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Anterograde Axonal Transport, Transcytosis, and Recycling of Neurotrophic Factors

The Concept of Trophic Currencies in Neural Networks

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Abstract

Traditional views of neurotrophic factor biology held that trophic factors are released from target cells, retrogradely transported along their axons, and rapidly degraded upon arrival in cell bodies. Increasing evidence indicates that several trophic factors such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF-2), glial cell-line derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-I), and neurotrophin-3 (NT-3), can move anterogradely along axons. They can escape the degradative pathway upon internalization and are recycled for future uses. Internalized ligands can move through intermediary cells by transcytosis, presumably by endocytosis via endosomes to the Golgi system, by trafficking of the factor to dendrites or by sorting into anterograde axonal transport with subsequent release from axon terminals and uptake by second- or third-order target neurons. Such data suggest the existence of multiple "trophic currencies," which may be used over several steps in neural networks to enable nurturing relationships between connected neurons or glial cells, not unlike currency exchanges between trading partners in the world economy. Functions of multistep transfer of trophic material through neural networks may include regulation of neuronal survival, differentiation of phenotypes and dendritic morphology, synapse plasticity, as well as excitatory neurotransmission. The molecular mechanisms of sorting, trafficking, and release of trophic factors from distinct neuronal compartments are important for an understanding of neurotrophism, but they present challenging tasks owing to the low levels of the endogenous factors.

Index Entries: Neurotrophic factor; anterograde axonal transport; transcytosis; neurotrophin; internalization; endocytosis; release; degradation; NGF; BDNF; NT-3; GDNF, IGF-I.

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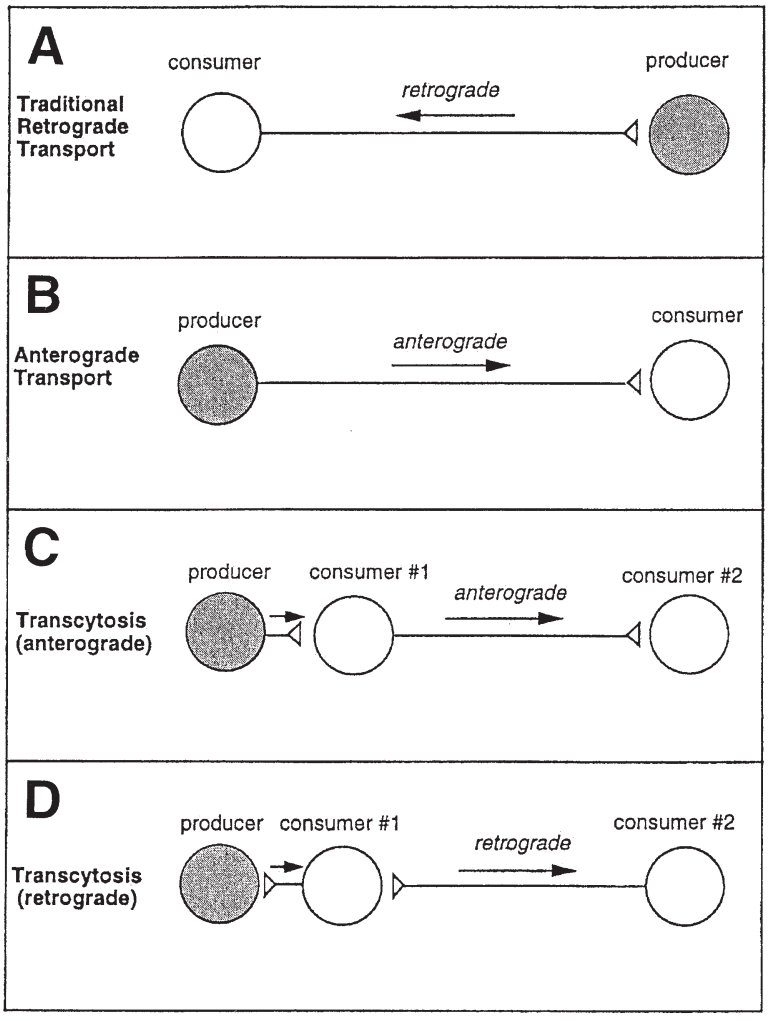

Fig. 1**(A–D).** Trafficking of neurotrophic factors in neural circuits. The main types of transfer between connected neurons are schematically depicted. The producing cells are indicated in gray, the consuming neurons in white. The direction of transport of trophic factors is indicated by arrows.

Historical Overview

Traditional views of neurotrophin biology are largely based on experiments with the prototypic growth factor, nerve growth factor (NGF). Studies with NGF led to the concept that trophic factors are produced by target cells, are released, and are internalized by innervating nerve terminals containing appropriate receptors (Fig. 1A). The internalized factors are transported retrogradely in a ligand-receptor complex to the cell body where signal-transduction cascades are initiated *(1,2)* and the transported trophic factors are degraded in lysosomes *(3–12)*.

Recent studies have shown that trafficking of many neurotrophic factors is more complex. In addition to retrograde signaling, trophic factors can move anterogradely along axons *(13–18)* (Fig. 1B), consistent with the idea that afferent-derived trophic influences are mediated via release of trophic molecules from axon terminals *(19–23)*. It now appears that there may be two fundamentally different types of neurotrophic factors. One type, such as NGF, is

exclusively transported in the retrograde direction along axons, and is not transcytosed after internalization. Another type of factor, such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor I (IGF-I), is targeted primarily to the regulated secretory pathway, can be transported retrogradely as well as anterogradely along axons, and can travel through neurons by transcytosis *(17,24–28).*

The anterograde axonal movement of some factors such as BDNF is thought to be more common than retrograde movement, at least in the adult nervous system *(29,30).* Furthermore, it has been shown that basic fibroblast growth factor (FGF-2), IGF-I, some neurotrophins, and glial cell line-derived neurotrophic factor (GDNF) can move through neurons or glial cells by transcytosis *(13,17,28,31–34).* Such data indicate that trophic factors can be used by more than one neuron and are "recycled" so that factors produced by neuron #1 (or glial cell #1) are released and taken up by neuron #2, and transported anterogradely in the axon to be released at synapses with neuron #3 (Fig. 1C) or released by neuron #2 to be retrogradely transported in neuron #3 (Fig. 1D). Indirect evidence indicates that such transcytosis occurs physiologically in the nervous system. Thus, it is possible that neurons use the same trophic factors for subsequent functions in a chain of connected neurons, as has been shown for other receptor ligands in other cell types *(35–40).*

This review focuses on the emerging evidence for anterograde transport, transcytosis, and recycling of neurotrophic factors in the nervous system, on possible mechanisms, and its functional significance. This aspect of neurotrophism has been much neglected relative to other systems and receptor ligands such as toxins, lectins, and immunoglobulins *(39,40)* and significantly expands the scope and extent of functions of trophic factors in neural circuits.

Transcytosis in Other Cell Types

Since systematic studies on transcytosis of neurotrophic factors have only very recently been implemented, we will first consider the substantial evidence for transcytosis of other receptor ligands in non-neuronal cell types. Such studies were pioneered by research on tetanus, shiga and ricin toxins, lectins, transferrin, polymeric immunoglobulin A, and epidermal growth factor (EGF) (*38;* for reviews, *see* refs. *37,39,40*). It was shown that these ligands can be endocytosed at the basolateral membranes of epithelial cells, travel in endosomes, reach the Golgi system, and a significant fraction of the peptide is released at the apical membrane. In secretory cells (such as PC12 cells), this may be a common pathway for several membrane proteins, including secretory granule proteins *(36,41,42).* More recently, Dotti and coworkers have shown that a similar pathway for transcytosis exists in neurons *(43),* and they suggest that this pathway may be significant for trafficking of neurotrophic factors *(37).*

When EGF is internalized by epithelial cells, between 5 and 30% of the receptor-bound ligand (and 10–50% of the internalized EGF) is transcytosed and recycled *(44–47).* The transcytosed EGF initially binds to the EGF receptor, but during endocytosis (presumably in early or late endosomes), the ligand dissociates from the receptor, while the receptor is recycled to the plasma membrane *(44,48).* The majority of the dissociated ligand is targeted to lysosomes for degradation *(45),* while another fraction (10–15%) is targeted via the Golgi system for transcytosis and release of intact EGF *(47,49,50).*

When trophic factors are internalized and transcytosed, at least their binding site must remain intact in order to retain function. There are examples for neurotrophin transcytosis in enteric cells (NGF; *see* ref. *52*), CHO cells (NGF; *see* ref. *53*), and in glial cells (BDNF and neurotrophin-4 [NT-4] *see* refs. *31,32*). BDNF and NT-4 can be internalized by astrocytes and Schwann cells in vitro, and after internalization the neurotrophins can be stored and subsequently released intact. This transcytosis of BDNF and NT-4 in glial cells is mediated by truncated trkB receptors *(31).* It is not yet clear what the physiological source of the neurotrophins may be in vivo and which compart-

Table 1
Anterograde Axonal Transport of Exogenous Neurotrophic Factors (Transcytosis)

Factor	Pathway	Species	Age	Technique	Year	Reference
bFGF	Retinal ganglion cell	Rat	Adult	^{125}I-radiolabel	1990	*(13)*
IGF-I	Inferior olive/climbing fibers	Rat	Adult	^{125}I-radiolabel	1993	*(28)*
BDNF	Neostriatal fibers	Finch	Juvenile	^{125}I-radiolabel	1997	*(26)*
	Retinal ganglion cell	Rat	Neonatal	^{125}I-radiolabel	1999	*(34)*
	Retinal ganglion cell	Rat	Adult, postnatal	Ligation, immunolabel	2000	*(72)*
NT-3	Retinal ganglion cell	Chick	Embryo	^{125}I-radiolabel	1996	*(17)*
	Neostriatal fibers	Finch	Juvenile	^{125}I-radiolabel	1997	*(26)*
NT-4	Retinal ganglion cell	Rat	Neonatal	^{125}I-radiolabel	1999	*(34)*
GDNF	Hypoglossal motoneuron	Rat	Neonatal	^{125}I-radiolabel	2001	*(91)*
	Dorsal-root ganglion	Rat	Adult	^{125}I-radiolabel	2001	*(91)*
TNF-α	Sciatic nerve (motor)	Rat	Adult	^{125}I-radiolabel	2001	*(209)*

ment(s) store the internalized neurotrophins, but it is clear that glial cells (and possibly neurons) can use receptors to internalize and release the ligands, and thus to control their bioavailability.

