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Anterograde Transport of Neurotrophic Factors

Possible Therapeutic Implications

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Abstract

The actions of neurotrophic factors are classically thought to be mediated by their retrograde transport from target tissues to the cell bodies. There is now evidence that specific trophic factors are trafficked anterogradely along peripheral and central axons and released to postsynaptic cells. This review focuses on recent experiments that demonstrate the involvement of the anterograde transfer of neurotrophic factors in various physiological processes, including the regulation of developmental neuronal death, the modulation of synaptic transmission, and the control of axonal and dendritic architecture. The authors also discuss whether anterograde transport of exogenous trophic factors can be exploited to protect damaged postsynaptic neurons and spare their function. This issue has clear implications for possible therapeutic applications of neurotrophic factors.

Index Entries: Anterograde axonal transport; neurotrophic factors; neurotrophin release; neuronal death; synaptic transmission; gene therapy; neuroprotection; functional sparing.

Introduction

The field of neurotrophic factors was inaugurated by the discovery and characterization of nerve growth factor (NGF) by Rita Levi-Montalcini and her coworkers. It is now known that NGF is the prototypical member of a family of secreted polypeptide growth factors known as the neurotrophins, and comprising in mammals also brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4. The neurotrophins exert their actions on target cells through the binding to two different receptor classes, the tropomyosin-related kinase (Trk) family of receptors and the p75 receptor, a member of the tumor necrosis factor receptor superfamily *(1–3)*. p75 and Trks activate autonomous intracellular pathways, but can also collaborate

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to mediate specific neurotrophin signals. The intracellular domain of p75 has no catalytic activity, and signal transduction occurs through recruitment of a variety of cytoplasmic adaptor proteins *(2).* Trks are transmembrane receptors with tyrosine kinase activity. Individual neurotrophins activate different Trk receptors, with NGF acting through TrkA, BDNF, and NT-4 interacting with TrkB, and NT-3 activating TrkC. Neurotrophin binding leads to Trk receptor dimerization followed by transautophosphorylation on intracellular tyrosine residues. These phosphorylated tyrosines act as docking sites for several adaptor and effector molecules that ultimately propagate the neurotrophin signal. This occurs by setting in motion three main signaling cascades, namely the Ras-MEK-Erk pathway, the PI3 kinase/Akt pathway, and the PLC-γ pathway *(1,4).* More information on the signal transduction cascades activated by neurotrophins is available in several recent reviews *(1–3).*

In addition to the neurotrophins, several different classes of neurotrophic factors have been identified, including the glial cell line-derived neurotrophic factor (GDNF) family of trophic factors and ciliary neurotrophic factor (CNTF). GDNF uses a receptor complex composed of the tyrosine kinase, Ret, and the glycosylphosphatidylinositol (GPI)-anchored protein, GFRα-1. Recently, it has been shown that the neural cell adhesion molecule, NCAM, can also function as a signaling receptor for members of the GDNF ligand family *(5).* Ciliary neurotrophic factor is a member of the neuropoietic cytokine family, that also includes leukemia inhibitory factor (LIF), interleukin-6, and oncostatin-M. CNTF elicits cellular responses via activation of a receptor complex consisting of CNTF receptor α, LIF receptor, and glycoprotein 130 ultimately leading to the activation of the Janus kinase (JAK/STAT) and Erk cascades *(6).*

Neurotrophic factors have many different functions in both the developing and adult nervous system. They play a central role in regulating neuronal birth, differentiation, survival, and connectivity during nervous system development *(7).* It is well known that developing postmitotic neurons compete for limited amounts of neurotrophic factors, and that those which fail to obtain sufficient trophic support are eliminated by a process called programmed cell death *(8).* At later developmental stages and in the adult, trophic factors have been implicated in the regulation of synaptic plasticity *(9,10).* Neurotrophic factors are also involved in the response to nervous system damage. In this respect, it is hoped that their survival-promoting effects may be exploited for the development of novel therapeutic approaches for the treatment of neurodegenerative conditions *(11,12).*

Traditional views of neurotrophic factor biology, largely based on experiments with NGF, held that trophic factors exert their effects by acting as target-derived molecules. The retrograde pathway comprises several steps: synthesis of neurotrophin in the target cell, secretion into the extracellular space, receptor-mediated uptake by the nerve terminal, and retrograde axonal transport towards the soma of the responsive neurons *(13).* More recent evidence indicates that neurotrophic factors can also act by paracrine and autocrine mechanisms *(14).* In addition, it is well established that trophic factors can move anterogradely along axons and be transferred to the postsynaptic target cells *(15).*

Here we focus on the functional roles of the anterograde transfer of trophic signals. Consideration of the anterograde propagation of neurotrophic signaling after stimulation of the cell soma with a growth factor is given first. We then concentrate on possible physiological roles of the anterograde transport of endogenous trophic factors. Finally, the issue of whether anterograde transport of exogenous factors can be exploited to provide trophic support to degenerating postsynaptic neurons and spare their function is addressed.

