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Glial cell line-derived neurotrophic factor (GDNF) gene therapy in an aged rat model of Parkinson's disease

Review Article

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Abbreviations: **GDNF**, glial cell line-derived neurotrophic factor; **BDNF**, brain-derived neurotrophic factor; **DA**, dopaminergic; **6-OHDA**, 6-hydroxydopamine; **Ad**, adenoviral; **SN**, substantia nigra pars compacta; **CNS**, central nervous system; **MFB**, medial forebrain bundle; **MPTP**, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; **MPP+**, 1-methyl-4-phenyl pyridinium; **MAO-B**, monoamine oxidase; **TH**, tyrosine hydroxylase; **AAV**, adeno-associated virus; **PSP**, persephin; **NTN**, neurturin; **IR**, immunoreactivity

Key Words: Glial cell line-derived neurotrophic factor, GDNF, gene therapy, ex-vivo, Parkinson's disease, neurotrophic factors, dopaminergic neurons, striatum, 6-OHDA, neurodegenerative disorders, brain-derived neurotrophic factor

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Summary

The chronic delivery of neuroprotective factors to specific regions of the CNS via gene therapy offers a new strategy for the treatment of neurodegenerative disorders. The neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF) is a potent dopaminergic (DA) trophic factor that ameliorates the behavioral and histological consequences of lesioning DA neurons in rodent and primate models of Parkinson's disease. GDNF gene therapy may therefore have potential use in the treatment of Parkinson's disease. We have observed previously that an adenoviral (Ad) vector harboring a GDNF minigene protects DA neurons from degeneration in the young rat brain. However, as Parkinson's disease occurs primarily in aged populations, we have also studied the effects of GDNF gene delivery in an aged rat model of Parkinson's disease. In the aged (20 month) Fischer 344 rat, an Ad vector was used to deliver GDNF either to the DA cell bodies in the SN or to the DA terminals in the striatum. One week following gene delivery, the neurotoxin 6-hydroxydopamine (6-OHDA) was injected unilaterally into the striatum to cause progressive degeneration of DA neurons. Injection of GDNF vector into either the striatum or SN provided significant cell protection against 6-OHDA. However, only striatal injections of Ad GDNF protected against the development of behavioral and neurochemical changes that occur in the DA-depleted brain. The results of this study are reviewed here and the behavioral and cellular effects of GDNF gene delivery into striatal versus mesencephalic sites are discussed.

I. Introduction

While neurotrophic factors are promising therapeutic agents in the treatment of neurodegenerative disorders, the delivery of these factors to the central nervous system (CNS) provides an interesting challenge. The identification of factors with potent dopaminergic (DA) trophic activities *in vitro* led to the concept of using neurotrophic factors as therapeutic agents for Parkinson's disease. The most potent of these factors is glial cell line-derived neurotrophic factor (GDNF) (Lin et al., 1993; Lin et al., 1994). There is much *in vivo* evidence demonstrating the effectiveness of GDNF protein in ameliorating neurodegeneration and maintaining behavioral function in animal models of Parkinson's disease. However, neurotrophic factors are labile substances that are unable to cross the blood-brain barrier in significant amounts. Therefore, the therapeutic use of factors such as GDNF in the treatment of Parkinson's disease will require the development of methods for delivering these factors to specific regions of the CNS in a continuous and regulatable manner that is safe, minimally invasive and does not result in side effects.

Parkinson's disease is a neurodegenerative disorder characterized by the progressive degeneration of DA neurons in the substantia nigra pars compacta (SN) that innervate the striatum. This degeneration results in the loss of DA terminals in the caudate and putamen leading to the clinical symptoms of bradykinesia, rigidity and resting tremor. Because Parkinson's disease is progressive, it is likely that long-term trophic support for degenerating DA neurons will be required. Repeated injections of recombinant neurotrophic factors into the human brain are unlikely to be practical, and are likely to elicit deleterious side effects over the long-term. In contrast, genetic approaches are ideal for delivery of neurotrophic factors to the CNS. Through the use of gene therapy techniques, increased levels of neurotrophic factor biosynthesis in the CNS might prevent neuronal cell death and enhance neuronal function. Furthermore, the development of vectors harboring genes driven by specific cellular promoters would provide the potential of limiting expression of a transgene to one typically defined cell population in the CNS, as well as regulating transgene expression.

Several research groups, using adenoviral (Ad) or adeno-associated viral vectors expressing GDNF, have reported that viral delivery of a GDNF gene protects DA neurons in the young adult rat brain from 6-OHDA-induced degeneration (Bilang-Bleuel et al., 1997; Choi-Lundberg et al., 1997; Choi-Lundberg et al., 1998; Mandel et al., 1997). However, Parkinson's disease is a neurodegenerative disorder that primarily affects the aging population. Age-related reductions in neurotransmitter synthesis and alterations in receptor levels may contribute to ongoing degenerative and behavioral deficits in Parkinson's disease. Multiple functional changes of the nigrostriatal DA system, similar to those observed in the Parkinsonian brain, have been described during normal aging in both animals and humans (Felten et al., 1992; Hubble, 1998; Morgan and Finch, 1988; Roth and Joseph, 1994). Compensatory events may be observed in the aged brain due to age-dependent degenerative changes occurring in the nigrostriatal and mesolimbic pathways. Alternatively, the aged brain may be unable to produce compensatory changes in response to either age-dependent or lesion-induced neuronal degeneration, worsening lesion-induced degenerative alterations in DA neuronal morphology and function in the aged brain (Demarest et al., 1980; Schallert, 1988; Unnerstall and Long, 1996; Zigmond et al., 1993). Therefore, while Ad GDNF has been observed to exhibit neuroprotective effects within the young rat brain, we believed it to be important to examine whether chronic biosynthesis of GDNF, achieved by Ad-mediated delivery of GDNF, is able to protect DA neurons and maintain DA function in an aged rat model of Parkinson's disease.

Here, we review the use of Ad to deliver a GDNF minigene to the aged (20 month) rat brain, prior to a partial, progressive lesion of the nigrostriatal pathway. The results show that Ad GDNF not only protects DA neurons from degeneration, but that functional consequences in DA target neurons following a lesion of the nigrostriatal pathway are prevented by Ad GDNF in a brain region specific manner.

II. Animal models of Parkinson's disease

Since Parkinson's disease does not occur naturally in animals, several well characterized models have been developed for stimulating the neuropathological and neurological features of Parkinson's disease in laboratory animals (reviewed in Bankiewicz et al., 1993). The most common animal model of Parkinson's disease involves the intracerebral injection of the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA), resulting in a reduction of dopaminergic phenotypic markers (i.e. tyrosine hydroxylase) and the selective death of DA neurons. 6-OHDA is a dopamine analog that is specifically taken up by the high-affinity dopamine transporter. 6-OHDA undergoes auto-oxidation, generating hydroxyl radical, hydrogen peroxide and superoxide anion, which causes damage to various cellular components. Injection of 6-OHDA into either the striatum, SN or the medial forebrain bundle (MFB), which contains dopaminergic axons from the ventral tegmental area and SN, results in specific loss of dopaminergic neurons and fibers. The neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) also causes Parkinsonian symptoms in humans, non-human primates and mice, following systemic administration. MPTP is oxidized to the toxic molecule, MPP+ (1-methyl-4-phenyl pyridinium) by the monoamine oxidase system (MAO-B). MPP+ enters dopaminergic neurons by high-affinity through the dopamine transporter and interferes with ATP production by inhibiting complex 1 of the mitochondrial electron transport chain.