The distinction between pathways of newly synthesized proteins and internalized proteins can be blurred. In hepatocytes, membrane proteins are trafficked to the basolateral cell surface, then endocytosed and targeted via an endocytic-Golgi pathway to the apical cell surface; the cells are not capable of directly moving newly synthesized membrane proteins to the apical-cell surface (reviewed in ref. *54*). Whether similar transcytosis of endogenous peptides occurs in neurons is not known *(55).*

Although there are several examples for anterograde transport of internalized neurotrophic factors in neurons (*see* Table 1), examination of the underlying pathways *(56)* and of mechanisms of their release from presynaptic axon terminals is sparse *(28,57,58).* The following section discusses which neurotrophic factors have been implicated in anterograde transport, both of newly synthesized endogenous factor as well as factors that can be anterogradely transported after internalization.

Anterograde Axonal Transport of Neurotrophic Factors

Endogenous Factors (Newly Synthesized by the Transporting Neuron)

Anterograde axonal transport has been demonstrated for IGF-I, the neurotrophins BDNF, neurotrophin-3 (NT-3) and NT-4, GDNF, and tumor necrosis factor-α (TNF-α), as summarized in Table 2. Virtually all studies used immunolabeling, combined with ligation of peripheral nerves or lesioning of fiber tracts, enzyme-linked immunosorbent assay (ELISA) for quantification of protein levels (*see* Table 2), or the comparison of transcript and protein localization to show translocation of the neurotrophic factor along the axon. It is clear from these and other studies that some, but not all, neurotrophic factors that are expressed in neurons can be anterogradely transported and released. No studies have shown this to date for NGF *(59),* except when NGF was overexpressed as a transgene *(60).* Platelet-derived growth factor (PDGF) is expressed and released from neuronal cell bodies, but not from axons *(61).*

A large variety of neuronal cell types in the peripheral nervous system (PNS) and central

Table 2
Anterograde Axonal Transport of Endogenous Neurotrophic Factors: Direct Evidence

Factor	Pathway	mRNA	Species	Age	Technique	Year	Reference
IGF-I	Sciatic nerve	(+)	Rat	Adult	Immunolabel, lesion	1987	*(14)*
	Olivo-cerebellar projection	+	Rat	Adult	Radioimmunoassay, lesion	1991	*(16)*
BDNF	Dorsal-root ganglion	+	Rat	Adult	Immunolabel, ligation	1996	*(18)*
	Dorsal-root ganglion	+	Rat	Adult	Immunolabel, ligation	1997	*(115)*
	Dorsal-root ganglion	+	Rat	Adult	Immunolabel, ligation	1998	*(67)*
	Dorsal-root ganglion	+	Rat	Adult	Immunolabel, ligation	1999	*(65)*
	Septo-habenular fibers, pontine-amygdala fibers	+	Rat	Adult	Immunolabel, lesion	1997	*(30)*
	Hippocampal mossy fibers	+	Rat	Adult	Immunolabel	1997	*(102)*
	Cortico-striatal fibers	+	Rat	Adult	Immunolabel, ELISA, lesion	1997	*(71)*
	Cortico-striatal fibers	+	Rat	Adult	Immunolabel, ELISA, ischemia	1998	*(64)*
	Noradrenergic septal afferents	+	Rat	Adult, neonatal	Immunolabel, lesion	2000	*(74)*
	Retinal ganglion cell	+	Rat	Adult, neonatal	Immunolabel, ligation	2000	*(72)*
NT-3	Retinal ganglion cell	+	Chick	Embryo	Immunolabel	2000	*(152)*
GDNF	Dorsal-root ganglion	–	Rat	Adult	Immunolabel, ligation, lesion	1998	*(15)*
	Dorsal-root ganglion	–	Rat	Adult	Immunolabel, lesion	1999	*(86)*
	Hypoglossal motor neuron	–	Rat	Adult	Immunolabel, ligation	2000	*(33)*
TNF-α	Sciatic nerve	(+)	Rat	Adult	Immunolabel, ligation	2001	*(209)*

nervous system (CNS) can anterogradely transport neurotrophic factors, both in the developing and mature nervous system (*see* Tables 2,3). These cell types include motoneurons (IGF-I, GDNF, TNF-α); sensory ganglion neurons (BDNF, GDNF); CNS projection neurons such as cortico-striatal, septohabenular, pontine-amygdalar, as well as noradrenergic brainstem neurons, hippocampal mossy fibers (all BDNF); retinal ganglion cells (BDNF, NT-3, NT-4); olivocerebellar climbing fibers (BDNF, IGF-I); and potentially cerebellar parallel fibers (NT-3, BDNF). The anterograde transport of IGF-I and TNF-α in axons of motor neurons is of particular interest, because these two factors are thought to have opposing effects *(62,63)* and may act in a push-pull fashion to finely balance trophic effects. Injury causes an increase in the production and the anterograde axonal transport of some trophic factors as shown for BDNF *(64–67)* and GDNF *(68–70).*

Exogenous Neurotrophic Factors (exogenous to the organism)

Several studies have shown that certain neuronal cell types can internalize exogenously supplied trophic factors, which then gain access to the anterograde axonal pathway. The cell bodies and dendrites possess receptors for the ligands, and a significant amount of the internalized ligand is targeted for anterograde

Table 3
Anterograde Axonal Transport of Endogenous Neurotrophic Factors: Overexpression Studies and Indirect Evidence

Factor	Pathway	mRNA	Species	Age	Technique	Year	Reference
NGF	Sympathetic neurons	+	Transgenic mouse	Not reported	Immunolabel, ligation	1993	*(60)*
NT-3	Cerebellar granule cell	+	Rat	Embryo	mRNA expression, function	1993	*(105)*
BDNF	Various CNS neurons	+	Rat	Adult	mRNA expression, immunolabel	1997	*(99)*
	Cerebellar granule cells	+	Mouse	Postnatal	mRNA expression, dendritic	1997	*(210)*
	Inferior olive: climbing fibers	+	Mouse	Postnatal	trk activation	1997	*(210)*
	CNS noradrenergic fibers	+	Rat	Adult	Immunolabel	1998	*(73)*
		+	Mouse	Neonatal	Overexpression, transgene	1998	*(73)*
	Cortical neurons in vitro	+	Mouse	Neonatal	Overexpression of GFP-BDNF	2001	*(116)*

axonal transport, apparently with subsequent release of the ligand from axon terminals. These studies are listed in Table 1. While many studies demonstrate anterograde transport of the trophic factor along the axon, only a few of them provide direct evidence that transcytosis of the endogenous or exogenous trophic factor occurs, i.e., that the factor is released at the axon terminus *(17,19,28,57,71–74).* Our survey of the literature shows the potential for neuronal transcytosis in several neuronal circuits when exposed to exogenous trophic factors, i.e., the receptors are in place and the cells possess the machinery for anterograde transport of internalized neurotrophic factors (*see* Table 1). But is there evidence for transcytosis of *endogenous* trophic factors in the PNS or CNS?

Potential Physiological Examples of Trophic Factor Transcytosis

GDNF Transport in DRG

An examination of the available literature on GDNF in dorsal root ganglion (DRG) cells shows that GDNF is expressed by cells in the sciatic nerve *(68–70,75,76);* primarily in Schwann cells and satellite glial cells in the DRG, but apparently not in DRG neurons *(68,69,75–79).* Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analyses showed GDNF transcripts in the sciatic nerve, but much less in DRG including glial cells, suggesting glial production of GDNF *(69,80).* A significant fraction of DRG neurons possess receptors for GDNF (Ret and GFRα *[81–83];* TrnR2 *[84]*). It is also known that DRG's central axons transport GDNF to their terminals where the GDNF is thought to be released in synaptic contacts with target neurons *(15,85,86).* Since the amount of GDNF seen by immunolabel in the terminals appears to be substantial, and the endogenous GDNF is clearly transported in the anterograde direction by DRG axons, one would expect to see robust message levels (GDNF mRNA) in the DRG neuronal perikarya. Since this is not the case, despite numerous studies *(68,75–79,87),* and several of the same studies show mRNA expression in the satellite cells, nerve (Schwann cells), muscle, and cells within the

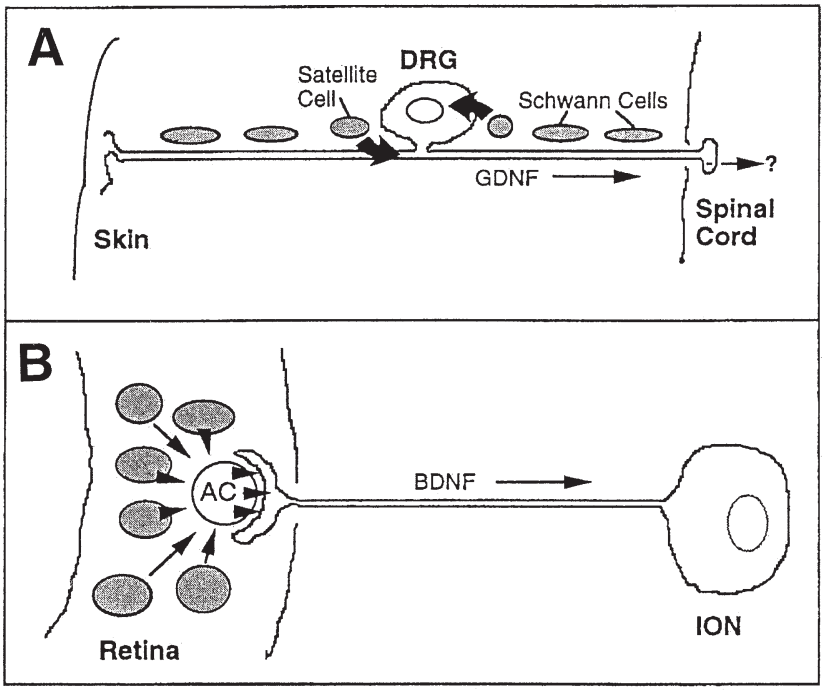

Fig. 2. Cartoons illustrate two likely physiological examples of transcytosis of trophic factors in neurons. **(A)** Dorsal-root ganglion (DRG) neurons obtain glial-cell line-derived neurotrophic factor (GDNF) from satellite and Schwann cells (GDNF producers, gray), and transport the internalized GDNF anterogradely along their axons into the spinal cord where the GDNF is believed to be released from the presynaptic axon terminals. **(B)** Amacrine cells (AC) in the avian retina obtain brain-derived neurotrophic factor (BDNF) from BDNF-producing cells (gray) in the retina and provide the internalized BDNF to axon terminals from neurons in the isthmo-optic nucleus (ION). The ION neurons retrogradely transport the retina-derived BDNF as an essential survival factor. For details and references, *see* "Potential Physiological Examples of Trophic Factor Transcytosis."

spinal cord *(68,69,75,76)*, it is most likely that DRG neurons acquire the GDNF by internalization and then traffic the internalized GDNF by anterograde transport to their central terminations in the spinal cord (Fig. 2A).