Anterograde Propagation of Neurotrophic Signaling

NGF represents the prototype of target-derived, retrogradely transported trophic fac-

tors. It binds to receptors on nerve terminals and transmits signals back to the cell body of the responsive neurons *(13).* In NGF-responsive sympathethic and sensory neurons, it is firmly established that propagation of the NGF signal is unidirectional—only retrograde. Indeed, phosphorylated TrkA and its downstream effectors, including activated Erk1/2 and Akt, appear in cell bodies following NGF stimulation of neuronal terminals, but activated Erk1/2 and Akt do not propagate anterogradely following NGF application at the soma *(16,17).* However, trophic signals do propagate in an anterograde direction after stimulation of the cell bodies in other experimental situations. For example, in one of the first studies describing effects of growth factors on synaptic function, Stoop and Poo *(18)* found that application of CNTF at the cell body of spinal motoneurons rapidly potentiated transmitter release at neuromuscular synapses in *Xenopus* cell cultures. When the factor was applied directly at the synapse, the onset of the potentiation was slower and required signaling within the cell soma. Remarkably, the potentiation occurred independent of new gene transcription or protein synthesis, indicating that the most likely somatic action of CNTF was a posttranslational modification (e.g., phosphorylation) of pre-existing components that are then ferried to the axon terminal. Interestingly, anterograde transport of activated signaling components such as phosphorylated Erk1/2, p38, and c-jun N-terminal kinase (JNK) has been detected in vivo in the sciatic nerve *(19–21).* These data suggest the possibility that pathway components activated by growth factor stimulation at the soma transmit neurotrophic signals in an anterograde direction by hitching a ride on the transport system running between cell bodies and neuronal terminals. What could be the role(s) played by this anterograde transfer of trophic signals generated at the cell body?

One possibility, suggested directly by the work of Stoop and Poo *(18),* is the regulation of synaptic function. In this respect, it is interesting to note that activated Erk1/2 can directly influence transmitter release through the phosphorylation of synapsin I *(22).* A second possible role for the anterograde movement of activated signaling molecules is the maintenance of the axonal compartment. Recent studies demonstrate that axons and synapses undergo regulated degeneration by active mechanisms that are akin to apoptosis. This axonal and synaptic apoptosis plays various roles in the reorganization of connectivity during development and in neurodegenerative disorders *(23,24).* The anterograde transfer of activated pathway components could provide a means for "remote" (i.e., somatic) control of axon and synapse integrity by growth factors. Thus, long-range anterograde transfer of neurotrophic signals emanating from the soma is likely to be integrated with trophic signaling locally generated in the axonal compartment and at the synapse to regulate various physiological processes.

Cellular Mechanisms of the Anterograde Axonal Transport and Release of Neurotrophic Factors

Anterograde trafficking of trophic factors appears to be widespread in both the peripheral and central nervous system (*see* ref. *15* for a comprehensive review). The neuronal pathways for which anterograde transport of *endogenous* trophic factors has been demonstrated are represented in Fig. 1.

For neurotrophins, the first demonstrations of anterograde transport date back to the mid 1990s *(25–28).* From anatomical studies, it was clear that specific neurotrophins, such as BDNF, are widely distributed in nerve terminals *(28).* Damage to specific fiber tracts followed by BDNF immunostaining clearly demonstrated accumulation of BDNF protein on the proximal side of the lesion, indicative of anterograde axonal transport. Concurrently, BDNF protein content was found to decrease in the brain regions deafferented by the lesion. Using these techniques, anterograde transport