The standard animal model of Parkinson's disease, based on unilateral injection of 6-OHDA into either the SN or the MFB, has been used extensively in Parkinson's disease research. Injection of 6-OHDA in either the SN or the MFB results in the rapid death of dopaminergic neurons, within 48 hours. However, due to the rapid and near-complete degeneration of nigral DA neurons, these lesion models do not closely reflect the clinical picture in which DA neurons die over a prolonged time. These models also are not optimal for studies on neuroprotection or regeneration of the nigrostriatal system (reviewed in Bjorklund et al., 1997). In contrast, intrastriatal delivery of 6-OHDA results in the rapid destruction of dopaminergic terminals in the striatum, followed by progressive degeneration of parent DA cell bodies in the SN over a period of several weeks (Sauer and Oertel, 1994). This degeneration is preceded and accompanied by cellular atrophy and a partial loss of tyrosine hydroxylase (TH) expression. This progressive lesion model yields an animal model which closely resembles the early stages of Parkinson's disease in humans, in which a portion of the nigrostriatal projection remains intact. These remaining neurons serve as a substrate for regeneration and functional recovery. The progressive lesion model is an ideal model for studying the neuroprotective or restorative properties of trophic factors (Bilang-Bleuel et al., 1997; Choi-Lundberg et al., 1997; Choi-Lundberg et al., 1998; Connor et al., 1999; Horger et al., 1998; Mandel et al., 1997; Milbrandt et al.,

1998; Rosenblad et al., 1999; Rosenblad et al., 1998; Sauer et al., 1995; Shults et al., 1996).

Unilateral physical and chemical lesions of the nigrostriatal pathway result in an imbalance in the level of DA and DA receptors between the two striatae, producing impairment in DA-dependent behavioral function. Lesion-induced behavioral impairment and the effects of experimental interventions can be quantified by several means (reviewed in (Schallert, 1995; Schwarting and Huston, 1996). Specifically, unilaterally lesioned animals exhibit rotational behavior in response to amphetamine or DA agonists, such as apomorphine, that is readily quantifiable (Schwarting and Huston, 1996; Ungerstedt and Arbuthnott, 1970; **Figure 1A**). Specifically, in animals with a unilateral lesion of the nigrostriatal DA system, the injection of drugs that act to release DA, such as amphetamine, will induce rotational behavior towards the denervated striatum due to an imbalance in striatal DA levels. Lesioned animals will turn away from the hemisphere where there is greater amphetamine-stimulated DA release and greater DA receptor stimulation (Ungerstedt, 1971). In contrast, injection of DA agonists, such as apomorphine, in animals exhibiting a < 90% loss of DA in the lesioned hemisphere, will induce rotational behavior away from the denervated striatum due to compensatory upregulation and supersensitivity of DA receptors in the unlesioned striatum (Ungerstedt and Arbuthnott, 1970). In addition, unilaterally lesioned animals exhibit deficits in contralateral limb use in several spontaneous behaviors (Olsson et al., 1995; Schallert, 1995; **Figure 1B**).

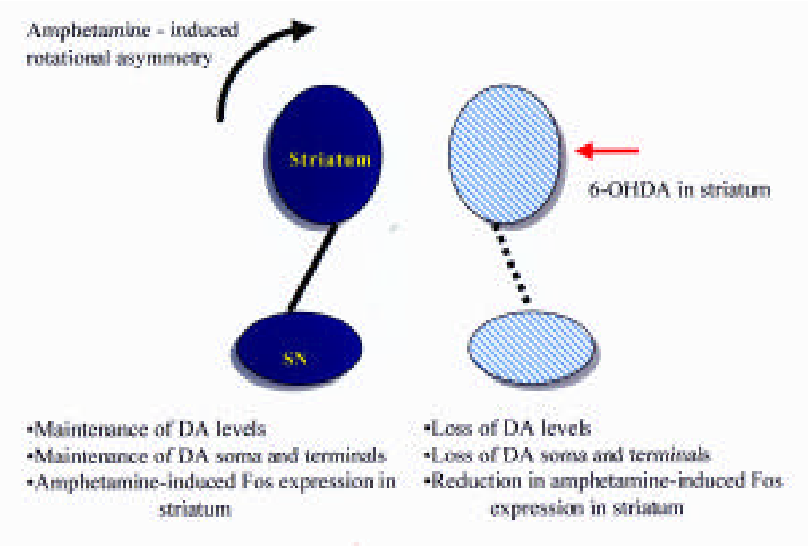

Figure 1A. - Diagram representing the behavioral and neurochemical changes that occur in the DA-depleted brain. In rats with a unilateral lesion of the nigrostriatal DA system, injection of amphetamine will induce rotational behavior towards the lesioned hemisphere. The lesioned rat turns away from the hemisphere where there is greater amphetamine-stimulated DA release and greater DA receptor stimulation. In the unlesioned striatum, injection of amphetamine also results in the induction of the transcription factor, *c-fos* in striato-nigral cells. However in the lesioned striatum, due to a loss of functional DA terminals and a reduction in striatal DA levels, amphetamine-induced Fos expression is reduced. Dashed lines indicates the degeneration and loss of the nigrostriatal pathway connecting the SN and the ST. Hatched area represents a loss of DA.

Figure 1B. - Rat exhibiting preferential use of the ipsilateral forelimb following a unilateral 6-OHDA-induced lesion of the nigrostriatal pathway. Spontaneous exploratory forelimb use is a non-drug induced test of forelimb locomotor function which has been shown to correlate with DA depletion in the lesioned hemisphere. Following a unilateral 6-OHDA lesion of the nigrostriatal pathway, rats preferentially use the forelimb ipsilateral to the side of the lesion to initiate and terminate weight-shifting movements during rearing and exploration along vertical surfaces.