Indeed, DRG neurons can internalize and retrogradely transport "significant levels" of ^{125}I-GDNF injected into the nerve or the peripheral target *(83,89,90)*, but these studies did not determine whether the internalized GDNF is also transported transganglionically, beyond the DRG cell bodies, and anterogradely into the spinal cord. Our own data indicate that this is the case *(91)*. Although most DRG neurons that express GDNF receptors appear to project into the inner lamina II in the dorsal horn *(92)*, a significant fraction of GDNF-receptor-positive (or ^{125}I-GDNF-labeled) DRG neurons (10–15% *[83]*; 15% *[89]*, express other markers such as trkA and would be expected to belong to the type of DRG neurons that project to lamina I and the outer part of lamina II, where most of the endogenous GDNF is found *(15,83,86,93)*. Thus it is possible that a fraction of DRG neurons with GDNF receptors anterogradely transport Schwann cell-derived GDNF to lamina I and the outer part of lamina II in the spinal cord, where it is presumably released, thus being a potential example of physiological transcytosis of an endogenous neurotrophic factor by neurons.

Alternative interpretations of these collective data are that: 1) the GDNF antibodies in

fact recognize a molecule other than GDNF (but presumably closely related), whose mRNA, in turn, is not recognized by the probes used for *in situ* hybridization; or that 2) the mRNA levels of GDNF in DRG neurons are below the level of detection, yet still produce the large amount of GDNF protein seen by immunolabel. Since the GDNF antibodies used for these studies have been extensively studied and characterized *(15,33),* the first interpretation is unlikely. The second interpretation seems to be the one currently favored by investigators, but the lack of GDNF mRNA in DRG neurons has been largely ignored *(15,86).* Thus, GDNF transport by DRG neurons likely is an example of physiological transcytosis of a neurotrophic factor *(91).*

BDNF Transport in Amacrine Cells

A second example of apparent transcytosis of a neurotrophic factor in neurons emerged from studies of BDNF trafficking in the retina and subsequent uptake of the factor by axon terminals of the isthmo-optic nucleus (ION *[94]*). The ≈ 10,000 neurons in the ION of chick embryos accumulate substantial amounts of BDNF (20–40 pg BDNF measured by electrochemiluminescent immuno assay (ECLIA), which is 30–50% of the total BDNF amount in the entire chick retina and 20 times more per cell or mg tissue than the retina), and they obtain most of their BDNF from sources in the retina. The target cells of ION neurons in the retina are an exclusive small subpopulation of amacrine cells (0.01% of the amacrine cells *[95]*). Since the ION neurons transport relatively large amounts of endogenous BDNF from the retina to the ION cell bodies (and with surprisingly high receptor occupancy *[96]*), one would expect their amacrine target cells to express significant amounts of BDNF mRNA. When von Bartheld and Johnson *(94)* examined this question, they found only low levels of BDNF mRNA in amacrine cells, but modest-robust levels of BDNF immunolabel. The most likely explanation for these results is that the amacrine target cells of the ION accumulate BDNF protein derived from other retinal cells, as demonstrated for retinal ganglion cells *(97),* and provide the internalized BDNF to ION axons for retrograde axonal transport to ION cell bodies (Fig. 2B). Thus, the retinal amacrine-ION trafficking of BDNF presents another likely example of physiological transcytosis of a neurotrophic factor.

GDNF Transport in Hypoglossal Motor Neurons

Endogenous GDNF is transported anterogradely in the axons of hypoglossal motoneurons *(33),* but hypoglossal motoneurons do not express levels of GDNF mRNA that can be detected by *in situ* hybridization *(76).* Since GDNF mRNA is expressed in samples of the hypoglossal nucleus that contain glial cells *(33),* and since glial cells can express GDNF mRNA abundantly *(68),* it is most likely that the source of GDNF for hypoglossal motor neurons are the glial cells. This conclusion is supported by the fact that GDNF was detected in the Golgi apparatus of Schwann cells, but not of motoneurons *(33),* and motoneurons in the ventral horn do not express GDNF mRNA *(77,98).* Furthermore, when exogenous GDNF is injected into the hypoglossal motor nucleus in neonatal rat brainstem slices, it is internalized in a receptor-mediated fashion and anterogradely transported in hypoglossal axons *(91).* These data support the notion that GDNF transport in hypoglossal motoneurons is a physiological example for neuronal transcytosis.

The interpretation of all three examples for neuronal transcytosis of neurotrophic factors depends on the assumption that protein levels seen by immunocytochemistry, ELISA or ECLIA assays require transcript levels that are detectable by *in situ* hybridization. A survey of the literature indeed indicates that in the large majority of cases, the cell bodies of neurons contain significant amounts of transcripts when that same neuron type transports the trophic factor anterogradely (*30,99; see* Tables 2,3). For example, BDNF mRNA can be readily detected in DRG neurons *(100,101),* granule

cells in the dentate gyrus *(30,102)*, septo-habenular neurons and pontine neurons *(30)*, cortical neurons *(30)*, noradrenergic neurons *(103)*, retinal ganglion cells (for review, *see 104*), and the inferior olive *(30)*. NT-3 is expressed in cerebellar granule cells *(105)*, IGF-I in sensory ganglion cells *(106)* and the inferior olive *(107)*, and TNF-α can be induced in motor neurons *(108)*. These examples contrast with the lack of transcripts for GDNF in DRG neurons and hypoglossal motoneurons (*see* Table 2, discussed earlier), which likely obtain the GDNF protein from glial cells.

Molecular Mechanisms of Anterograde Transport and Transcytosis

This section considers transcytosis of neurotrophic factors after uptake into dendritic/somal compartments and subsequent axonal trafficking and release from the axon terminus. This may not be the only mode of transcytosis in neurons, as it is possible that target-derived factors are retrogradely transported from the axon terminus and subsequently targeted to dendrites for release. The "transperikaryal transport" of endogenous proteins (from axons to dendrites) has been described in neurons *(109)*, but there is as yet no evidence for retrograde transcytosis of neurotrophic factors, unlike other ligands such as tetanus toxin *(110–112)*. Therefore, we will focus on transcytosis from dendrites or from the soma to the axon for which compelling evidence exists for several trophic factors (Table 1). This transcytosis requires: 1) receptor-mediated internalization, 2) sorting from endosome to Golgi, 3) packaging into presumptive large dense-core vesicles (LDCVs) for anterograde axonal transport, and 4) release of the neurotrophic factor from the presumptive LDCVs at the nerve terminal. In support of such mechanisms for neurotrophic factor transcytosis; 1) anterograde transport of exogenous neurotrophins (NT-3) is receptor-mediated *(17,56);* 2) sorting of NT-3 appears to require trkC activity and passage through the Golgi system *(56);* 3) anterograde BDNF, GDNF, and NT-3 appear to be packaged at least in part in presumptive LDCVs *(58,93,113–115);* and 4) axo-dendritic transfer of neurotrophic factors occurs across the synaptic cleft of olivo-cerebellar contacts (IGF-I *[28,57]*), retinotectal contacts (NT-3 *[17,58]*), noradrenergic cortical synapses (BDNF *[19]*), and cortical synapses in vitro (BDNF *[116]*).

Receptor Distribution and Receptor-Mediated Internalization: Neurotrophins

Because little is known about receptor mechanisms of anterograde transport and transcytosis of other neurotrophic factors, we will focus here on the neurotrophins. Neurotrophins can bind to full-length and truncated receptors, isoforms whose functions remain to be explored *(117–121)*. Internalization of neurotrophins has mostly been studied in PC12 cells (for reviews *see 122,123)*) and, for retrograde signaling, in sympathetic or sensory PNS neurons in vitro *(9,11)*. Both types of neurotrophin receptors, the "common" neurotrophin receptor p75 and trk receptors, alone can mediate internalization *(53,124)*. When both p75 and the relevant trk receptors are expressed, axonal internalization of NGF in vivo appears to be mediated by trk receptors, but that of BDNF and NT-4 by p75 receptors *(125)*. When p75 is expressed without trk receptors, the p75 receptor can mediate internalization of NGF *(124)*, indicating that trkA suppresses the ability of p75 to internalize NGF. It is not clear whether p75 may influence the internalization of NGF via trkA receptors *(126,127)*. When internalized at the axon terminal, there seems to be a delay before retrograde transport of the neurotrophin occurs *(10)*.

Internalized neurotrophin receptors recycle rapidly back to the plasma membrane with little degradation of the neurotrophin or the receptor *(5)*. When trkA-bound NGF is internalized in CHO cells, up to 60% of the NGF is re-released *(53)*. NGF was initially thought not to be internalized by p75 receptors, however, it

now seems that NGF is internalized by this receptor but rapidly re-released *(128).* This may explain why initial early studies failed to show internalization; it was not clear whether p75 reduced the rate of internalization or increased the rate of recycling.

It is now being appreciated that depending on the localization, route, or "speed" of internalization of trk receptors, neurotrophins can activate different signaling pathways, e.g., survival vs neuritogenesis *(129,130).* Full-length and truncated trk receptors are expressed throughout dendritic and axonal compartments *(131),* and an ultrastructural analysis shows that full-length trkB is concentrated in axon terminals as well as dendritic spines *(132).* TrkA is internalized by clathrin-mediated endocytosis *(122),* remains phosphorylated upon internalization, and NGF (and presumably trkA) are moved towards the perisomal region within elongated membraneous profiles *(133),* small transport vesicles *(123),* and multivesicular bodies *(134).* The transport of phosphorylated trkA (and thus presumably an NGF/trkA ligand/receptor complex) from the axon terminus to the cell body is required for NGF-induced signal transduction at the cell body *(9,11).* Internalized trophic factors and their receptors presumably move from the early to the late endosome. Within the endosomal network, recycling proteins are separated from material destined for degradation *(110).* TrkB receptors are degraded by the proteasome degradation system, regulated by motifs in the cytoplasmic and juxtamembrane domains *(135).* It can be assumed that neurotrophins are trafficked similarly when they are internalized by dendrites (after axo-dendritic transfer or uptake from glial cells) rather than axons, but to our knowledge there is no data for this as yet.