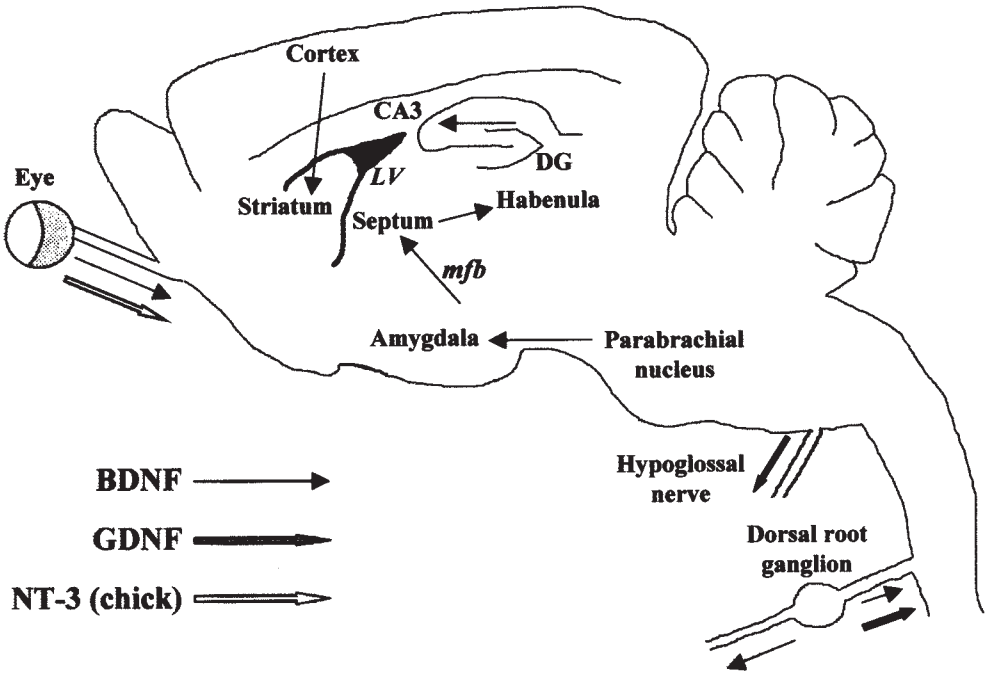


Fig. 1. Schematic sagittal section through the rat CNS summarizing the anterograde routes used by endogenous trophic factors. LV, lateral ventricle; DG, dentate gyrus of the hippocampus; mfb, medial forebrain bundle. Other endogenous factors for which anterograde transport has been documented are insulin-like growth factor I and tumor necrosis factor-α (not shown in the figure; *see* ref. *15*).

of endogenous BDNF has been demonstrated in both the peripheral and central projections of sensory neurons *(26,29)*, in the cortico-striatal projection *(27)*, in hippocampal mossy fibers *(30)*, in afferents of the parabrachial pontine nucleus to the amygdala *(28)*, in the septo-habenular pathway *(28)*, in catecholaminergic nerve fibers *(31,32)*, and in retinal ganglion cell axons *(33)*. Other endogenous trophic factors are trafficked in an anterograde direction along axons (Fig. 1). Endogenous NT-3 is transported to the optic tectum by retinal ganglion cells in chick embryos *(34)*. Like BDNF, GDNF travels anterogradely in primary sensory afferents to the dorsal horn of the spinal cord *(35)*. Anterograde transport of GDNF has also been shown in motoneurons *(36)*.

It is not clear what are the cellular mechanisms that determine the anterograde destination of a trophic factor once it is produced by a neuron. Studies with BDNF have demonstrated that this neurotrophin can display axonal as well as dendritic targeting in several CNS neurons *(37)*, and BDNF release can occur from either location *(38,39)*. In any case, neurotrophic factors destined to anterograde trafficking are believed to be packaged in large dense core vesicles (LDCVs). Evidence for ultrastructural localization of BDNF in LDCVs has been obtained in the optic nerve *(40)*. BDNF-containing LDCVs are also found in axon terminals of dorsal root ganglion nociceptive neurons in the dorsal horn of the spinal cord *(29,41)*. Therefore, the available data point to a storage of BDNF within LDCVs in both axons and presynaptic structures. Consistent with this subcellular localization, the characteristics of BDNF secretion from nerve terminals are similar, albeit not identical, to those of classical neuropeptides (reviewed in ref. *42*).

Using dorsal horn slices isolated in vitro, Lever et al. *(43)* have directly analyzed BDNF release from central axon terminals of sensory neurons. This system is particularly advantageous, since in the dorsal horn BDNF is derived primarily by anterograde transport from a population of TrkA-expressing nociceptive neurons *(26,29,44,45).* It was found that BDNF secretion is induced by specific stimulation patterns applied to the dorsal roots. BDNF was released after short bursts of high-frequency stimulation, while neither constant low frequency nor tetanic high-frequency stimulation delivering the same number of pulses as burst stimulation were effective *(43).* Peripheral noxious stimulation in vivo, which produces bursting activity in nociceptive fibers, is also able to induce BDNF release in the dorsal horn, as demonstrated by increased levels of phosphorylated TrkB in somatotopically appropriate spinal segments *(46).* Activity-dependent release of anterogradely transported neurotrophins has also been demonstrated for CNS neurons. In cultured cortical cells transfected with a BDNF–GFP chimera, fluorescent puncta representing BDNF moved anterogradely and were transferred to postsynaptic cells in an activity-dependent manner *(38).* Indeed, the transfer was blocked or enhanced, respectively, when network activity was silenced by tetrodotoxin application or enhanced via blockade of GABAergic inhibition with picrotoxin *(38).* A study by von Bartheld and colleagues *(47)* examined the release of anterogradely transported radiolabeled NT-3 from retinal afferents in the chick optic tectum. Secretion of NT-3 was induced by depolarization with high K^+ and by stimulation of retinal ganglion cells with kainic acid *(47).* Importantly, it appears that the functions of trophic factors do not end with their release and uptake by the nearby consumer cells. After initial endocytosis, trophic factors can escape degradation and undergo re-exocytosis into the extracellular space *(48,49).* These data underline the complexity of trophic interactions, which appear to be mediated via both newly synthesized and "recycled" factors *(15).* Further adding to this complexity, it is known that neurotrophins can induce release of other neurotrophins *(50).* Therefore, the possibility must be considered that some of the effects of anterogradely transported neurotrophins might be secondary to mobilization of additional trophic factors from postsynaptic neurons.