III. Neurotrophic factors for dopaminergic neurons

The discovery and characterization of neurotrophic factors that promote the survival, neurite outgrowth and phenotypic differentiation of DA neurons has been an area of intense research in recent years. Early studies of DA neurons in culture showed glia from different brain regions and glial cell lines produce factors that influence the survival and differentiation of embryonic DA neurons in culture (Engele and Bohn, 1991; Engele et al., 1991; Rousselet et al., 1988). These studies prompted the search for specific DA neurotrophic factors. To date, more than 20 neurotrophic factors have been identified for DA neurons (**Table 1**). These include members of several growth factor families, operating through different intracellular signalling mechanisms, including the TGF-β superfamily, the neurotrophins, cytokines and mitogenic growth factors. The first neurotrophic factor shown to act directly on DA neurons was brain-derived neurotrophic factor (BDNF) (Hyman et al., 1991). Subsequently, GDNF was purified from the glial cell line, B49, and shown to be a very potent DA factor that enhances survival and neurite outgrowth of embryonic DA neurons *in vitro* (Lin et al., 1993; Lin et al., 1994). Recently, three additional members of the GDNF family have been identified - neurturin (Kotzbauer et al., 1996), persephin (Milbrandt et al., 1998) and artemin (Baloh et al., 1998). These factors have been shown to exhibit various degrees of trophic support to DA neurons.

A wealth of *in vivo* evidence has been accumulated in animal models of Parkinson's disease, supporting the potential therapeutic use of several DA neurotrophic factors, in particular GDNF, in the treatment of Parkinson's disease. A summary of these studies is shown in **Table 2**. GDNF has been shown to ameliorate the behavioral and pathological consequences of lesioning DA neurons in rodent and primate models of Parkinson's disease when administered to the adult nigrostriatal DA system (reviewed in Bjorklund et al., 1997; Gash et al., 1998). Specifically, in animal models of Parkinson's disease, single or continuous injection of recombinant GDNF protein has been shown to rescue injured or axotomized DA neurons when administered before or shortly after insult, and to preserve injured atrophic DA neurons during chronic neurodegeneration (Beck et al., 1995; Bowenkamp et al., 1995; Gash et al., 1996; Hoffer et al., 1994; Kearns and Gash, 1995; Lapchak et al., 1997; Sauer et al., 1995; Tomac et al., 1995). In addition, GDNF stimulates regenerative growth or axonal sprouting after partial lesions of the DA system and stimulates metabolism and function of lesioned DA neurons (Lindner et al., 1995; Rosenblad et al., 1998; Shults et al., 1996).

Table 1. Dopaminergic Neurotrophic Factors (updated from Bohn and Choi-Lundberg, 1997)

TGF-β Superfamily	
GDNF Family	
GDNF	(Lin et al., 1993; Lin et al., 1994)
Neurturin	(Kotzbauer et al., 1996)
Persephin	(Milbrandt et al., 1998)
Artemin	(Baloh et al., 1998)
Others	
TGF-β-1	(Krieglstein et al., 1995)
TGF-β-2, 3	(Krieglstein et al., 1995; Poulsen et al., 1994)
GDF-5	(Krieglstein et al., 1995)
Activin A	(Krieglstein et al., 1995)
Neurotrophins	
BDNF	(Hyman et al., 1991)
NT-3	(Hyman et al., 1994)
NT-4/5	(Hyman et al., 1994)
Mitogenic Growth Factors	
TGF-α	(Alexi and Hefti, 1993)
aFGF and bFGF	(Beck et al., 1993; Engele and Bohn, 1991; Ferrari et al., 1989; Otto and Unsicker, 1990)
EGF	(Casper et al., 1991)
Insulin	(Knusel et al., 1990)
IGF-I	(Beck et al., 1993)
IGF-2	(Liu and Lauder, 1992)
PDGF	(Othberg et al., 1995)
Midkine	(Kikuchi et al., 1993)
Cytokines	
CNTF	(Hagg and Varon, 1993; Magel et al., 1993)
IL-1β	(Akaneya et al., 1995)
IL-6	(Hama et al., 1991; von Coelln et al., 1995)
IL-7	(von Coelln et al., 1995)
Cardiotrophin-1	(Pennica et al., 1995)

Comparable findings for BDNF have been reported in both the 6-OHDA lesioned rat and the MPTP primate model in which BDNF improved DA levels or DA-dependent behavior in the absence of an effect on the density of DA fibers in the striatum (Altar et al., 1994; Galpern et al., 1996; Levivier et al., 1995; Lucidi-Phillipi et al., 1995; Tsukahara et al., 1995; Yoshimoto et al., 1995). In addition, both neurturin and persephin have been reported to protect mature DA neurons from cell death induced by 6-OHDA in the absence of striatal reinnervation and DA-dependent behavioral improvement (Akerud et al., 1999; Horger et al., 1998; Milbrandt et al., 1998; Rosenblad et al., 1999; Tseng et al., 1998).

While many studies have demonstrated the efficacy of DA neurotrophic factors, such as GDNF, in animal models of Parkinson's disease, these studies have utilized infusion of protein into the striatum, near the SN or into the lateral ventricles. In addition, large quantities of proteins are infused in these studies (typically 10μg or more), and repeated injections are typically required to maintain effects. Repeated injections of recombinant neurotrophic factors into the human brain are unlikely to be practical, and are likely to elicit deleterious side effects over the long term. In contrast, genetic approaches are ideal for delivering GDNF to the CNS.

Several laboratories have studied viral vector mediated GDNF gene delivery in animal models of Parkinson's disease (**Table 2**). In young rats, we have observed that injection of an Ad vector harboring human GDNF into the SN one week prior to a progressive 6-OHDA lesion significantly protects DA neurons in the lesioned SN (Choi-Lundberg et al., 1997; Choi-Lundberg et al., 1998). In addition, intrastriatal injection of Ad GDNF in this model was effective in protecting DA cell bodies and also prevented the onset of DA-dependent behaviors that occurred in control rats as a consequence of the unilateral 6-OHDA lesion (Choi-Lundberg et al., 1998). Similar effects of GDNF gene delivery have been confirmed by other groups using either adenoviral or adeno-associated viral vectors (**Table 2**). In addition, our laboratory has recently observed that injection of Ad GDNF into the SN 1 week after 6-OHDA induced damage has commenced, not only rescues DA neurons, but also protects against alterations in DA target cells in the striatum (Kozlowski et al, Submitted).