Sorting of Neurotrophins: An Endocytic Route to Golgi

Newly synthesized neurotrophic factors enter the anterograde axonal transport pathway presumably after passage through the endoplasmic reticulum, processing in the Golgi system, and packaging in LDCVs. N-linked glycosylation is thought to be important for neurotrophin folding as well as trafficking *(136,137).* All proteins destined for the axon terminal are thought to be targeted via the Golgi system *(138),* and this rule likely applies to neurotrophic factors.

Studies on the distribution of internalized NGF in neurons and PC12 cells have concluded that NGF accumulates in endosomes, multivesicular bodies, and lysosomes, but not in the Golgi system *(133,139–142),* even in the presence of lysosomotropic agents *(133).* The lack of NGF in the Golgi system was confirmed in a recent study in which accumulation of internalized NGF, BDNF, and NT-3 was compared within the same cell type *(56).* Unlike NGF, however, internalized NT-3, and to a lesser degree BDNF, accumulated transiently in the Golgi system, consistent with the notion that these neurotrophins are sorted in the Golgi prior to their anterograde axonal transport. The internalized neurotrophins were diverted away from the Golgi pathway and into a lysosomal pathway by treatment with the tyrosine kinase inhibitor K252a *(56),* indicating that the tyrosine motif or tyrosine kinase activity may be a sorting signal as suggested previously for other receptor ligands *(39,115,143,144).*

The sorting of internalized proteins is thought to occur in two main steps: early endosomes can recycle proteins back to the membrane *(36)* and late endosomes can fuse with the Golgi *(36,40,145).* There appear to be multiple endocytic pathways to the Golgi *(146)* and multiple sorting signals may determine the fate of internalized ligands. These include peptide targeting motifs, acidification, glycosylation, tyrosine kinase activity, signal sequences, and receptor binding as well as organelle characteristics such as size, shape, pH, lipid composition, and vesicle docking or fusion proteins in the membranes *(39,137,143,147,148).* Tyrosine kinases may aid in exposing cryptic internalization domains upon ligand binding *(149).* Acidification enhances ligand-receptor dissociation, and it has been shown that the pH in trafficking organelles is complexly regulated in

neurons and their processes *(150).* The ubiquitin-proteasome system may be involved in the internalization and transport of trophic factors and receptors to lysosomes *(151).* Research in the area of molecular sorting or "address tags" for internalized ligands and receptors in neurons is increasing *(37,135).*

Anterograde Axonal Transport

Little is known about receptor-binding during internalization and anterograde axonal transport of neurotrophic factors. Several different possibilities exist: The ligand may remain associated with its receptor and travel along the axon or within the cell body in a receptor-ligand complex, as is generally assumed for transport in the retrograde direction *(4,11,31,122,123).* Alternatively, the internalized ligand may bind to the receptor only during endocytosis, and during transport in endosomes the ligand dissociates from its receptor. Dissociation may occur due to acidification in endosomes. The receptor may be modified as shown for the internalized pIgA receptor (for review, *see 39*). It is also possible that the ligand dissociates from one receptor and may bind to another receptor present within the same vesicle, or after budding and fusion with other membrane components, e.g., in the Golgi complex. Thus, it is not certain whether the ligand remains in the same ligand-receptor complex during transcytosis, throughout its journey from the site of internalization to the site of release.

Indeed, our recent studies suggest that NT-3 is internalized predominantly via trkC receptors in retinal ganglion cells (RGCs), but subsequently appears to dissociate from trkC and gains access to p75 receptor, which it binds during anterograde axonal transport in the optic chiasm *(56).* This may be explained by the hypothesis that the endosome with the internalized NT-3 fuses with the Golgi system where the NT-3, dissociated from trkC, may gain access to p75 receptors, which are known to be transported anterogradely by chick RGCs *(152).*

It is assumed that neurotrophic factors are packaged in secretory granules or LDCVs when they follow the regulated secretory pathway *(19,25,27,58,93,113,115,153–155).* In cultured hippocampal neurons, however, transfected myc-labeled neurotrophin 6 (NT-6myc) was localized in smooth endoplasmic reticulum-like compartments rather than LDCVs at the ultrastructural level *(156).* Due to the low levels of endogenous neurotrophic factors in axon terminals, and the relatively low abundance of LDCVs in most terminals, convincing evidence is difficult to obtain at the ultrastructural level in intact tissue.

Once the vesicle that contains neurotrophins is properly packaged and loaded onto the anterograde transport machinery, presumably the kinesin-microtubule system *(157),* it is transported to the axon terminus, analogous to the binding and retrograde transport of trk receptors via dynein *(158).* Neurotrophins travel with the "fast axonal transport speed" which is similar to the fast retrograde transport (about 12 mm/h *[159]*). Some signaling molecules appear to be released from the axon shaft (and act on glial cells *[22]*), and NT-6 can be secreted from neurites, apparently including axons in vitro *(156).* Release of NGF does not seem to occur "in transit" when it travels along neurites in the retrograde direction *(10).* It is not known whether anterogradely transported trophic factors can be released from axon shafts in vivo or are exclusively secreted at synaptic contacts, as seems to be the case for some ligands such as tetanus toxin *(111).*

Multiple reasons exist for the paucity in knowledge about anterograde axonal transport of neurotrophic factors. First, most neurotrophic factors are highly potent but very low-abundance peptides, therefore they are difficult to detect and to quantify. Second, anterograde transport of exogenous neurotrophic factors often involves relatively smaller amounts and thus is less robust than retrograde axonal transport. For example, about 100 RGCs transport anterogradely the amount of exogenous NT-3, which is retrogradely transported by a single isthmo-optic neuron *(94,160).* Unless a system is used that provides amplification of the neurotrophin

protein, such as accumulation after ligation of nerves, or convergence of a large number of afferent fibers onto a relatively small target area (such as the retinotectal system *[17]*) anterograde transport of trophic factors can escape detection due to the small, but nevertheless functionally significant, amounts. This may be different in the case of IGF-I anterograde transport from the inferior olive to the cerebellum, which appears to involve a more substantial amount *[16].* Finally, it can be difficult to distinguish retrograde and anterograde transport in neural circuits where bi-directional transport occurs in the same fibers or within the same fiber tract.

It is not known whether or not newly synthesized neurotrophins bind to receptors and are trafficked in a ligand-receptor complex within the producing neuron. When cells express the ligand as well as the receptor for the ligand, this is often interpreted in terms of "autocrine" regulation *(100).* Alternatively, co-expression of ligands and receptors may present a mechanism for sophisticated regulation of intracellular trafficking of neurotrophic factors *(56,114,152).*

Release of Neurotrophins from Axon Terminals

Although several neurotrophic factors have been demonstrated in terminals or in secretory granules in numerous cell types and by various methods, including confocal immunocytochemistry, biochemistry, and ultrastructural studies *(17,19,25,27,93,113–115,154,155,161,162),* very few studies have provided direct evidence for the release of anterograde neurotrophic factors from presynaptic terminals. Progress has been slowed for multiple reasons: the source of the neurotrophic factors has to be determined by tagging; the amounts released are very small and difficult to quantify; and the release is assumed to be linked to an observed function of the neurotrophic factors, which is difficult to prove directly because there could be co-release of another factor that is responsible for the function. Nevertheless, functional studies have been conducted that demonstrate release of endogenous IGF-I *(28,57,163,164)* and BDNF *(19,71–74).* Other studies have demonstrated that exogenous neurotrophins such as NT-3 are released from presynaptic axon terminals by employing ultrastructural autoradiography of tissue sections or gamma-counting of biochemically enriched compartments *(17,58)* or demonstrated that overexpressed BDNF-GFP is released from presumptive axon terminals in vitro *(116).* Together, these studies provide strong evidence that anterogradely transported trophic factors are released and that the release of such factors is functionally significant (*see* below).

Mechanisms of Release

What are the mechanisms of release of anterogradely transported neurotrophic factors from axon terminals? If these factors reside in LDCVs, as generally assumed for neuropeptides, one would expect them to be released according to the regulated pathway *(165),* by strong depolarizing stimuli *(166).* Studies on the release of IGF-I and NT-3 from presynaptic axon terminals indeed show that release is induced by depolarization either with electrical stimulation or with high potassium, and that release requires extracellular calcium *(28,57,58).* Studies on NT-3 release revealed the significance of the mobilization of calcium from internal stores *(58),* confirming results obtained for neurotrophin release from whole cells *(153,161,167).*

The mechanisms of intracellular neurotrophin targeting and release have recently become the subject of intense controversy *(25,58,153,162,167,168):* 1) Does overexpression of trophic factors, due to overloading of sorting capacities, target them into nonphysiological pathways and sites of release? 2) Are internalized, anterogradely transported trophic factors targeted to the same transport and secretion pathways as newly synthesized endogenous trophic factors? 3) Do mechanisms of release from presynaptic terminals differ from the release from other cellular compartments, and can the release specifically from presynaptic terminals be measured reliably?

A Uniform Mechanism for Neurotrophin Release?

Protein targeting follows two distinct types of pathways for secretion, either constitutive or regulated release *(165).* While Thoenen, Shooter, and their coworkers found that NGF and BDNF were released by both constitutive and regulated mechanisms in cell lines and neurons *(153,155,161,167,169–171),* Murphy and coworkers found that targeting of newly synthesized neurotrophins was determined by proteolytic cleavage of the neurotrophin precursor, and thus directed the mature neurotrophin either into a constitutive or into a regulated secretory pathway *(25,27,172).* NGF and NT-3 were released constitutively, unless overexpressed, while only BDNF was released in a regulated fashion. They concluded that at physiological levels of expression, only BDNF was targeed to the regulated pathway of release, but not NGF or NT-3 (*25,27 see* also ref. *60*). On the other hand, Heymach et al. *(170)* concluded that the mature neurotrophin moiety of NGF (and likely of BDNF and NT-3) determined sorting into regulated secretion, and Thoenen and coworkers argued that, in their hands, there were no differences in the release properties of NGF, BDNF, and NT-6 *(153,156).* Obviously, this important issue awaits clarification.

A second issue of contention is whether depolarization-evoked neurotrophin release requires extracellular calcium *(153,162),* and whether or not neurotrophin release is mediated by an unconventional, extracellular sodium-dependent mechanism *(58,167,168).*

Sorting of Exogenous and Endogenous Trophic Factors in the Same Pathway?