Possible Functions of the Anterograde Transport of Trophic Factors

Following release from nerve terminals, an anterogradely transported factor might bind to receptors on the presynaptic membrane or activate receptors residing on postsynaptic neurons and nearby glial cells. It is difficult to determine precisely which kind of trophic interactions take place in the intact tissue. Evidence has been obtained indicating a direct action of anterogradely transported neurotrophins on postsynaptic neurons in vivo. For example, Fawcett et al. *(31)* reported anterograde transport of BDNF protein in noradrenergic nerve fibers. Using transgenic mice overexpressing BDNF in noradrenergic neurons, they were able to demonstrate functional effects of the anterograde BDNF, namely an increased activation of TrkB receptors in noradrenergic nuclei target fields. Another study has examined expression of immediate-early gene products, such as c-Fos, in retinal target fields following an anterograde supply of BDNF from the eye in the rat *(33).* This work demonstrates that after injection into the vitreous, BDNF is efficiently internalized by retinal ganglion cells and transported anterogradely to retinorecipient structures, joining a route followed by endogenous BDNF *(33,51).* c-Fos was chosen as a reporter to map postsynaptic effects of the anterograde BDNF because the levels of this immediate-early gene product are low basally, and they increase rapidly and robustly upon neurotrophin stimulation. Indeed, it was found that intraocular injections of exogenous BDNF rapidly triggered c-Fos expression in target neurons in the superior colliculus and lateral geniculate nucleus. This

effect was dependent upon anterograde transport and release of BDNF, as it was blocked by co-injection of colchicine into the eye and by infusion of a functional blocking antiBDNF antibody into the targets *(33).*

The data reported above clearly demonstrate activation of signal transduction cascades in target cells by anterograde factors. These signaling events may impinge on several physiological processes. In the following sections, we discuss the potential role played by anterograde trophic factors in the developing and adult nervous system.

Regulation of Neuronal Survival During Development

Endogenous anterograde factors have been shown to regulate the process of programmed cell death, consistent with the idea that afferent control of cell survival is mediated via release of trophic factors from nerve terminals *(52).* Endogenous BDNF is anterogradely transported along the rat retinotectal pathway, and neutralization of endogenous BDNF within the developing superior colliculus increases the rate of programmed neuronal death *(33).* In keeping with these data, anterograde supply of BDNF from the eye reduces naturally occurring death of tectal neurons *(53).* BDNF is anterogradely transported along the corticostriatal pathway and almost all of the BDNF in the striatum is derived from afferent sources in rodents *(27,54).* In mice with deletions of the BDNF gene, the number of striatal parvalbuminergic neurons is reduced, consistent with anterogradely supplied BDNF having a role in survival or phenotypic specification of these cells *(27).* BDNF is normally present in terminals of noradrenergic neurons and mice that overexpress BDNF in noradrenergic neurons display increased numbers of tyrosine hydroxylase-positive neurons in the midbrain. This effect is attributed to the rescue of nigral dopaminergic neurons from developmental death as a consequence of increased availability of BDNF via afferent noradrenergic projections from the locus coeruleus *(55).*

Control of Dendritic Architecture

It is well known that trophic factors, and particularly neurotrophins, can regulate dendritic structure *(56–58).* Remarkably, the location of stimulation is a key variable in determining the net effects of neurotrophins on dendritic growth and branching. For example, in developing Xenopus retinal ganglion cells, BDNF stimulation of axon terminals increases dendritic arborization, while direct BDNF application to cell bodies and dendrites reduces arborization *(59).* These differential effects may be explained by the activation of distinct signaling pathways, depending on the spatial source of the neurotrophin *(60).* Therefore, it really matters for a responsive neuron if the same trophic factor is received via retrograde transport or anterogradely via afferent axons. A recent paper employing a "chimera culture" system has allowed to establish a role for anterogradely supplied BDNF in controlling the dendritic growth of cortical inhibitory neurons *(61).* In this work, GABAergic cortical neurons from a *BDNF–/–* mouse were cocultured with neurons from another mouse whose neurons expressed both BDNF and green fluorescent protein (GFP). BDNF was detected only in those inhibitory neurons that received presynaptic input from a GFP-labeled excitatory neuron, suggesting an anterograde transfer of the trophic factor. These BDNF-accumulating neurons had more abundant dendritic branches than nearby inhibitory neurons not contacted by BDNF-containing presynaptic terminals. This difference was selectively ascribable to BDNF release, as it was abolished by an anti-BDNF antibody applied to the culture medium *(61).*

Control of Connectivity Patterns and Synapse Maturation During Development

A recent study suggests a role for the anterograde transport of BDNF from the retina in shaping the retino-geniculate connectivity during development *(62).* The authors used intraocular injections of antisense oligonu-

cleotides to suppress selectively BDNF synthesis within the retina of rat pups. They found that in the absence of endogenous BDNF, retinal ganglion cell axons retracted from their target cells in the geniculate. Blockade of BDNF action at the retinal level failed to produce this effect, suggesting an anterograde action of the endogenous BDNF. Interestingly, the effects of BDNF removal on retinal fibers were evident only during a narrow temporal window coincident with the critical period for the retinothalamic refinement, indicating a role for BDNF in growth and elaboration of retinal axons rather than in their maintenance *(62).* A second paper demonstrates that anterogradely transported NT-3 influences synapse structure in the optic tectum of chick embryos *(63).* It was found that blocking endogenous NT-3 with function-blocking antibodies decreased the number of tectal synapses, synaptic vesicle density, and number of docked vesicles. These alterations could be selectively attributed to depletion of anterograde NT-3, as endogenous NT-3 is exclusively derived from presynaptic retinotectal nerve terminals in this system *(63).* In both these studies, the anterogradely transported neurotrophins could conceivably exert their effects by acting either directly on the presynaptic fibers or on the postsynaptic cells. In the latter case, it must be postulated that the anterotrophin induces some retrograde messenger(s) in target cells that is responsible for the presynaptic effect. The presence of neurotrophin receptors at both presynaptic and postsynaptic sites does not allow to determine the site of action of the anterograde factor in these examples.