Table 2 Delivery of neurotrophic factors in animal models of Parkinson's disease (selected references)

Paradigm	Delivery site, Factor	Biological Effects	Reference
Neurotrophic factor protein infusion			
6-OHDA - complete	Intranigral, GDNF	↗ in nigral DA levels Improvement in behavioral function	Hoffer et al., 1994
6-OHDA - partial striatal	Intranigral, BDNF / NT-3	↗ in striatal DA metabolites Improvement in behavioral function No change in striatal DA levels No reinnervation of lesioned striatum	Altar et al., 1994
6-OHDA - complete	Intranigral, GDNF	↗ survival of DA cell bodies in SN Improvement in behavioral function No reinnervation of lesioned striatum	Bowenkamp et al., 1995
MPTP - mouse model	Intrastriatal, GDNF Intranigral, GDNF	↗ in nigral and striatal DA / metabolite levels Protection of striatal innervation (intrastriatal only)	Tomac et al., 1995
6-OHDA - complete	Intranigral, GDNF	Protection of DA cell bodies in SN Improvement in behavioral function	Beck et al., 1995
MPTP - monkey model	Intrathecal, BDNF	Protection of DA cell bodies in SN Improvement in behavioral function	Tsukahara et al., 1995
6-OHDA - complete	Intranigral, GDNF Intraventricular, GDNF	↗ in striatal DA / metabolite levels Protection of DA uptake sites Improvement in behavioral function	Opacka-Juffry et al., 1995
6-OHDA - complete or partial	Intranigral, GDNF	Protection of DA cell bodies in SN	Kearns and Gash, 1995
6-OHDA - partial striatal	Intranigral ,GDNF	Protection of DA cell bodies in SN	Sauer et al., 1995
6-OHDA - partial striatal	Intrastriatal ,GDNF	Protection of DA cell bodies in SN Protection of striatal innervation Improvement in behavioral function	Shults et al., 1996

6-OHDA - complete	Intranigral, GDNF	Protection of DA cell bodies in SN No reinnervation of lesioned striatum No improvement in behavioral function	Winkler et al., 1996
MPTP - monkey model	Intracerebral, GDNF	↗ DA cell body size ↗ fiber density ↗ in nigral and striatal DA levels Improvement in behavioral function	Gash et al., 1996
6-OHDA - complete	Intraventricular, GDNF	↗ in nigral DA / metabolite levels Protection of DA cell bodies in SN Improvement in behavioral function	Bowenkamp et al., 1997
6-OHDA - complete	Intranigral, GDNF	Protection of DA cell bodies in SN Did not restore TH-IR in nigral neurons	Lu and Hagg, 1997
6-OHDA - complete	Intranigral ,GDNF	Partial restoration of nigral DA levels Improvement in behavioral function	Hoffman et al., 1997
6-OHDA - complete	Intraventricular, GDNF Intranigral, GDNF	↗ expression of TH in SN Improvement in behavioral function Intranigral GDNF prevented lesion-induced increase in dynorphin A	Lapchak et al., 1997
6-OHDA - partial striatal	Intranigral, NTN	Protection of DA cell bodies in SN	Horger et al., 1998
6-OHDA - complete	Intranigral, GDNF Intraventricular, GDNF	Prevented loss of DA re-uptake sites Prevented loss of striatal DA / metabolites Prevented loss of DA cell bodies in SN Improvement in behavioral function	Sullivan et al., 1998
6-OHDA - partial striatal	Intranigral, PSP	Protection of DA cell bodies in SN	Milbrandt et al., 1998
MPTP - mouse model	Intrastriatal ,GDNF	Protection of nigral and striatal DA levels Improvement in behavioral function	Cheng et al., 1998
6-OHDA - partial striatal	Intrastriatal, GDNF	Restored DA uptake sites Protection of DA cell bodies in SN Improvement in behavioral function	Rosenblad et al., 1998
6-OHDA - partial striatal	Intrastriatal, GDNF / NTN Intraventricular, GDNF / NTN	GDNF - Protection of DA cell bodies in SN - No reinnervation of lesioned striatum - No improvement in behavioral function NTN - Partial protection of DA cell bodies in SN (intrastriatal only) - No reinnervation of lesioned striatum - No improvement in behavioral function	Rosenblad et al., 1999
MPTP - monkey model	Intraventricular, GDNF	↗ in nigral DA / metabolite levels Improvement in behavioral function	Gerhardt et al., 1999
Ex vivo gene delivery			
MPP+ - rat model	Fibroblasts, BDNF	Protection of DA cell bodies in SN	Frim et al., 1994
6-OHDA - complete	BHK, GDNF	TH-IR fiber ingrowth to BHK-GDNF capsules No improvement in behavioral function	Lindner et al., 1995
6-OHDA - partial SN	Astrocytes, BDNF	No effect on TH-IR fibers in ipsilateral striatum Improvement in behavioral function	Yoshimoto et al., 1995
6-OHDA - partial striatal	Fibroblasts, BDNF	Prevented loss of nerve terminals Protection of DA cell bodies in SN	Levivier et al., 1995
6-OHDA - complete	Fibroblasts, BDNF	↗ in nigral DA / metabolite levels Did not induce fiber sprouting No improvement in behavioral function	Lucidi-Phillipi et al., 1995

MPP+ - rat model	Fibroblasts, BDNF	↗ in nigral DA levels	Galpern et al., 1996
6-OHDA - complete	BHK, GDNF	Protection of DA cell bodies in SN Improvement in behavioral function Did not prevent loss of striatal DA	Tseng et al., 1997
6-OHDA - complete	BHK, NTN	Protection of DA cell bodies in SN No improvement in behavioral function	Tseng et al., 1998
6-OHDA - complete	BHK, GDNF BHK, NTN	Both GDNF and NTN protected DA cell bodies in SN GDNF induced TH-IR, sprouting or hypertrophy of DA neurons	Akerud et al., 1999
In vivo gene delivery			
6-OHDA - partial striatal	Intranigral, Ad GDNF	Protection of DA cell bodies in SN	Choi-Lundberg et al., 1997
6-OHDA - partial striatal	Intrastriatal, Ad GDNF	Protection of DA cell bodies in SN Protected innervation of striatum Improvement in behavioral function	Bilang-Bleuel et al., 1997
6-OHDA - complete	Intranigral, Ad GDNF	Restored nigral DA levels Improvement in behavioral function	Lapchak et al., 1997
MPTP - mouse model	Intrastriatal, Ad GDNF	↗ in striatal DA levels	Kojima et al., 1997
6-OHDA - partial striatal	Intranigral, AAV GDNF	Protection of DA cell bodies in SN	Mandel et al., 1997
6-OHDA - partial striatal	Intrastriatal, Ad GDNF	Protection of DA cell bodies in SN Improvement in behavioral function	Choi-Lundberg et al., 1998

IV. The differential effects of GDNF gene delivery in the striatum and SN of the aged Parkinsonian rat

In order to extend our initial observations in the young rat, we recently examined whether Ad GDNF was able to restore compensatory events, protect DA neurons and maintain DA function in the aged rat brain following a partial lesion of the nigrostriatal pathway. It has also become apparent that the site of GDNF administration is important in determining the degree of functional recovery observed in the damaged or degenerating nigrostriatal DA system. Previous studies have demonstrated that, while repeated injections of GDNF near cell bodies in the SN following a partial 6-OHDA lesion of the nigrostriatal pathway protects or restores DA neuronal function, intranigral injection of GDNF does not prevent impairment of behavioral function in the unilaterally lesioned Parkinsonian animal (Sauer et al., 1995; Winkler et al., 1996). This suggests that long-lasting functional recovery in the intrastriatal 6-OHDA lesion model may require reinnervation and restoration of DA neurotransmission in the denervated striatum. Supporting this proposal, several studies examining the effect of intrastriatal injections of GDNF have reported that, in addition to preventing the loss of dopaminergic neurons in the lesioned SN, GDNF injected into the striatum also induces partial axonal regeneration or reinnervation of the denervated striatum and prevents behavioral impairment caused by 6-OHDA-induced depletion of striatal DA (Bilang-Bleuel et al., 1997; Choi-Lundberg et al., 1998; Rosenblad et al., 1998; Shults et al., 1996).