It is not known whether transcytosed (recycled) trophic factors are sorted into the same secretion pathway as newly synthesized factors, or if they may utilize different pathways. Since transcytosing NT-3 appears in presumptive LDCVs in retinotectal terminals *(58),* and internalized NT-3 is processed in the Golgi system *(56),* it is likely that this internalized NT-3 merges at the level of the Golgi system with newly synthesized NT-3, where it is packaged into LDCVs for anterograde axonal transport (*see* Fig. 3). However, if pro-NT-3 indeed is sorted into the constitutive release pathway *(27),* how could NT-3 be transported anterogradely in several neuronal cell types *(17,26,105,152)* and be released from the axon terminus in an activity-dependent manner indicating regulated secretion *(58)?*

Several possible scenarios may explain these apparently contradictory results. First, it is possible that the efficiency of cleavage by furin (a secretory pathway endoprotease that catalyses the maturation of proprotein substrates) differs between cell types, or conditions may exist in some cell types that protect pro-NGF and pro-NT-3 from cleavage. Second, NT-3 may form heterodimers with other neurotrophins such as BDNF, which then can be processed in the regulated pathway *(25).* Third, there may exist two fundamentally different pathways of secretion, one for newly synthesized trophic factors, and another one for recycled mature trophic factors which are sorted and targeted for regulated secretion after internalization through an endocytic Golgi pathway (*see* Fig. 3). Fourth, there may be principal differences in release mechanisms between trophic factors in LDCVs released from terminals and trophic factors in other storage pools released from dendrites or the cell body.

Release of IGF-I, NT-3, and BDNF from Axon Terminals

None of the techniques used in conventional assays for release of trophic factors *(25,27,153,161,162,167)* were sufficiently sensitive to measure the release exclusively from axon terminals. It is likely that release mechanisms differ between presynaptic axon terminals and other cellular compartments. LDCVs and secretory granules are expected to be released via the regulated pathway. Neurotrophic factors that have been transported anterogradely to terminals are generally believed to be packaged in LDCVs. LDCVs are rare in most terminals compared with small synaptic vesicles (SSVs), they are not concen-

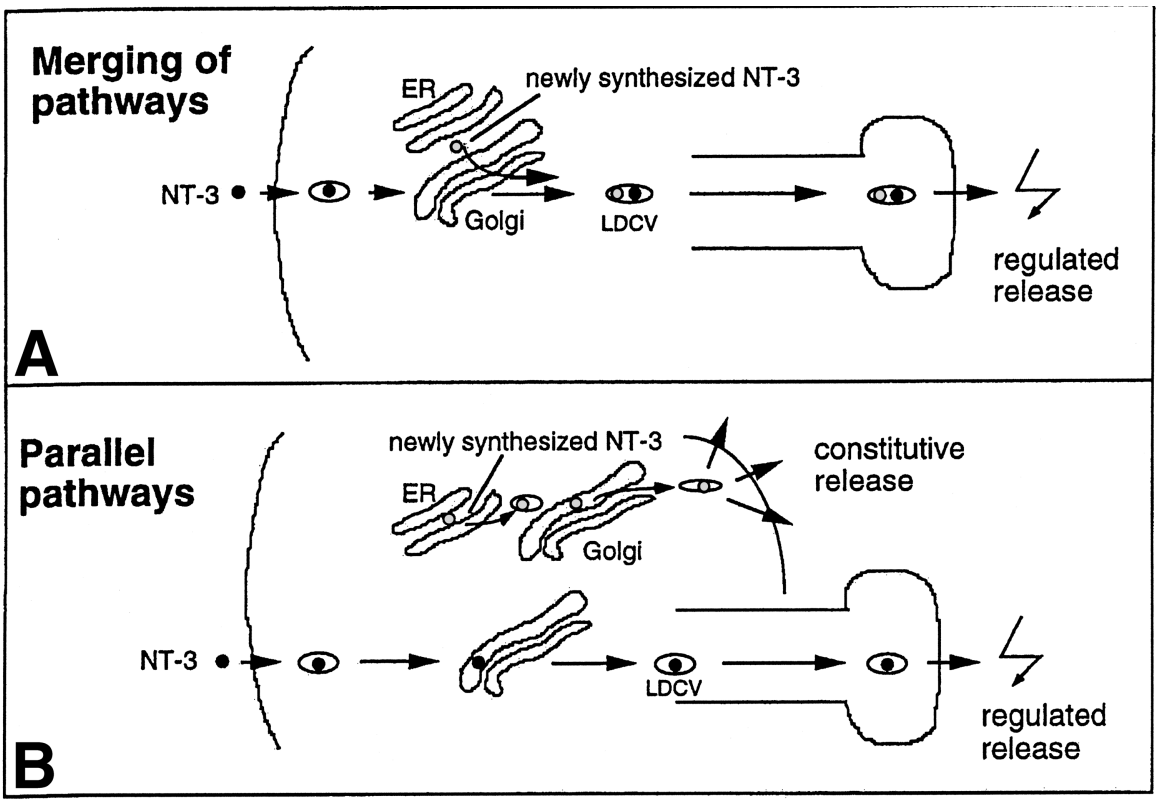

Fig. 3. Intracellular pathways of internalized and newly synthesized trophic factors may merge in the Golgi **(A)**, or they may follow parallel pathways **(B)**. If pathways merge, then internalized trophic factors may provide an advantageous system to analyze release mechanisms of anterogradely transported trophic factors. If pathways are parallel, this may allow for additional functions of internalized trophic factors (such as regulated release), other than those of newly synthesized factors. ER, endoplasmic reticulum; LDCV, large dense-core vesicle; NT-3, neurotrophin 3.

trated at the active site, and they are released by different mechanisms than SSVs *(166,173).* Different neuropeptides are released by a variety of different mechanisms in different systems *(174,175),* and therefore it can not be inferred which parameters would release anterogradely transported neurotrophic factors from presynaptic axon terminals.

Release of trophic factors specifically from axon terminals was first studied by measuring the release of IGF-I from climbing fibers (olivary-cerebellar projection *[28,57]*). It is thought that 40–50% of the IGF-I in the cerebellum is derived by anterograde axonal transport from the inferior olive *(16).* Since the afferent source of IGF-I can be abolished by selective lesions of the inferior olive, it is possible to measure IGF-I release with radioimmunoassays and compare the release of IGF-I from cerebellar slices containing or lacking IGF-I-containing climbing fibers. Release of IGF-I from cerebellar slices showed a basal (constitutive) component and a regulated component. Release in the cerebellum was increased when neurons in the inferior olive were stimulated electrically in vivo *(57).* Release of IGF-I from cerebellar slices was also increased by high potassium or veratridine, but only when the climbing fibers were present, and this release was partially abolished in the absence of extracellular calcium *(28).*

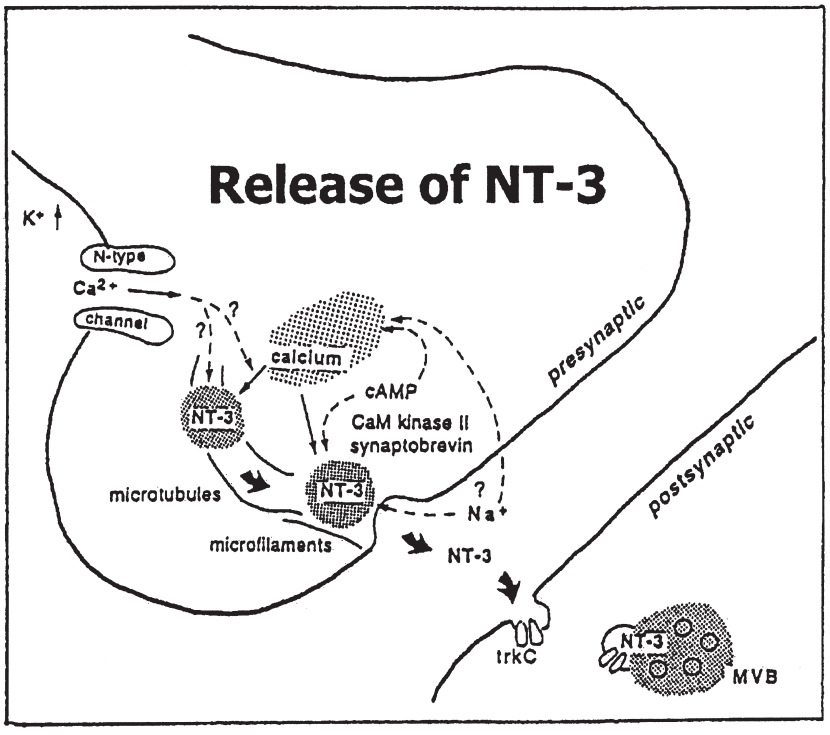

Fig. 4. Synopsis of mechanisms of the release of anterogradely transported NT-3 from presynaptic axon terminals. Adapted with permission from ref. *(58).* Depolarization of the axon terminal by high potassium induces calcium influx via N-type channels. The increase in intracellular calcium may mobilize calcium from internal stores and trigger movement of vesicles containing NT-3 via microtubules and microfilaments to the synaptic cleft. Local calcium increases may also require elevated cAMP and CaM kinase II activity. The vesicle containing NT-3 fuses with the plasma membrane via synaptobrevin-mediated exocytosis, possibly aided by the influx of extracellular sodium. The NT-3 is released into the synaptic cleft and binds to postsynaptic trkC receptors, is internalized and accumulates in multivesicular bodies (MVBs).

