:** 276–284, 1995.
18. Matheson C, Carnahan J, Urich J, Bocangel D, Zhang T and Yan Q, Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophic factor for sensory neurons: Comparison with the effects of the neurotrophins. *J Neurobiol* **32:** 22–32, 1997.
19. Moore MW, Klein RD, Fariñas I, Sauer H, Armanini M, Phillips H, Reichardt LF, Ryan AM, Carver-Moore K and Rosenthal A, Renal and neuronal abnormalities in mice lacking GDNF. *Nature* **382:** 76–79, 1996.
20. Pichel JG, Shen L, Shenq HZ, Granholm A-C, Drago J, Grinberg A, Lee EJ, Huang SP, Saarma M, Hoffer BJ, Sariola H and Westphal H, Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* **382:** 73–76, 1996.
21. Sánchez MP, Silos-Santiago I, Frisén J, He B, Lira SA and Barbacid M, Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* **382:** 70–73, 1996.
22. Tomac A, Lindqvist E, Lin L-FH, Ögren SO, Young D, Hoffer BJ and Olson L, Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*. *Nature* **373:** 335–339, 1995.
23. Beck KD, Valverde J, Alexi T, Poulsen K, Moffat B, Vandlen RA, Rosenthal A and Hefti F, Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain. *Nature* **373:** 339–341, 1995.
24. Kearns CM and Gash DM, GDNF protects nigral dopamine neurons against 6-hydroxydopamine *in vivo*. *Brain Res* **672:** 104–111, 1995.
25. Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, Russell D, Martin D, Lapchak PA, Collins F, Hoffer BJ and Gerhardt GA, Functional recovery in parkinsonian monkeys treated with GDNF. *Nature* **380:** 252–255, 1996.
26. Oppenheim RW, Housenou LJ, Johnson JE, Lin L-FH, Li L, Lo AC, Newsome AL, Prevette DM and Wang S, Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. *Nature* **373:** 344–346, 1995.
27. GFRα Nomenclature Committee, Nomenclature of GPI-linked receptors for the GDNF ligand family. *Neuron* **19:** 485, 1997.
28. Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, Tamir R, Antonio L, Hu Z, Cupples R, Louis J-C, Hu S, Altrock BW and Fox GM, GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-α, a novel receptor for GDNF. *Cell* **85:** 1113–1124, 1996.
29. Trupp M, Arenas E, Fainzilber M, Nilsson A-S, Sieber B-A, Grigoriou M, Kilkenny C, Salazar-Grueso E, Pachnis V, Arumae U, Sariola H, Saarma M and Ibanez CF, Functional receptor for GDNF encoded by the c-*ret* proto-oncogene. *Nature* **381:** 785–789, 1996.
30. Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, Sariola H and Pachnis V, GDNF signalling through the ret receptor tyrosine kinase. *Nature* **381:** 789–793, 1996.
31. Engele J and Bohn MC, The neurotrophic effects of fibroblast growth factors on dopaminergic neurons *in vitro* are mediated by mesencephalic glia. *J Neurosci* **11:** 3070–3078, 1991.
32. Kotzbauer P, Lampe P, Heuckeroth R, Golden J, Creedon D and Johnson E Jr, Neurturin, a relative of glial-cell-like-derived neurotrophic factor. *Nature* **384:** 467–470, 1996.
33. Rousselet A, Felter L, Chamak B and Prochiantz A, Rat mesencephalic neurons in culture exhibit different morphological traits in the presence of media conditioned on mesencephalic or striatal astroglia. *Dev Biol* **129:** 495–504, 1988.
34. Hoffman PC, Hemmendinger LM, Kotake C and Heller A, Enhanced dopamine cell survival in reaggregates containing telencephalic target cells. *Brain Res* **274:** 275–281, 1983.
35. Bohn MC, Marciano F, Cupit L and Gash DM, Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. *Science* **237:** 913–916, 1987.
36. Hudson J, Granholm A-C, Gerhardt GA, Henry MA, Hoffman A, Biddle P, Leela NS, Mackerlova L, Lile JD, Collins F and Hoffer BJ, Glial cell line-derived neurotrophic factor augments midbrain dopaminergic circuits *in vivo*. *Brain Res Bull* **36:** 425–432, 1995.
37. Sauer H, Rosenblad C and Björklund A, Glial cell line-derived neurotrophic factor but not transforming growth factor β3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. *Proc Natl Acad Sci USA* **92:** 8935–8939, 1995.
38. Bilang-Bleuel A, Revah F, Colin P, Locquet I, Robert J-J, Mallet J and Horellou P, Intrastriatal injection of an adenoviral vector expressing glial-cell-line-derived neurotrophic

factor prevents dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson disease. *Proc Natl Acad Sci, USA* **94:** 8818–8823, 1997.