In order to fully examine the differential effect of injecting GDNF either near DA cell bodies in the SN or at dopaminergic terminals in the striatum, we compared the behavioral and cellular effects of GDNF gene delivery into striatal and mesencephalic sites in aged (20 month) Fischer-344 rats with progressive lesions of the nigrostriatal pathway (Connor et al., 1999). In this study, a subpopulation of DA neurons was prelabelled by bilateral intrastriatal injection of the retrograde tracer fluorogold (FG) so that the fate of those DA neurons that projected specifically to the lesion site could be assessed without having to rely on DA phenotypic markers. Following FG injection, one group of rats was injected unilaterally in the striatum, while a second group of rats was injected unilaterally into the SN with Ad vectors encoding either GDNF, a mutant form of GDNF lacking bioactivity (muGDNF) or LacZ. An additional group of rats underwent surgery, but received no vector injection into either the striatum or the SN. One week later, rats received a unilateral intrastriatal injection of 6-OHDA on the same side as the vector injection and at the same coordinates as the FG injection.

Thirty-five days after lesioning, we observed that injections of Ad GDNF into either the striatum or the SN provided significant cell protection against 6-OHDA (Connor et al., 1999). As shown in **Figure 2**, Ad GDNF injected in the SN protected an average of about 55% of

the FG labeled DA neurons while Ad GDNF injected in the striatum protected an average of about 65% of FG labeled neurons. In contrast, only an average of about 30% of the neurons remained in the control groups (Connor et al., 1999). Overall, injections of Ad GDNF into either the striatum or the SN were similarly effective in protecting DA neurons in the lesioned SN of the aged rat brain. This indicates that the site of GDNF injection (striatum versus SN) does not affect the degree of neuronal protection seen in the lesioned SN.

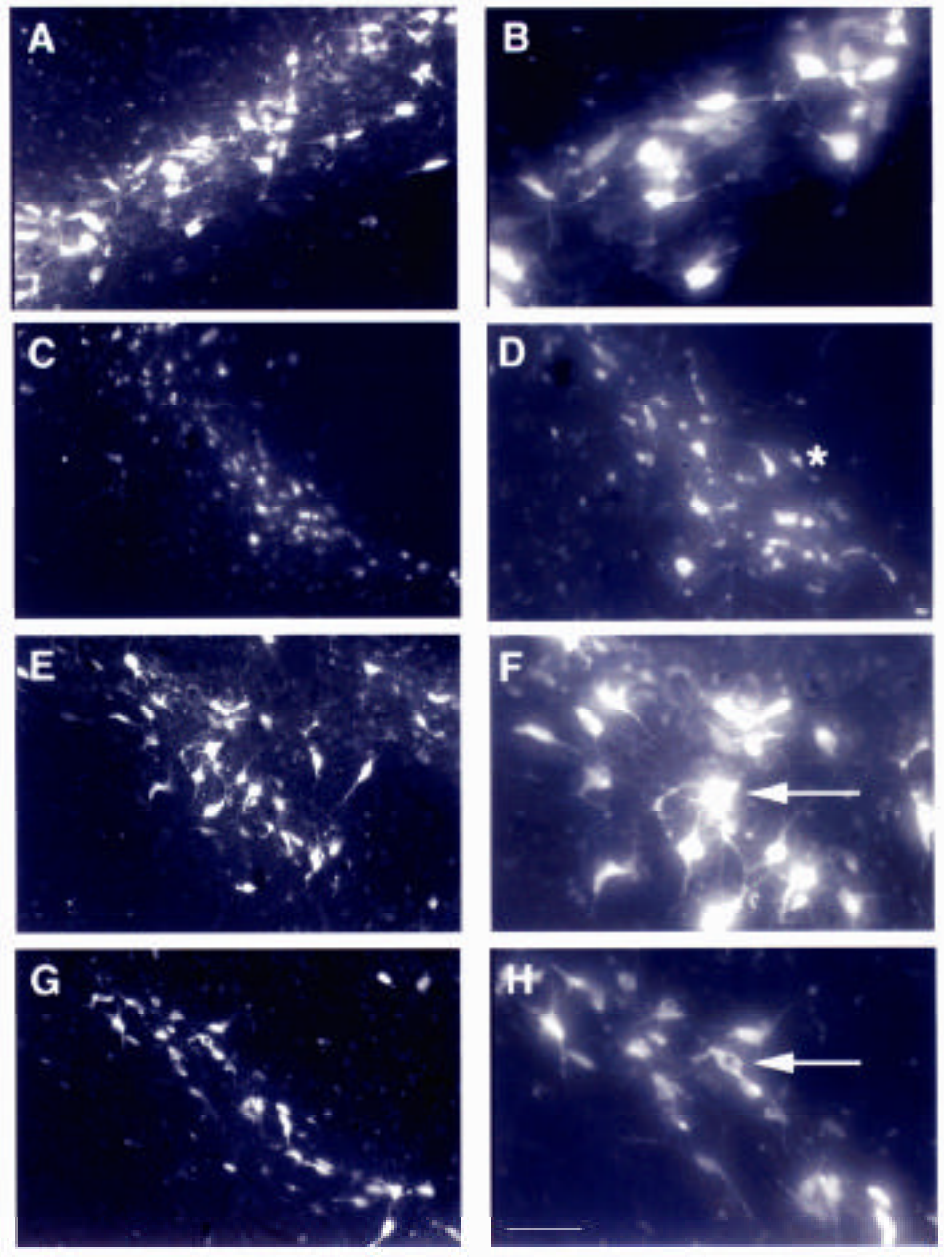

Figure 2A: Injection of Ad GDNF in either the striatum or the SN prevents the degeneration of FG-positive DA neurons in the SN five weeks after 6-OHDA. - Five weeks following intrastriatal injection of 6-OHDA many large FG-positive cells (i.e.: DA neurons - arrows) remain in the SN on the unlesioned side (A & B) and on the lesioned side in rats injected with Ad GDNF in either the striatum (E & F) or the SN (G & H). In contrast, fewer large FG-positive neurons, but many small secondarily labeled FG-positive cells (microglia and other non-neuronal cells - *) were observed in the lesioned SN in control rats (C & D). Scale bars: A, C, E & G = 100μm; B, D, F & H = 50μm. Reprinted with permission from Connor et al, 1999. Copyright 1999 Stockton Press.