Regulation of Synaptic Transmission

There are several reports indicating that trophic factors can modulate synaptic transmission at central and peripheral synapses (reviewed in ref. *9*). While most effects of growth factors are accounted by presynaptic modification of transmitter secretion (e.g., ref. *64*), there are also data for actions at postsynaptic sites. For example, Konnerth and colleagues *(65)* have recently demonstrated that the facilitating action of BDNF on long-term potentiation (LTP) induction in the hippocampus involves postsynaptic mechanisms. The same group has shown that application of BDNF and NT-4 to several types of CNS neurons causes membrane depolarization within a few milliseconds, and as a function of the dose and time of application, results in trains of action potentials *(66).* The rapid BDNF-induced neuronal excitation is mediated through TrkB-dependent activation of the sodium channel Na_v 1.9, a TTX-insensitive member of the family of voltage-gated sodium channels *(67).* Thus, an anterogradely transported neurotrophin such as BDNF may act not only as a synaptic modulator, but also as a fast transmitter.

A particularly advantageous system for the study of the synaptic effects of the endogenous, anterograde BDNF is represented by the dorsal horn of the spinal cord. Here, dorsal root ganglion (DRG) fiber afferents represent the primary source of BDNF, that is concentrated at synaptic terminals and released onto postsynaptic dorsal horn neurons *(26,29,42).* BDNF released from sensory afferents exerts a complex neuromodulatory role in pain processing (*see* ref. *45* for a review). First, endogenous BDNF appears to contribute to the increased excitability of pain transmission neurons (central sensitization) in models of inflammatory pain. Procedures that mimic peripheral inflammatory states, such as systemic NGF treatment, raise BDNF levels in sensory neurons and their terminations within the spinal cord *(29),* and increase nociceptive spinal reflex excitability *(68).* This increased central excitability is partly ascribable to release of anterograde BDNF, as it is reduced by sequestration of BDNF with TrkB receptor bodies (TrkB-IgG), a BDNF scavenger consisting of the extracellular domain of TrkB fused to the constant portion of an IgG molecule *(68).* Application of TrkB-IgG in vivo reduces behavioral nociceptive responses, consistent with a role for endogenous BDNF in central sensitization. Endogenous BDNF may contribute to central sensitization via induction

of Erk phosphorylation in dorsal horn neurons. It is indeed known that noxious stimulation induces BDNF release and subsequent Erk activation in spinal neurons *(46)*, and phosphorylated Erk is critically involved in inflammatory pain hypersensitivity *(69)*. On the other hand, application of exogenous BDNF has also been reported to depress nociceptive processing within the spinal cord. This is accomplished by an indirect mechanism that involves the release of GABA from dorsal horn interneurons and the activation of $GABA_B$ receptors located on the terminals of sensory neurons *(70)*. Thus, anterograde BDNF can regulate bidirectionally synaptic gain in the dorsal horn.

BDNF is not the only anterograde factor acting as a central pain modulator. Like BDNF, GDNF is anterogradely transported by sensory afferents to the dorsal horn *(35)*. It appears that primary afferent fibers acquire the GDNF protein from nearby glial cells and then traffic the internalized GDNF by anterograde transport to their central terminations in the spinal cord *(15,71)*. Injection of a functional blocking antibody to GDNF has been reported to decrease the delayed hyperalgesia induced in response to peripheral inflammation, suggesting that the endogenous, anterograde GDNF supports biochemical changes that contribute to hyperalgesia *(72)*.

Effects on Glial Cells

Anterograde transport of trophic factors may serve to mediate interactions between neurons and glial cells located along the axon and in the target. In support of this idea, Lisovoski et al. *(73)* have demonstrated that CNTF triggers phenotypic alterations in astrocytes of the adult brain when supplied via anterograde transport through the afferent axons. These authors made use of a recombinant adenovirus vector expressing CNTF to obtain local overexpression of CNTF in striatal cells. The transgene product was anterogradely transported to striatal projection areas, such as the globus pallidus and substantia nigra pars reticulata, that indeed displayed increased CNTF immunolabeling. In these regions, astrocytes appeared hypertrophied and showed enhanced staining for glial fibrillary acidic protein (GFAP; ref. *73*). Thus, an anterogradely transported factor may induce morphological and biochemical changes in astrocytes. Whether this effect is directly mediated via interaction of the released CNTF with target astrocytes is not known. Other potential targets for anterotrophins are the myelin-forming cells. Several studies have demonstrated that trophic factors, in particular neurotrophins and CNTF, are essential components of the myelination program in both the CNS and PNS. Trophic factors control various steps in this program, including proliferation, survival and differentiation of myelin-forming cells and their precursors, and myelin synthesis *(74–77)*. Whether the trophic factors acting on oligodendrocytes and Schwann cells are derived anterogradely from axons or from other sources remains to be determined *(75,77,78)*.