To measure the release of NT-3 from presynaptic axon terminals, von Bartheld and colleagues designed a protocol in which retinotectal terminals were loaded with anterogradely transported radiolabeled NT-3, and they measured release of NT-3 by synaptosomal fractionation after incubation of intact tissue with various pharmacological agents *(58).* These studies shed first light on the mechanisms of release of an anterogradely transported neurotrophin (albeit not newly synthesized) from presynaptic axon terminals in vivo. NT-3 was released by depolarization, by mobilization of calcium from internal stores, and by cAMP; and release required CaM kinase II, intact microfilaments, and microtubules (*see* Fig. 4). The implications of these results are that neurotrophins are released only in certain "contexts" of strong calcium signals; tetanic stimulation, as in long-term potentiation; or their release may require multiple influences, not just "normal activity" *(19,176,177).* Consistent with this idea, anterogradely transported BDNF appears to be released from central terminals of DRG neurons and alters membrane excitability of postsynaptic neurons only when these neurons are made hypersensitive (by low-intensity tactile stimulation), but not when the neurons maintain their normal or basal sensitivity *(173).*

Despite the notion that BDNF is likely the most abundant anterograde neurotrophic fac-

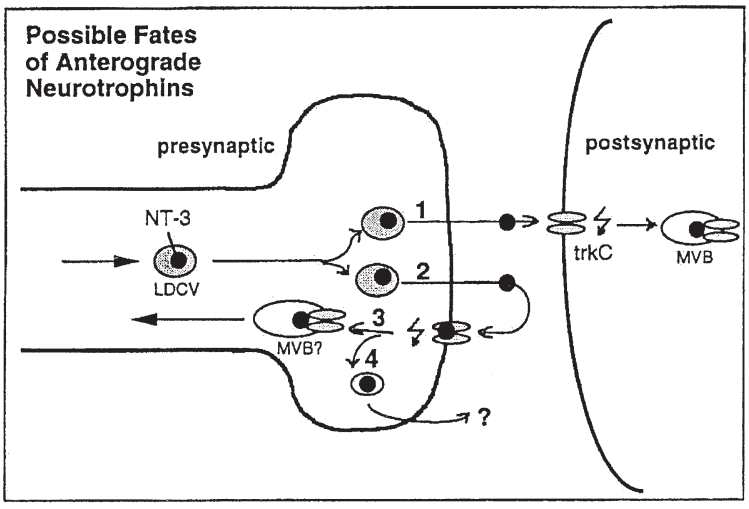

Fig. 5. Possible fates of anterogradely transported neurotrophins after release from presynaptic axon terminals, shown for NT-3. The NT-3, presumed to reside in LDCVs, may be released to diffuse across the synaptic cleft **(1)** and activate trkC receptors located on postsynaptic membranes, and eventually accumulates in MVBs. Alternatively, the released NT-3 may not traverse the synaptic cleft, but bind to receptors on the presynaptic membrane **(2)**. When internalized by presynaptic receptors, the NT-3 may be returned to the cell body by retrograde axonal transport **(3)**, presumably in small transport vesicles *(123)* or MVBs *(134)*. Alternatively, the internalized neurotrophin may enter a storage pool within the terminal from which it can be returned to the synaptic cleft *(178)* by yet another release cycle **(4)**.

tor *(29,30)*, virtually nothing is known about the mechanisms of release of BDNF from presynaptic terminals in intact tissue, apparently due to the lack of a suitable model system. Release of BDNF from synaptosomes has been examined *(178)*. In this study the synaptosomes were incubated with radiolabeled BDNF, and with this protocol neurotrophins are not internalized into the same pool in which they would be present after anterograde axonal transport (LDCVs *[58]*), since LDCVs, unlike SSVs, are not recycled at the terminal. Rather, BDNF internalized by synaptosomes is likely in a pool of presumptive vesicles which in vivo either are destined for retrograde axonal transport (#3 in Fig. 5) or for re-release into the synaptic cleft (#4 in Fig. 5). Such release of BDNF internalized in synaptosomes was enhanced by depolarization and depended on intracellular calcium *(178)*. The functional significance of such release is unclear, as it is not known whether neurotrophins internalized by presynaptic receptors are stored for re-release, or if they are removed from the terminal by retrograde axonal transport *(4,8)*.

Overexpressed BDNF-GFP was shown to be anterogradely transported in vitro, and its release and postsynaptic accumulation was reduced by reduced electrical activity, while enhanced by increased electrical discharges *(116)*.

Findings of presynaptic release of anterogradely transported neurotrophins are of particular interest in light of the recent identification of neurotrophins, and especially BDNF and NT-3, as the most potent excitatory neurotransmitters discovered to date *(179)*. If mechanisms of release of NT-3 from retinotectal terminals can be generalized for other neurotrophic factors and other neurons, then this will allow a better understanding of when and how neurotrophic factors are released from axon terminals with implications for synaptic plasticity, long-term potentiation, shaping of neuronal architecture, and other functions in which anterogradely transported neurotrophic factors are believed to play important roles.

Functions of Anterogradely Transported Neurotrophic Factors

When neurotrophic factors are released from axon terminals, they can either act on the postsynaptic site (axo-dendritic transfer, #1 in Fig. 5), or they can act back on the presynaptic site (re-uptake, #2 in Fig. 5). When acting back on presynaptic receptors, neurotrophic factors may be retrogradely transported (#3 in Fig. 5) or recycled for another release cycle (#4 in Fig. 5). There are numerous examples of receptors for neurotrophic factors on dendrites as well as on presynaptic terminals *(132,180–184).* When anterogradely transported neurotrophins are released from the presynaptic site, they are primarily expected to act on postsynaptic receptors, and we will focus here on such effects.

Since neurotrophins can enhance the survival of neurons *(185),* and afferents can provide trophic support *(23),* this was one of the first parameters that was examined. Anterogradely transported BDNF and NT-3 indeed can increase neuronal survival of postsynaptic neurons *(17,26,34,72–74).* Anterogradely transported IGF-I and neurotrophins also influence the differentiation program and the phenotype of postsynaptic neurons *(163),* but such effects can be difficult to distinguish from effects on survival *(71).*

Neurotrophic factors can have effects on synapse plasticity, efficacy of neurotransmission, and modulation of transmitter release *(57,164,177,186–192),* properties that have led to the notion of "synaptotrophins" *(193).* While at least in some cases synaptic transmission is modified by neurotrophic factors released from postsynaptic sites *(194),* release from presynaptic sites also plays a role in synaptic plasticity *(19,102,164,173).* Anterogradely transported IGF-I in olivocerebellar fibers mediates long-term depression in the cerebellum *(57).* The recent identification of BDNF and NT-3 as the most potent excitatory neurotransmitters *(179)* further emphasizes the importance of an understanding of the function of neurotrophins in synapses and their sources. Neurotrophins can acutely increase the number and vesicle density of synapses *(195,196).* In retinotectal synapses, morphological changes were induced by exogenous NT-3 *(196),* which is known to have an exclusive presynaptic source in this in-vivo system *(152).* Since BDNF is thought to be transferred from postsynaptic to presynaptic sites in these terminals, the effect of presynaptic NT-3 may be due to stimulation of the release of postsynaptic BDNF. It is known that NT-3 can stimulate the release of BDNF in other systems *(197,198).* Thus, synaptic plasticity regulated by neurotrophins may involve the bidirectional transfer of neurotrophins across the synaptic cleft. This is consistent with the original idea that both pre- and postsynaptic factors are necessary for the development or stabilization of synapses *(19,199).*

In addition to effects on synapses, presynaptic release of neurotrophic factors may regulate dendritic morphology. It has been shown that afferent-derived IGF-I affects the spine size of Purkinje neurons *(164).* Neurotrophins can have significant effects on the branching and growth of cortical dendrites *(200),* but it is not known whether the neurotrophins that are thought to regulate dendritic morphology in vivo are derived from the presynaptic neuron, the postsynaptic neuron, or from glial cells.

The Concept of Trophic Currencies

Over the past 10–15 years, our understanding of trophic mechanisms in the developing and mature nervous system has advanced remarkably. Numerous new trophic factors and their receptors have been identified, their distribution mapped, and many functions have been elucidated. On the other hand, some areas of neurotrophin biology have received relatively little attention, such as the trafficking of trophic factors in neural circuits at the systems level *(104,201–204),* as well as their intracellular pathways, and mechanisms of release from identified compartments. In part, this can be attributed to the prevailing, but simplistic concept that trophic factors are produced in target cells, released, and rapidly consumed by

innervating neurons. If this was the only way that neurotrophins work, one need not worry about trafficking and multiple functions in neural circuits. However, it turned out that neurotrophins behave in complex ways *(205).* Transcript and protein levels do not necessarily correlate *(203,206),* neurotrophins can move anterogradely along axons, and they can even move through neurons by transcytosis. It now appears that trophic factors are not necessarily produced exclusively by the neuron or glial cell when a connecting neuron has an immediate need for the factor, and released by the producing cell upon an appropriate stimulus. Rather, it is likely that trophic factors are produced in subpopulations of cells, which then distribute their products among a variety of connected neurons within neural circuits, and whenever a neuron requires trophic substances to conduct business with its trading partners, it relies on pools of acquired savings, trophic molecules stored in various compartments, some of them newly synthesized, but others recycled after they were received from other sources.

What is the advantage of recycling trophic factors? First, it may be economical; many receptors and other membrane proteins and lipids are recycled. Recycling pathways are generally more efficient than the lysosomal degradation pathways *(39,47,53,148,207).* Many receptors recycle 100–300 times and 40% of internalized insulin is recycled and released *(208).* Second, use of recycled trophic factors may allow for a simple regulation of survival and maintenance of properly connected and functional neurons. When neurons perform well among their peers in terms of receiving and sending appropriate signals through the neural network, they should be rewarded with "currencies" and other trophic material or resources, for them to spend as they see fit. If each neuron could "print its own money" or trophic material, the temptation may be to pump itself full with inflationary bills in an autocrine loop *(100)* that may not serve any meaningful purpose "in society" other than to perpetuate a systemically meaningless life. Only if your work is considered valuable by your peers will you receive your share of trophic material, and you will be diligent in the distribution and release of such precious goods.

From a more cybernetic/economic viewpoint, the need to accumulate trophic material from outside sources may provide for an instant measure of how successful the neuron was in its recent past in communicating with connected partners. Thereby, the recent history of a neuron becomes a factor in its present performance and allows a historical dimension to enter the picture. The past performance may provide important information for the allotment of trophic resources. The trafficking, storing, and release of trophic material may be for the neuron what in our world is the cash and the credit card, and the currency exchanges that drive the world economy among trading partners.

Many important issues remain to be resolved. We do not yet understand how trophic factors are targeted to either dendritic, axonal, or degradation pathways, and how they may be sorted into either constitutive or regulated secretion pathways. The sources and functions of neurotrophic factors in the synaptic cleft need to be clarified. We still lack clear evidence for physiological transcytosis of trophic factors in neurons. It is not known how many times an individual neurotrophic molecules is functionally used (activates a signal-transduction cascade) before it is degraded, i.e., the rate of recycling remains to be determined. To understand the role of trophic factors in synaptic plasticity, it is important to elucidate the physiological stimuli that induce their release into the synaptic cleft from defined compartments, either pre- or postsynaptically, and to follow the fate and functions of such released trophic molecules.