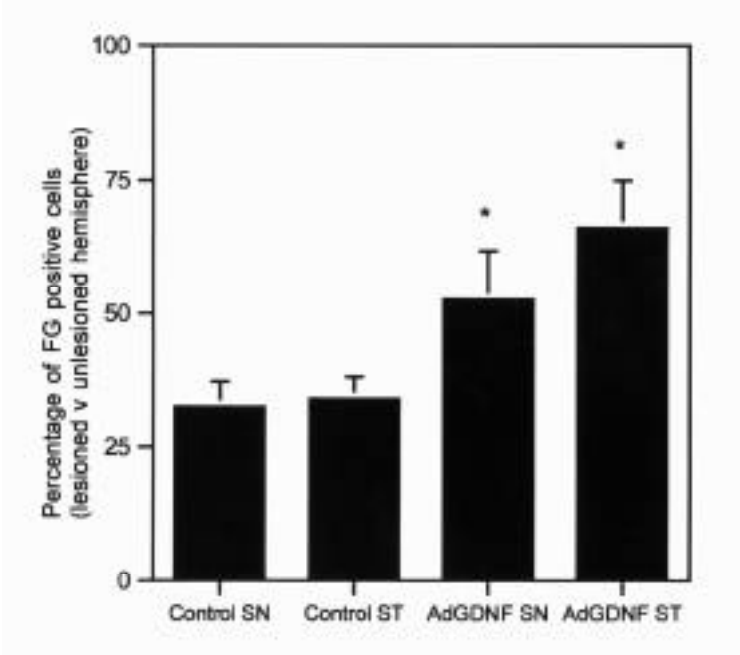

Figure 2B: (Cont.) - A significant increase in the percentage of FG-positive DA neurons was observed in the lesioned SN in rats injected with Ad GDNF in either the striatum or the SN compared to control groups (* $p \leq 0.01$). There was no significant difference in the percentage of FG-positive neurons between rats injected with Ad GDNF in the striatum versus the SN ($p > 0.05$). Reprinted with permission from Connor et al, 1999. Copyright 1999 Stockton Press.

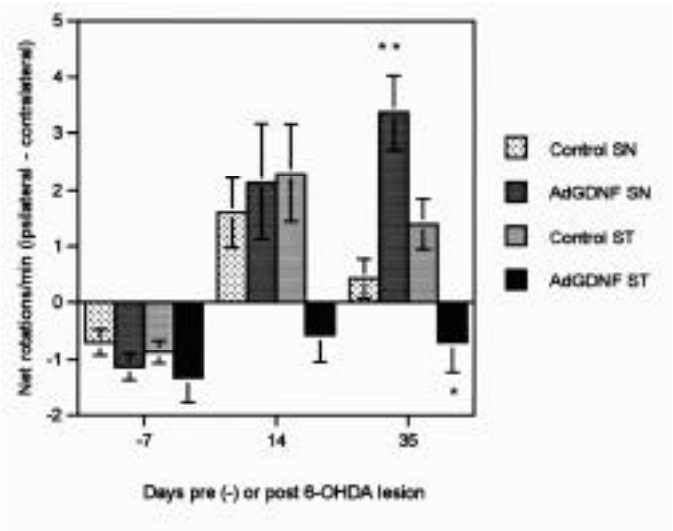

Figure 3: Striatal injections of Ad GDNF prevents the development of amphetamine-induced ipsilateral rotational behavior observed in control rats after 6-OHDA lesioning. Rats were injected with dl-amphetamine (5mg/kg i.p), placed in a rotation chamber and their behavior recorded for 60 minutes. Baseline amphetamine rotation tests were performed 7 days before lesioning (-7) and the results used to assign the side of 6-OHDA lesioning. Five weeks after lesioning, a significant reduction in ipsilateral rotational behavior was observed in rats injected with Ad GDNF in the striatum compared to control groups and rats injected with Ad GDNF in the SN (* $p \leq 0.05$). In contrast, rats injected with Ad GDNF into the SN exhibited a significant increase in ipsilateral rotational behavior 35 days after lesioning compared to control groups (** $p \leq 0.05$). Reprinted with permission from Connor et al, 1999. Copyright 1999 Stockton Press.

The differential effects of the vector placed into these two sites became evident however, when we examined DA dependent behaviors and cellular changes in DA target neurons. Only striatal injections of Ad GDNF protected against the development of behavioral impairment characteristic of unilateral DA-dependent deficits (Connor et al., 1999). Thirty-five days after lesioning, rats injected with Ad GDNF in the striatum exhibited a significant reduction in amphetamine-induced rotational asymmetry compared to control groups and rats injected with Ad GDNF in the SN (**Figure 3**, Connor et al., 1999). In addition, rats injected with Ad GDNF in striatum exhibited a significant

reduction in the preferential use of the ipsilateral forelimb 21 days and 28 days after 6-OHDA lesioning compared to control groups and rats injected with Ad GDNF in the SN (Connor et al., 1999). Maintenance of DA-dependent behavioral function requires the presence of functional DA terminals and/or preservation of striatal DA levels. Our observations suggest that when GDNF biosynthesis is increased near the DA terminals prior to 6-OHDA delivery, the terminals are protected against degeneration and striatal DA levels persist. In contrast, when GDNF biosynthesis is increased near DA cell bodies in the aged rat brain, levels are sufficient to inhibit cell death, but insufficient to increase striatal DA levels or to stimulate terminal sprouting. This apparently results in the development of behaviors indicative of unilateral DA deficiency.

These behavioral observations are further supported by morphological data on the response of striatal target neurons and DA fiber density. In the unlesioned striatum, injection of indirect DA agonists, such as amphetamine results in the expression of Fos protein (**Figure 1A**; Graybiel et al., 1990; Robertson et al., 1989). Amphetamine is thought to induce Fos in the striatum primarily by its ability to release endogenous DA which, via activation of DA D1 receptors, triggers a transduction cascade culminating in the expression of the *c-fos* gene in striato-nigral cells (Berretta et al., 1992; Konradi et al., 1994; Paul et al., 1992; Robertson et al., 1992). Therefore in the striatum, amphetamine-induced Fos expression requires the presence of functional DA terminals and is used as a marker of DA-mediated postsynaptic responses (Cenci et al., 1992; Labandeira-Garcia et al., 1996; Morgan and Curran, 1991). We observed that striatal, but not SN, injections of Ad GDNF increased amphetamine-induced Fos expression in the lesioned striatum above that in the unlesioned contralateral striatum, indicating protection of DA terminals (Connor et al., 1999). As shown in **Figure 4**, a reduction in the percentage of Fos-immunopositive neurons, reflecting a decrease in functional DA terminals was seen in both control groups and rats injected with Ad GDNF in the SN at the lesion site and 700µm posterior to the lesion site (Connor et al., 1999). In contrast, rats injected with Ad GDNF in the striatum exhibited a significant increase in the percentage of Fos-immunopositive neurons at both the lesion site and 700µm posterior to the lesion site compared to control groups and rats injected with Ad GDNF in the SN (**Figure 4**; Connor et al., 1999). This suggests that increased levels of GDNF near the terminals of DA neurons increased DA levels available to target neurons. This increase could result from either an increase in DA release per terminal or an increase in the number of DA terminals. In support of this latter possibility, we observed that striatal, but not SN, injections of Ad GDNF reduced tyrosine hydroxylase fiber (TH) loss in the lesioned striatum. The area of TH-immunoreactive fiber denervation (lesion size) was significantly decreased in rats injected with Ad GDNF in the striatum compared to control groups (Connor et al., 1999), suggesting that Ad GDNF injection in the striatum protects TH-immunopositive fibers or stimulates neuronal sprouting of fibers in the denervated striatum.