Neuroprotective Potential of the Anterograde Transport of Trophic Factors

As discussed above, anterograde and retrograde neurotrophic factors play critical roles in controlling neuronal survival during the developmental phase of programmed cell death. Whether adult neurons remain dependent upon trophic factors for their survival is less clear. There are data suggesting that a failure of trophic factor retrograde transport and/or signaling is implicated in neuronal death and dysfunction in certain neurodegenerative disorders *(79)*. Defects in the anterograde trafficking of trophic factors could also be conceivably involved in the pathogenesis of neurodegenerative diseases. In particular, a shortage in the anterograde supply of BDNF from the cortex to striatal targets may lead to striatal cell loss in Huntington's disease *(54)*. Irrespective of whether a lack of trophic support is causally related to neurodegeneration, neurotrophic factors have been proposed as symptomatic treatments in cases of brain injury

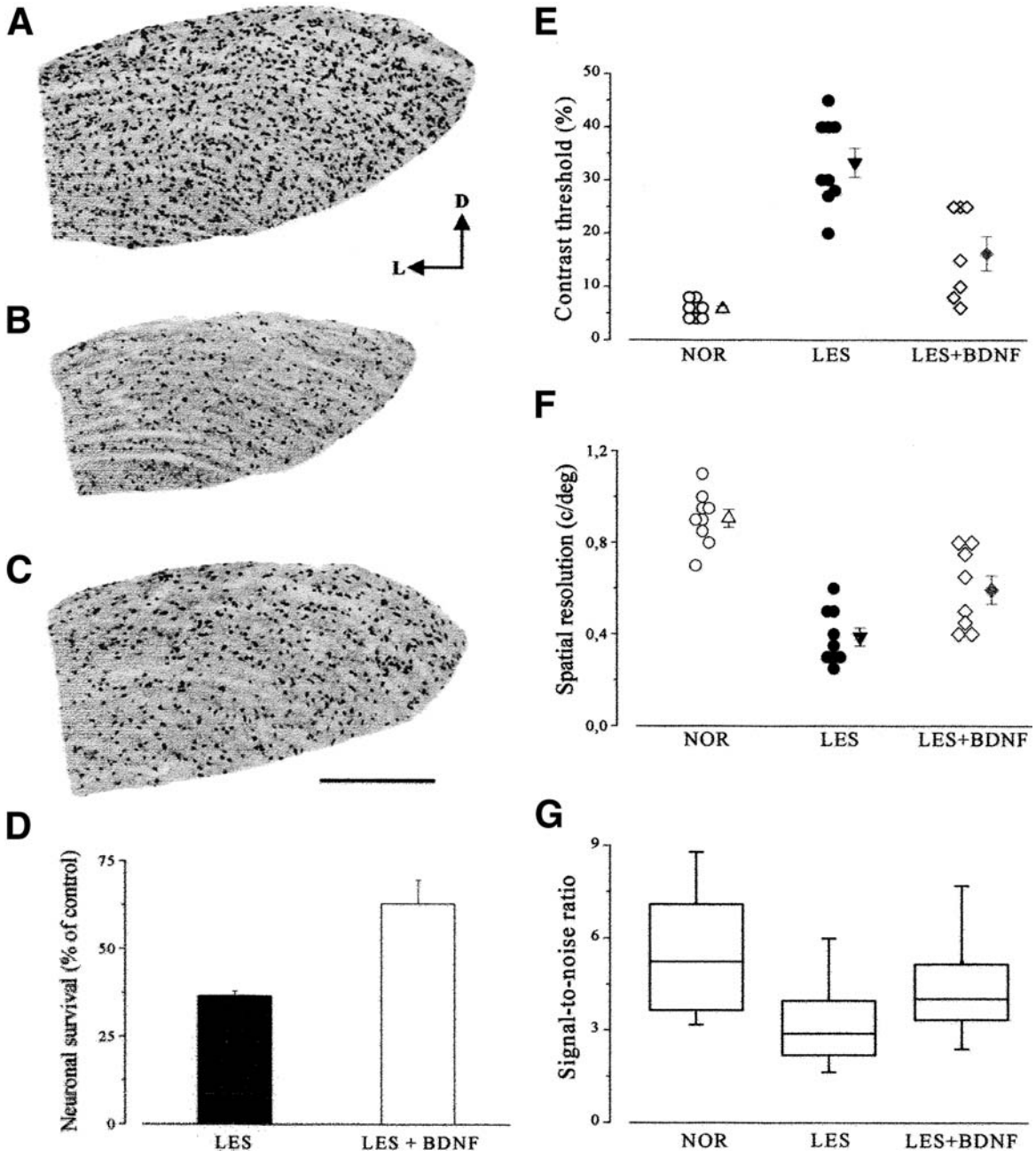
and disease due to their neuroprotective activity. Indeed, specific factors display survival-promoting actions on select populations of damaged adult neurons. For example, BDNF and CNTF can rescue motoneurons from death *(80,81),* and NGF is a powerful trophic factor for basal forebrain cholinergic cells *(82).* GDNF was first characterized as a specific survival factor for dopaminergic nigrostriatal neurons *(83),* and several trophic factors support retinal ganglion cells after optic-nerve injury *(84).* These animal data have prompted the launch of clinical trials testing the safety and efficacy of trophic-factor delivery in several neurodegenerative pathologies, from amyotrophic lateral sclerosis to Huntington's and Alzheimer's disease. These clinical trials failed because of treatment-related side effects and the absence of efficacy. The possible reasons for these failures have been discussed recently *(12).* First, the subcutaneous route of administration chosen for most trials was inadequate to deliver functionally relevant amounts of trophic factors to the relevant destinations. In other cases, delivery via the cerebrospinal fluid resulted in indiscriminate flooding of the brain with the growth factor and occurrence of unsustainable side effects. Side effects are not unexpected in view of the plethora of functions controlled by trophic factors in the nervous system. These failures call for the development of methods that allow delivery of trophic factors to specific populations of neurons in effective doses and in a precisely timed and localized fashion. In this regard, adeno-associated virus and lentivirus vectors have been demonstrated to provide efficient expression of neurotrophic factor genes in the brain and spinal cord *(85,86).* Another therapeutic option is the transplantation of engineered cells overexpressing trophic factors *(87).* Future steps will include the optimization of controllable delivery systems that can either turn on or turn off neurotrophic factor expression through the use of regulatable promoters *(88,89).* This could allow intermittent release of trophic factors to avoid drawbacks associated with continuous infusion and terminate the treatment in the case of unwanted side effects. In the case of viral vectors, advances are also expected that restrict transgene expression to specific cell types *(90),* thus limiting possible side effects of the factor. Another issue to be considered is the spatial source of the trophic factor. In this respect, provision of trophic factors through an anterograde route may present some advantages with respect to local supply to a population of neurons. Anterograde transport may ensure specific delivery to cells that are synaptically connected to the transduced ones and dispersed in a vast brain volume. There are now evidences (described later) that validate anterograde supply of trophic factors as a neuroprotective strategy in the adult nervous system.