Our survey of the literature shows that transcytosis of trophic factors can occur in several neural circuits. Future studies will test how abundant recycled trophic factors are in the developing and mature nervous system, and thus how significant the concept of trophic currencies is within neural networks.

Acknowledgements

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Note Added in Proof

While this manuscript was set in print, the following relevant articles have been published or brought to our attention:

Tuffereau C., Benejean J., Blondel D., Kieffer B., and Flamand A. (1998) Low-affinity nerve-growth factor receptor (P75NTR) can serve as a receptor for rabies virus. *EMBO J.* **17,** 7250–7259.

This study shows that the p75 neurotrophin receptor can bind rabies virus, and suggests that p75-binding is a mechanism by which this virus (and by analogy other ligands) may propagate at synapses between connected neurons.

Kojima M., Takei N., Numakawa T., Ishikawa Y., Suzuki S., Matsumoto T., et al. (2001) Biological characterization and optical imaging of brain-derived neurotrophic factor-green fluorescent protein suggest an activity-dependent local release of brain-derived neurotrophic factor in neurites of cultured hippocampal neurons. *J. Neurosci. Res.* ***64,*** 1–10.

This study shows that BDNF is released from synaptic sites in response to depolarization.

References

1. Kaplan D. R. and Miller F. D. (2000) Neurotrophin signal transduction in the nervous system. *Curr. Opin. Neurobiol.* **10,** 381–391.
2. Segal R. A. and Greenberg M. E. (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu. Rev. Neurosci.* **19,** 463–89.
3. Barde Y.-A. (1989) Trophic factors and neuronal survival. *Neuron* **2,** 1525–1534.
4. Curtis R. and DiStefano P. S. (1994) Neurotrophic factors, retrograde axonal transport, and cell signalling. *Trends Cell. Biol.* **4,** 383–386.
5. Eveleth D. D. and Bradshaw R. A. (1988) Internalization and cycling of nerve growth factor in PC12 cells: interconversion of type II (fast) and type I (slow) nerve growth factor receptors. *Neuron* **1,** 929–936.
6. Johnson E. M. Jr., Andres R. Y., and Bradshaw R. A. (1978) Characterization of the retrograde transport of nerve growth factor (NGF) using high specific activity [^{125}I] NGF. *Brain Res.* **150,** 319–331.
7. Layer P. G. and Shooter E. M. (1983) Binding and degradation of nerve growth factor by PC12 pheochromocytoma cells. *J. Biol. Chem.* **258,** 3012–3018.
8. Oppenheim R. W. (1996) The concept of uptake and retrograde transport of neurotrophic molecules during development: history and present status. *Neurochem. Res.* **21,** 769–777.
9. Riccio A., Pierchala B. A., Ciarallo C. L., and Ginty D. D. (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. *Science* **277,** 1097–1100.
10. Ure D. R. and Campenot R. B. (1997) Retrograde transport and steady-state distribution of 125I-nerve growth factor in rat sympathetic neurons in compartmented cultures. *J. Neurosci.* **17,** 1282–1290.
11. Watson F. L., Heerssen H. M., Moheban D. B., Lin M. Z., Sauvageot C. M., Bhattacharyya A., et al. (1999) Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. *J. Neurosci.* **19,** 7889–7900.
12. Yuen E. C., Howe C. L., Li Y., Holtzman D. M., and Mobley W. C. (1996) Nerve growth factor and the neurotrophic factor hypothesis. *Brain Dev.* **18,** 362–368.
13. Ferguson I. A., Schweitzer J. B., and Johnson E. M. Jr. (1990) Basic fibroblast growth factor: receptor-mediated internalization, metabolism, and anterograde transport in retinal ganglion cells *J. Neurosci.* **10,** 2176–2189.
14. Hansson H. A., Rozell B., and Skottner A. (1987) Rapid axoplasmic transport of insulin-like growth factor I in the sciatic nerve of adult rats. *Cell Tissue Res.* **247,** 241–247.

15. Holstege J. C., Jongen J. L., Kennis J. H., van Rooyen-Boot A. A., and Vecht C. J. (1998) Immunocytochemical localization of GDNF in primary afferents of the lumbar dorsal horn. *Neuroreport* **9,** 2893–2897.
16. Torres-Aleman I., Pons S., and Garcia-Segura L. M. (1991) Climbing fiber deafferentation reduces insulin-like growth factor I (IGF-I) content in cerebellum. *Brain Res.* **564,** 348–351.
17. von Bartheld C. S., Byers M. R., Williams R., and Bothwell M. (1996) Anterograde transport and axo-dendritic transfer of neurotrophins in the developing visual system. *Nature* **379,** 830–833.
18. Zhou X.-F. and Rush R. A. (1996) Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. *Neuroscience* **74,** 945–951.
19. Aloyz R., Fawcett J. P., Kaplan D. R., Murphy R. A., and Miller F. D. (1999) Activity-dependent activation of TrkB neurotrophin receptors in the adult CNS. *Learn. Mem.* **6,** 216–231.
20. Catsicas M., Péquignot Y., and Clarke P. G. H. (1992) Rapid onset of neuronal death induced by blockade of either axoplasmic transport or action potentials in afferent fibers during brain development. *J. Neurosci.* **12,** 4642–4650.
21. Galli-Resta L., Ensini M., Fusco E., Gravina A., and Margheritti B. (1993) Afferent spontaneous electrical activity promotes the survival of target cells in the developing retinotectal system of the rat. *J. Neurosci.* **13,** 243–250.
22. Kunes S. (2000) Axonal signals in the assembly of neural circuitry. *Curr. Opin. Neurobiol.* **10,** 58–62.
23. Linden R. (1994) The survival of developing neurons. A review of afferent control. *Neuroscience* **58,** 671–682.
24. Conner J. M., Lauterborn J. C., and Gall C. M. (1998) Anterograde transport of neurotrophin proteins in the CNS: a reassessment of the neurotrophic hypothesis. *Rev. Neurosci.* **9,** 91–103.
25. Farhadi H. F., Mowla S. J., Petrecca K., Morris S. J., Seidah N. G., and Murphy R. A. (2000) Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. *J. Neurosci.* **20,** 4059–4068.
26. Johnson F., Hohmann S. E., DiStefano P. S., and Bottjer S. W. (1997) Neurotrophins suppress apoptosis induced by deafferentation of an avian motor-cortical region. *J. Neurosci.* **17,** 2101–2111.
27. Mowla S. J., Pareek S., Farhadi H. F., Petrecca K., Fawcett J. P., Seidah N. G., et al. (1999) Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *J. Neurosci.* **19,** 2069–2080.
28. Nieto-Bona M. P., Garcia-Segura L. M., and Torres-Aleman I. (1993) Orthograde transport and release of insulin-like growth factor I from the inferior olive to the cerebellum. *J. Neurosci. Res.* **36,** 520–527.
29. Altar C. A. and DiStefano P. S. (1998) Neurotrophin trafficking by anterograde transport. *Trends Neurosci.* **21,** 433–437.
30. Conner J. M., Lauterborn J. C., Yan Q., Gall C. M., and Varon S. (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J. Neurosci.* **17,** 2295–2313.
31. Alderson R. F., Curtis R., Alterman A. L., Lindsay R. M., and DiStefano P. S. (2000) Truncated TrkB mediates the endocytosis and release of BDNF and neurotrophin-4/5 by rat astrocytes and Schwann cells in vitro. *Brain Res.* **871,** 210–222.
32. Rubio N. (1997) Mouse astrocytes store and deliver brain-derived neurotrophic factor using the non-catalytic gp95trkB receptor. *Eur. J. Neurosci.* **9,** 1847–1853.
33. Russell F. D., Koishi K., Jiang Y., and McLennan I. S. (2000) Anterograde axonal transport of glial cell line-derived neurotrophic factor and its receptors in rat hypoglossal nerve. *Neuroscience* **97,** 575–80.
34. Spalding K. L., Tan M. M. L., Hendry I. A., and Harvey A. L. (1999) Cell death and the transport of neurotrophins in the rat visual system. *Soc. Neurosci. Abstr.* **25,** 767.
35. Gonatas N. K., Kim S. U., Stieber A., and Avrameas S. (1977) Internalization of lectins in neuronal GERL. *J. Cell Biol.* **73,** 1–13.
36. Green S. A. and Kelly R. B. (1992) Low density lipoprotein receptor and cation-independent mannose 6-phosphate receptor are transported from the cell surface to the Golgi apparatus at equal rates in PC12 cells. *J. Cell Biol.* **117,** 47–55.
37. Hemar A., Olivo J. C., Williamson E., Saffrich R., and Dotti C. G. (1997) Dendroaxonal transcytosis of transferrin in cultured hippocampal and sympathetic neurons. *J. Neurosci.* **17,** 9026–9034.
38. Joseph K. C., Kim S. U., Stieber A., and Gonatas N. K. (1978) Endocytosis of cholera