39. Choi-Lundberg DL, Lin Q, Bardwayj L, Qian J, Davidson BL, Schallert T, Crippens D, Chang Y-N, Chiang YL and Bohn MC, Behavioral and cellular protection of rat dopaminergic (DA) neurons elicited by a GDNF adenoviral vector (Ad). *Exp Neurol* **154:** in press, 1998.
40. Bowenkamp KE, Hoffman AF, Gerhardt GA, Henry MA, Biddle PT, Hoffer BJ and Granholm A-CE, Glial cell line-derived neurotrophic factor supports survival of injured midbrain dopaminergic neurons. *J Comp Neurol* **355:** 479–489, 1995.
41. Gash DM, Zhang Z, Cass WA, Ovadia A, Simmerman L, Martin D, Russell D, Collins F, Hoffer BJ and Gerhardt GA, Morphological and functional effects of intranigrally administered GDNF in normal rhesus monkeys. *J Comp Neurol* **363:** 345–358, 1995.
42. Lindner MD, Winn SR, Baetge EE, Hammang JP, Gentile FT, Doherty E, McDermott PE, Frydel B, Ullman MD, Schallert T and Emerich DF, Implantation of encapsulated catecholamine and GDNF producing cells in rats with unilateral dopamine depletions and Parkinsonian symptoms. *Exp Neurol* **132:** 62–76, 1995.
43. Rosenblad C, Martinez-Serrano A and Björklund A, Intrastriatal glial cell line-derived neurotrophic factor promotes sprouting of spared nigrostriatal dopaminergic afferents and induces recovery of function in a rat model of Parkinson's disease. *Neuroscience* **82:** 129–137, 1998.
44. Tseng JL, Baetge EE, Zurn AD and Aebischer P, GDNF reduces drug-induced rotational behavior after medial forebrain bundle transection by a mechanism not involving striatal dopamine. *J Neurosci* **17:** 325–333, 1997.
45. Perez-Navarro E, Arenas E, Reiriz J, Calvo N and Alberch J, Glial cell line-derived neurotrophic factor protects striatal calbindin-immunoreactive neurons from excitotoxic damage. *Neuroscience* **75:** 345–352, 1996.
46. Gage FH, Wictorin K, Rischer W, Williams LR, Varon S and Bjorklund A, Retrograde cell changes in medial septum and diagonal band following fimbria-fornix transection: Quantitative temporal analysis. *Neuroscience* **19:** 241–255, 1986.
47. Azmitia EC and Liao B, Dexamethasone reverses adrenalectomy-induced neuronal de-differentiation in midbrain raphe-hippocampus axis. *Ann NY Acad Sci* **746:** 180–193, 1994.
48. Wang T, Lin S, Chiou A, Williams L and Hoffer B, Glial cell line-derived neurotrophic factor protects against ischemia-induced injury in the cerebral cortex. *J Neurosci* **17:** 4341–4348, 1997.
49. Treanor JJS, Goodman L, de Sauvage F, Stone DM, Poulsen KT, Beck CD, Gray C, Armanini MP, Pollock RA, Hefti F, Phillips HS, Goddard A, Moore MW, Buj-Bello A, Davies AM, Asai N, Takahaski M, Vandlen R, Henderson CE and Rosenthal A, Characterization of a multicomponent receptor for GDNF. *Nature* **382:** 80–83, 1996.
50. Creedon DJ, Tansey MG, Baloh RH, Osborne PA, Lampe PA, Fahrner TJ, Heuckeroth RO, Milbrandt J and Johnson EM Jr, Neurturin share receptors and signal transduction pathways with glial cell line-derived neurotrophic factor in sympathetic neurons. *Proc Natl Acad Sci USA* **94:** 7018–7023, 1997.
51. Olson L, Nordberg A, von Holst H, Bäckman L, Ebendal T, Alafuzoff I, Amberla K, Hartvig P, Herlitz A, Lilja A, Viitanen M, Winblad B and Seiger Å, Nerve growth factor effects ^{11}C-nicotine binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient (case report). *J Neural Transm Park Dis Dement Sect* **4:** 79–95, 1992.
52. Olson L, Bäckman L, Ebendal T, Eriksdotter-Jönhagen M, Hoffer B, Humpel C, Freedman R, Giacobini M, Meyerson B, Nordberg A, Seiger A, Strömberg I, Sydow O, Tomac A, Trok K and Winblad B, Role of growth factors in degeneration and regeneration in the central nervous system; clinical experiences with NGF in Parkinson's and Alzheimer's diseases. *J Neurol* **242**(Suppl 1)**:** S12–S15, 1994.