The first study reporting rescue of damaged target neurons by an anterograde factor was provided by von Bartheld et al. in the chick visual system *(25).* It was found that anterograde transport of exogenous NT-3 from the eye to the optic tectum rescued developing tectal cells that would otherwise die after administration of pertussis toxin. A subsequent study from Freda Miller's group employed the abovementioned transgenic mice with increased BDNF synthesis in noradrenergic neurons *(31).* These authors focused on the axotomy-induced loss of neonatal facial motoneurons, since these cells are responsive to BDNF and receive a substantial noradrenergic innervation. They found that BDNF supplied via the noradrenergic input resulted in enhanced neuronal survival after facial nerve section in the transgenic mice *(31).* Another paper has examined the effects of CNTF gene therapy in a rat model of progressive striatal degeneration *(91).* Adenovirus vectors carrying a CNTF gene were injected into the rat striatum a few days before starting a neurotoxic treatment with systemic 3-nitropropionic acid. The authors were able to demonstrate a neuroprotective effect of CNTF that extended well beyond the transfected striatal area. In particular, a robust rescue effect was observed in the globus pallidus, that receives the bulk of its afferents from the striatum, suggesting enhanced survival by anterograde transport of CNTF *(91).* Finally, we have

Fig. 2. **(A–C)** Coronal sections through the rat lateral geniculate nucleus immunostained for neurons using antiNeuN antibodies. Fourteen days after a visual cortex lesion, the geniculate of control-lesioned animals is shrunken (B), and the number of geniculate neurons is reduced dramatically with respect to normal (A). Many more neurons survive in animals that received one intraocular BDNF injection at the time of the lesion (C). D, dorsal; L, lateral. Scale bar = 300 μm. **(D)** Stereological evaluation of neuronal survival in the lateral geniculate nucleus after lesion of the visual cortex. Survival is expressed as the percentage of NeuN-positive cells counted in the lesioned geniculate with respect to those present on the contralateral intact side. Intraocular BDNF (LES + BDNF) results in significant protection with respect to control lesions (LES; *t* test, $p < 0.01$). Error bars indicate SE. **(E–G)** Summary of the physiological data gained by visual evoked potential recordings from the lateral geniculate nucleus. Contrast threshold (E), spatial resolution (F; expressed as cycles per degrees of visual angle) and signal-to-noise ratio (G) are measured from the geniculate of normal rats (NOR), control lesioned rats (LES), and lesioned rats with BDNF administration into the eye (LES + BDNF). All lesioned animals are recorded 2 wk after surgery. It is clear that BDNF delivery maintains nearly normal functional responses in the geniculate. In (E) and (F), each point represents one animal. Mean ± SE is also shown for each experimental group. In (G), non-normally distributed signal-to-noise ratio data are summarized with a box chart. The horizontal lines in the box denote the 25th, 50th, and 75th percentile values, while the error bars denote the 5th and 95th percentile values. (Data reproduced from ref. *51.* Copyright 2003 by the Society for Neuroscience.)