In a partial 6-OHDA lesion model, GDNF could exert its neurotrophic effects both at the level of DA cell bodies in the SN or at the level of DA axon terminals in the striatum. Injection of Ad GDNF into the striatum will produce GDNF expression at both the terminals and the somata of the nigrostriatal pathway via retrograde transport (Choi-Lundberg et al., 1998). In contrast, Ad GDNF injected into the SN will produce GDNF mainly near DA somata. Striatal injections of Ad GDNF prior to lesioning may have prevented the initial loss of DA terminals in the striatum, or, though less likely, may have interfered with the up-take of 6-OHDA so that cells were not susceptible to degeneration. Our results suggest that increased levels of striatal GDNF biosynthesis prevents DA neuronal loss and protects DA terminals from 6-OHDA-induced damage, thereby maintaining nigrostriatal function in the aged rat brain (Connor et al., 1999; **Figure 5**).

V. Conclusion

GDNF gene therapy has a potential use in the clinical treatment of Parkinson's disease. As Parkinson's disease is a progressive disorder, we feel that both protective and rescue paradigms are important approaches to this disease. The protective GDNF paradigm aims at preventing further loss of DA neurons and function, while the rescue paradigm aims at reversing damage to DA neurons. To date, our results indicate that, with regard to preventing DA neuronal loss and protecting DA terminals from degeneration, the striatum is a more desirable site for GDNF therapy than the SN. This finding is relevant for the application of gene therapy to patients with Parkinson's disease. However, further technological advances are required to realize the potential of gene therapy for Parkinson's disease. Currently, there is no clinically safe vector available that provides long-term, stable gene expression in the CNS in absence of cytotoxic effects. Therefore, the development of new generation vectors will hopefully lead to stable gene expression and a reduction in host cellular and humoral responses.

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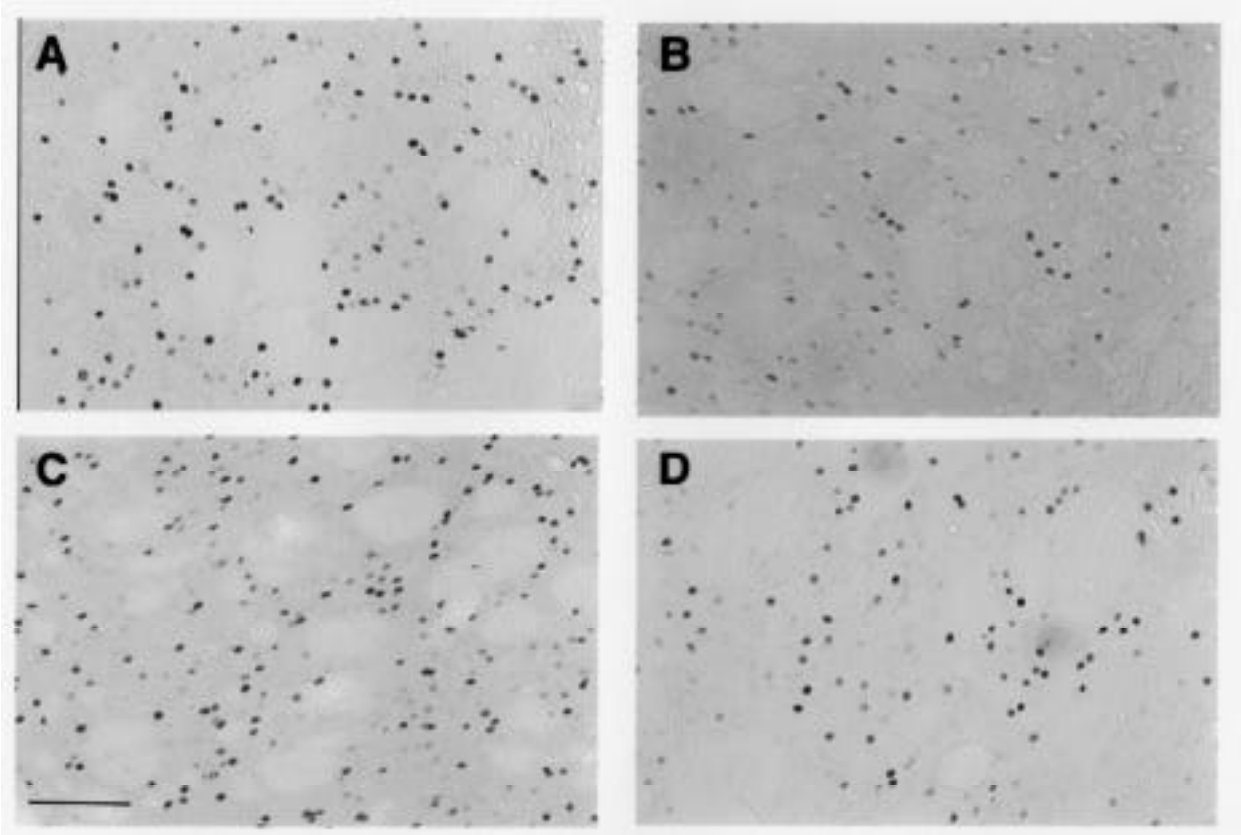

Figure 4A: The level of amphetamine-induced Fos expression is significantly increased in rats injected with Ad GDNF in the striatum. -Rats were injected with DL-amphetamine (5mg/kg i.p) 2 hours prior to sacrifice. In the unlesioned striatum, amphetamine-induced Fos expression is observed in striato-nigral cells (A). In contrast, a reduction in the level of Fos-immunoreactivity is observed in the lesioned striatum in both control rats (B) and rats injected with Ad GDNF in the SN (D). In rats injected with Ad GDNF in the striatum (C), the level of Fos-immunoreactivity in the lesioned striatum is greatly increased compared to the unlesioned hemisphere. Scale bar = 100μm. Reprinted with permission from Connor et al, 1999. Copyright 1999 Stockton Press.