53. ALS CNTF Treatment Study Group, A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. *Neurology* **46:** 1244–1249, 1996.
54. Miller RG, Petajan JH, Bryan WW, Armon C, Barohn RJ, Goodpasture JC, Hoagland RJ, Parry GJ, Ross MA, Stromatt SC and rhCNTF ALS Study Group, A placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis. *Ann Neurol* **39:** 256–260, 1996.
55. Zhang Z, Miyoshi Y, Lapchak PA, Collins F, Hilt D, Lebel C, Kryscio R and Gash DM, Dose response to intraventricular glial cell line-derived neurotrophic factor administration in parkinsonian monkeys. *J Pharmacol Exp Ther* **282:** 1396–1401, 1997.
56. Martin D, Miller G, Fischer N, Diz D, Cullen T and Russell D, Glial cell line-derived neurotrophic factor: The lateral cerebral ventricle as a site of administration for stimulation of the substantia nigra dopamine system in rats. *Eur J Neurosci* **8:** 1249–1255, 1996.
57. Davidson BL and Bohn MC, Recombinant adenovirus. A gene transfer vector for study and treatment of CNS diseases. *Exp Neurol* **144:** 125–130, 1997.
58. Choi-Lundberg DL, Lin Q, Chang Y-N, Chiang YL, Hay CM, Mohajeri H, Davidson BL and Bohn MC, Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science* **275:** 838–841, 1997.
59. Choi-Lundberg DL and Bohn MC, Applications of gene therapy to neurological diseases and injuries. In: *Stem Cell Biology and Gene Therapy* (Eds. Quesenberry PJ, Stein GS, Forget B and Weissman S), pp. 503–553. John Wiley, New York, NY, 1998.
60. Bohn MC and Choi-Lundberg DL, Gene therapies for Parkinson's disease. In: *Gene Therapy for Neurological Disorders and Brain Tumors* (Eds. Chiocca EA and Breakefield XO), pp. 377–395. Humana Press, Totowa, NJ, 1998.
61. Forno LS, DeLanney LE, Irwin I and Langston JW, Similarities and differences between MPTP-induced parkinsonism and Parkinson's disease. Neuropathologic considerations. *Adv Neurol* **60:** 600–608, 1993.
62. Langston JW, The etiology of Parkinson's disease with emphasis on the MPTP story. *Neurology* **47**(Suppl 3)**:** S153–S160, 1996.
63. Davidson BL, Doran SE, Shewach DS, Latta JM, Hartman JW and Roessler BJ, Expression of *Escherichia coli* β-galactosidase and rat HPRT in the CNS of *Macaca mulatta* following adenoviral mediated gene transfer. *Exp Neurol* **125:** 258–267, 1994.
64. Bohn MC, Choi-Lundberg DL, Lawrence MS, Leranth C, Smith JC, Davidson BL, O'Banion MK and Redmond DE Jr, Prolonged transgene expression from an adenoviral vector injected into primate striatum. *Soc Neurosci Abstr* **23:** 295, 1997.
65. Bankiewicz KS, Leff SE, Nagy D, Jungles S, Rokovich J, Spratt K, Cohen L, Libonati M, Snyder RO and Mandel RJ, Practical aspects of the development of *ex vivo* and *in vivo* gene therapy for Parkinson's disease. *Exp Neurol* **144:** 147–156, 1997.
66. Houenou LJ, Oppenheim RW, Li L, Lo AC and Prevette D, Regulation of spinal motoneuron survival by GDNF during development and following injury. *Cell Tissue Res* **286:** 219–223, 1996.
67. Yan Q, Matheson C and Lopez OT, *In vivo* neurotrophic

effects of GDNF on neonatal and adult facial motor neurons. *Nature* **373:** 341–344, 1995.
68. Sagot Y, Tan SA, Hammang JP, Aebischer P and Kato AC, GDNF slows loss of motoneurons but not axonal degeneration or premature death of *pmn/pmn* mice. *J Neurosci* **16:** 2335–2341, 1996.
69. Munson JB and McMahon SB, Effects of GDNF on axotomized sensory and motor neurons in adult rats. *Eur J Neurosci* **9:** 1126–1129, 1997.
70. Matheson CR, Wang J, Collins FD and Yan Q, Long-term survival effects of GDNF on neonatal facial motoneurons after axotomy. *Neuroreport* **8:** 1739–1742, 1997.
71. Verma IM and Somia N, Gene therapy—Promises, problems and prospects. *Nature* **389:** 239–242, 1997.
72. Anderson WF, McGarrity GJ and Moen RC, Report to the NIH Recombinant DNA Advisory Committee on murine replication-competent retrovirus (RCR) assays. *Hum Gene Ther* **4:** 311–321, 1993.