recently provided evidence that anterograde supply of BDNF from the eye is a potent stimulator of neurons the survival of adult lateral geniculate nucleus following removal of their cortical target in the rat (*51; see* Fig. 2A–D). This study was designed to address a central question that remains unanswered in most neuroprotection studies, namely whether the inhibition of neuronal death by trophic factors results in healthy, normally functioning neurons. Studies with other death inhibitors have clearly indicated that anatomical rescue by no means guarantees sparing at the physiological level *(92,93).*

Our own studies of geniculate physiology in animals with cortical lesions also demonstrate that cell dysfunction precedes the onset of neuronal death, implying that an assessment of neuronal numbers is not predictive of functional performance *(51).* We therefore analyzed whether neuroprotection by anterograde BDNF translates into sparing of function. Several physiological parameters, including contrast threshold, spatial resolution (acuity), and signal-to-noise ratio were measured from the geniculate in vivo by visual evoked potential recordings 2 wk after the cortical damage. The results were clear in indicating that the functional responses in the geniculate of control lesioned rats were dramatically altered and that BDNF administration resulted in a substantial improvement of all the parameters tested (*51; see* Fig. 2E–G). This preservation of function in adult injured neurons suggests possible therapeutic applications of anterograde delivery of trophic factors. However, studies in other systems indicate that this is not always the case. For example, work by two groups has demonstrated that viral vector-mediated expression of GDNF in either striatum or substantia nigra protects nigral dopaminergic neuron cell bodies from Parkinson's disease-like damage induced by 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *(94,95).* However, only GDNF overexpression in the striatum promoted reinnervation of the striatum by dopaminergic axons and functional recovery. Despite anterograde transport of GDNF along the nigrostriatal pathway, transduction of the substantia nigra alone was completely ineffective in inducing sprouting of dopaminergic axons *(94,96).* Thus, only target-derived GDNF is able to induce remodeling of the damaged nigrostriatal tract. Another study has examined the ability of BDNF anterogradely sup-



plied from the nigra to protect striatal cells in a model of focal ischemia in rats *(97)*. Nigral cells were transduced with an adeno-associated virus vector carrying the BDNF gene. This resulted in remarkably elevated BDNF levels in the ipsilateral striatum, due to anterograde transport of the factor. This continuous release of excess BDNF induced abnormalities in body posture and movement and exaggerated the loss of specific classes of striatal interneurons

following stroke. Interestingly, it was previously demonstrated that direct BDNF gene transfer to striatal neurons produces the opposite effect; i.e., it mitigates neuronal death after stroke *(98).* To explain these discordant results, it has been proposed that patchy transduction of striatal neurons by the virus vector results in low-to-moderate levels of exogenous BDNF throughout the striatum, and that these small BDNF amounts are neuroprotective *(96).* In contrast, nigral transduction results in much higher BDNF titers, so that the whole striatum appears to be flooded with the growth factor *(97).* According to several reports, this excess BDNF can reduce neuronal survival. Adverse effects of BDNF are partly ascribable to an increased formation of free radicals, particularly nitric oxide *(99).* Moreover, it is known that BDNF acts as a potent neuroexcitant in various brain areas *(66,67).* Thus, in pathological conditions with a significant excitotoxic component, such as ischemia or epilepsy, direct neuronal excitation by BDNF might override neuroprotective effects of the factor and increase cell vulnerability. This interpretation is supported by studies in animal models of epilepsy that demonstrate that BDNF directly contributes to the generation of epileptiform activity *(100).* Altogether, these data are direct proof of how levels of trophic factor expression, duration of exposure, and route of delivery are critical variables in determining neuroprotective efficacy and side effects.

Concluding Remarks

Anterograde transport of trophic factors appears to be a widespread phenomenon in both the peripheral and central nervous systems. Several roles for this transport have been demonstrated, including the control of developmental cell death, the regulation of synaptic transmission, and the shaping of dendritic and axonal arbors. From the therapeutic point of view, several studies have demonstrated the feasibility of exploiting natural anterograde routes to provide trophic support to degenerating neurons. However, negative results have also been reported, in which anterograde supply of a trophic factor had no neuroprotective efficacy, exacerbated neuronal loss, or resulted in unacceptable side effects. It appears that route and timing of trophic-factor administration are critical variables for effective neuroprotection. We have also emphasized the importance of functional endpoints in evaluating neuroprotective strategies based on delivery of neurotrophic factors. Indeed, neuronal dysfunction may be initiated before neuronal degeneration, and a successful therapy requires not only neuronal survival but also the maintenance of normal physiological responses of the rescued cells. Evidence for functional sparing is a necessary component of trophic factor-based neuroprotective approaches as these molecules, besides regulating survival, are involved in activity-dependent plasticity and structural rearrangements in the adult brain. Therefore, the design of effective therapeutic strategies will require a deeper basic understanding of the physiological effects of trophic factors on single-cell types and circuits in the nervous system, in addition to the optimization of delivery systems.

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