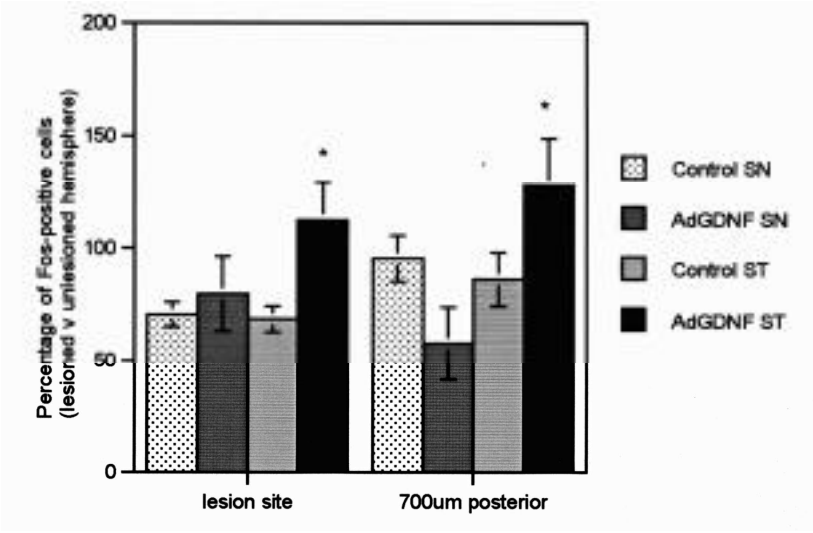

Figure 4B - Rats injected with Ad GDNF in the striatum exhibit a significant increase in the percentage of Fos-immunoreactive cells at both the lesion site and 700μm posterior to the lesion site compared to rats injected with Ad GDNF in the SN and control groups (* $p \leq 0.05$). Reprinted with permission from Connor et al, 1999. Copyright 1999 Stockton Press.

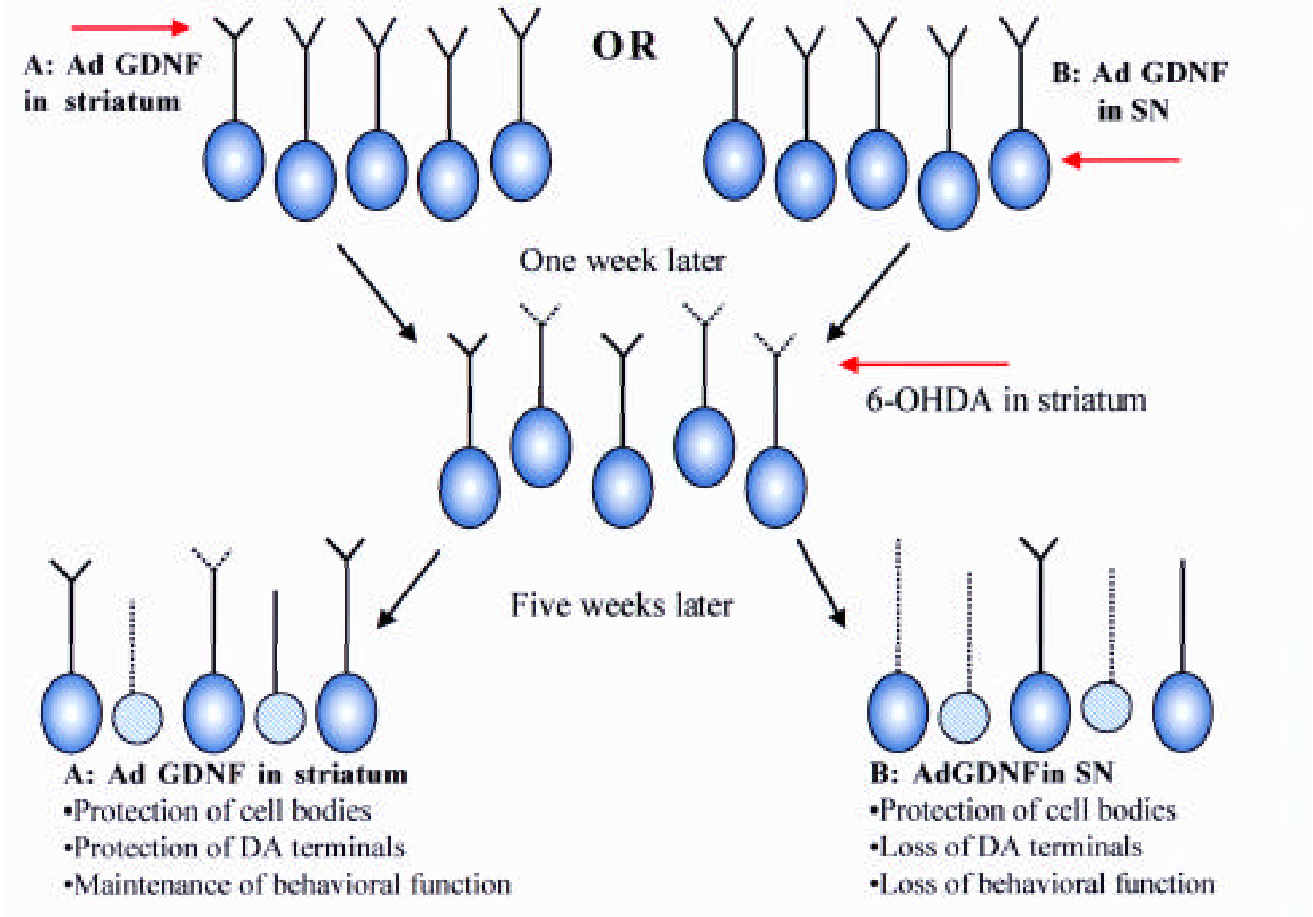

Figure 5: The differential effects of Ad GDNF in the striatum and the SN of the aged Parkinsonian rat. The site of GDNF administration is important in determining the degree of functional recovery observed in the damaged or degenerating nigrostriatal DA system. We compared the behavioral and cellular effects of GDNF gene delivery into striatal and mesencephalic sites in aged (20 month) rats with progressive lesions of the nigrostriatal pathway. Ad GDNF was injected either at dopaminergic terminals in the striatum (**A**) or near dopaminergic cell bodies in the SN (**B**). One week following gene delivery, the neurotoxin 6-OHDA was injected unilaterally into the striatum to cause progressive degeneration of DA neurons. Five weeks after lesioning, we observed that injections of Ad GDNF into either the striatum (A) or the SN (B) significantly protected DA cell bodies in the lesioned SN. However, only striatal injections of Ad GDNF (A) protected against the development of behavioral deficits characteristic of unilateral DA depletion. Furthermore, we observed that striatal (A), but not SN (B), injections of Ad GDNF prevented the loss of DA terminals as indicated by a reduction in tyrosine hydroxylase fiber loss and an increase in amphetamine-induced striatal Fos expression. This indicates that increased levels of striatal (A), but not nigral (B), GDNF biosynthesis prevents DA neuronal loss and protects DA terminals from 6-OHDA-induced damage, thereby maintaining DA function in the aged rat. Dead axons and terminals are denoted by dashed lines. Down-regulation of tyrosine hydroxylase and loss of DA neurons is indicated by smaller cell bodies and hatched areas.

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