toxin into neuronal GERL. *Proc. Natl. Acad. Sci. USA* **75,** 2815–2819.
39. Mukherjee S., Ghosh R. N., and Maxfield F. R. (1997) Endocytosis. *Physiol. Rev.* **77,** 759–803.
40. Sandvig K. and van Deurs B. (1996) Endocytosis, intracellular transport, and cytotoxic action of Shiga toxin and ricin. *Physiol. Rev.* **76,** 949–966.
41. Green S. A. and Kelly R. B. (1990) Endocytic membrane traffic to the Golgi apparatus in a regulated secretory cell line. *J. Biol. Chem.* **265,** 21,269–21,278.
42. Hurtley S. M. (1993) Recycling of a secretory granule membrane protein after stimulated secretion. *J. Cell Sci.* **106,** 649–655.
43. de Hoop M., von Poser C., Lange C., Ikonen E., Hunziker W., and Dotti C. G. (1995) Intracellular routing of wild-type and mutated polymeric immunoglobulin receptor in hippocampal neurons in culture. *J. Cell Biol.* **130,** 1447–1459.
44. Brändli A. W., Adamson E. D., and Simons K. (1991) Transcytosis of epidermal growth factor. The epidermal growth factor receptor mediates uptake but not transcytosis. *J. Biol. Chem.* **266,** 8560–8566.
45. Kozu A., Kato Y., Shitara Y., Hanano M., and Sugiyama Y. (1997) Kinetic analysis of transcytosis of epidermal growth factor in Madin-Darby canine kidney epithelial cells. *Pharm. Res.* **14,** 1228–1235.
46. Maratos-Flier E., Kao C. Y., Verdin E. M., and King G. L. (1987) Receptor-mediated vectorial transcytosis of epidermal growth factor by Madin-Darby canine kidney cells. *J. Cell Biol.* **105,** 1595–1601.
47. Sorkin A., Kornilova E., Teslenko L., Sorokin A., and Nikolsky N. (1989) Recycling of epidermal growth factor-receptor complexes in A431 cells. *Biochim. Biophys. Acta* **1011,** 88–96.
48. Dunn W. A. and Hubbard A. L. (1994) Receptor-mediated endocytosis of epidermal growth factor by hepatocytes in the perfused rat liver: ligand and receptor dynamics. *J. Cell Biol.* **98,** 2148–2159.
49. Miskimins W. K. and Shimizu N. (1982) Involvement of multiple subcellular compartments in intracellular processing of epidermal growth factor. *J. Cell. Biochem.* **20,** 41–50.
50. Shitara Y., Kato Y., and Sugiyama Y. (1998) Effect of brefeldin A and lysosomotropic reagents on intracellular trafficking of epidermal growth factor and transferrin in Madin-Darby canine kidney epithelial cells. *J. Control. Release* **55,** 35–43.
51. Sorkin A. and Waters C. M. (1993) Endocytosis of growth factor receptors. *Bioessays* **15,** 375–382.
52. Siminoski K., Gonnella P., Bernanke J., Owen L., Neutra M., and Murphy R. A. (1986) Uptake and transepithelial transport of nerve growth factor in suckling rat ileum. *J. Cell Biol.* **103,** 1979–1990.
53. Zapf-Colby A. and Olefsky J. M. (1998) Nerve growth factor processing and trafficking events following TrkA-mediated endocytosis. *Endocrinology* **139,** 3232–3240.
54. Simons K. and Wandinger-Ness A. (1990) Polarized sorting in epithelia. *Cell* **62,** 207–210.
55. Craig A. M. and Banker G. (1994) Neuronal polarity. *Annu. Rev. Neurosci.* **17,** 267–310.
56. Butowt R. and von Bartheld C. S. (2001) Sorting of internalized neurotrophins into an endocytic transcytosis pathway via the Golgi system: ultrastructural analysis in retinal ganglion cells. *J. Neurosci.* **21,** 8915–8930.
57. Castro-Alamancos M. A. and Torres-Aleman I. (1993) Long-term depression of glutamate-induced gamma-aminobutyric acid release in cerebellum by insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **90,** 7386–7390.
58. Wang X. X., Butowt R., Vasko M. R., and von Bartheld C. S. (2001) Mechanisms of the release of anterogradely transported NT-3 from presynaptic axon terminals. *J. Neurosci.* (in press).
59. Acsady L., Pascual M., Rocamora N., Soriano E., and Freund T. F. (2000) Nerve growth factor but not neurotrophin-3 is synthesized by hippocampal GABAergic neurons that project to the medial septum. *Neuroscience* **98,** 23–31.
60. Hoyle G. W., Mercer E. H., Palmiter R. D., and Brinster R. L. (1993) Expression of NGF in sympathetic neurons leads to excessive axon outgrowth from ganglia but decreased terminal innervation within tissues. *Neuron* **10,** 1019–1034.
61. Fruttiger M., Calver A. R., and Richardson W. D. (2000) Platelet-derived growth factor is constitutively secreted from neuronal cell bodies but not from axons. *Curr. Biol.* **10,** 1283–1286.
62. Loddick S. A. and Rothwell N. J. (1999) Mechanisms of tumor necrosis factor alpha action on neurodegeneration: interaction with insulin-like growth factor-1. *Proc. Natl. Acad. Sci. USA* **96,** 9449–9451.

63. Venters H. D., Dantzer R., and Kelley K. W. (2000) A new concept in neurodegeneration: TNF alpha is a silencer of survival signals. *Trends Neurosci.* **23,** 175–180.
64. Kokaia Z., Andsberg G., Yan Q., and Lindvall O. (1998) Rapid alterations of BDNF protein levels in the rat brain after focal ischemia: evidence for increased synthesis and anterograde axonal transport. *Exp. Neurol.* **154,** 289–301.
65. Michael G. J., Averill S., Shortland P. J., Yan Q., and Priestley J. V. (1999) Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. *Eur. J. Neurosci.* **11,** 3539–3551.
66. Tonra J. R. (1999) Classical and novel directions in neurotrophin transport and research: anterograde transport of brain-derived neurotrophic factor by sensory neurons. *Microsc. Res. Tech.* **45,** 225–232.
67. Tonra J. R., Curtis R., Wong V., Cliffer K. D., Park J. S., Timmes A., et al. (1998) Axotomy upregulates the anterograde transport and expression of brain-derived neurotrophic factor by sensory neurons. *J. Neurosci.* **18,** 4374–4383.
68. Hammarberg H., Piehl F., Cullheim S., Fjell J., Hokfelt T., and Fried K. (1996) GDNF mRNA in Schwann cells and DRG satellite cells after chronic sciatic nerve injury. *Neuroreport* **7,** 857–860.
69. Höke A., Cheng C., and Zochodne D. W. (2000) Expression of glial cell line-derived neurotrophic factor family of growth factors in peripheral nerve injury in rats. *Neuroreport* **11,** 1651–1654.
70. Naveilhan P., ElShamy W. M., and Ernfors P. (1997) Differential regulation of mRNAs for GDNF and its receptors Ret and GDNFR alpha after sciatic nerve lesion in the mouse. *Eur. J. Neurosci.* **9,** 1450–1460.
71. Altar C. A., Cai N., Bliven T., Juhasz M., Conner J. M., Acheson A. L., et al. (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature* **389,** 856–860.
72. Caleo M., Menna E., Chierzi S., Cenni M. C., and Maffei L. (2000) Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system. *Curr. Biol.* **10,** 1155–1161.
73. Fawcett J. P., Bamji S. X., Causing C. G., Aloyz R., Ase A. R., Reader T. A., et al. (1998) Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *J. Neurosci.* **18,** 2808–2821.
74. Fawcett J. P., Alonso-Vanegas M. A., Morris S. J., Miller F. D., Sadikot A. F., and Murphy R. A. (2000) Evidence that brain-derived neurotrophic factor from presynaptic nerve terminals regulates the phenotype of calbindin-containing neurons in the lateral septum. *J. Neurosci.* **20,** 274–282.
75. Trupp M., Ryden M., Jornvall H., Funakoshi H., Timmusk T., Arenas E., et al. (1995) Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. *J. Cell Biol.* **130,** 137–148.
76. Trupp M., Belluardo N., Funakoshi H., and Ibanez C. F. (1997) Complementary and overlapping expression of glial cell line-derived neurotrophic factor (GDNF), c-ret protooncogene, and GDNF receptor-alpha indicates multiple mechanisms of trophic actions in the adult rat CNS. *J. Neurosci.* **17,** 3554–3567.
77. Golden J. P., DeMaro J. A., Osborne P. A., Milbrandt J., and Johnson E. M. Jr. (1999) Expression of neurturin, GDNF, and GDNF family-receptor mRNA in the developing and mature mouse. *Exp. Neurol.* **158,** 504–528.
78. Nosrat C. A., Tomac A., Lindqvist E., Lindskog S., Humpel C., Stromberg I., et al. (1996) Cellular expression of GDNF mRNA suggests multiple functions inside and outside the nervous system. *Cell Tissue Res.* **286,** 191–207.
79. Widenfalk J., Widmer H. R., and Spenger C. (1999) GDNF, RET and GFRalpha-1-3 mRNA expression in the developing human spinal cord and ganglia. *Neuroreport* **10,** 1433–1439.
80. Yamamoto M., Sobue G., Yamamoto K., Terao S., and Mitsuma T. (1996) Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues. *Neurochem. Res.* **21,** 929–938.
81. Bennett D. L., Michael G. J., Ramachandran N., Munson J. B., Averill S., Yan Q., et al. (1998) A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for these neurons after nerve injury. *J. Neurosci.* **18,** 3059–3072.
82. Bennett D. L., Boucher T. J., Armanini M. P., Poulsen K. T., Michael G. J., Priestley J. V., et al. (2000) The glial cell line-derived neurotrophic factor family receptor components are differ-

entially regulated within sensory neurons after nerve injury. *J. Neurosci.* **20,** 427–447.
83. Molliver D. C., Wright D. E., Leitner M. L., Parsadanian A. S., Doster K., Wen D., et al. (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron* **19,** 849–861.
84. Baloh R. H., Tansey M. G., Golden J. P., Creedon D. J., Heuckeroth R. O., Keck C. L., et al. (1997) TrnR2, a novel receptor that mediates neurturin and GDNF signaling through Ret. *Neuron* **18,** 793–802.
86. Jongen J. L., Dalm E., Vecht C. J., and Holstege J. C. (1999) Depletion of GDNF from primary afferents in adult rat dorsal horn following peripheral axotomy. *Neuroreport* **10,** 867–871.
87. Wright D. E. and Snider W. D. (1996) Focal expression of glial cell line-derived neurotrophic factor in developing mouse limb bud. *Cell Tissue Res.* **286,** 209–217.
88. Springer J. E., Seeburger J. L., He J., Gabrea A., Blankenhorn E. P., and Bergman L. W. (1995) cDNA sequence and differential mRNA regulation of two forms of glial cell line-derived neurotrophic factor in Schwann cells and rat skeletal muscle. *Exp. Neurol.* **131,** 47–52.
89. Leitner M. L., Molliver D. C., Osborne P. A., Vejsada R., Golden J. P., Lampe P. A., et al. (1999) Analysis of the retrograde transport of glial cell line-derived neurotrophic factor (GDNF), neurturin, and persephin suggests that in vivo signaling for the GDNF family is GFRalpha coreceptor-specific. *J. Neurosci.* **19,** 9322–9331.
90. Matheson C. R., Carnahan J., Urich J. L., Bocangel D., Zhang T. J., and Yan Q. (1997) 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.
91. Rind H. B. and von Bartheld C. S. (2001) Anterograde axonal transport of internalized GDNF in sensory and motor neurons. *Soc. Neurosci. Abstr.* **27,** 801–807 .
92. Snider W. D. and McMahon S. B. (1998) Tackling pain at the source: new ideas about nociceptors. *Neuron* **20,** 629–632.
93. Holstege J. C., Rooijen-Boot A. v., Jongen J. L. M., Haasdijk E., Neuteboom R. F., and Vecht C. J. (1999) Localization of BDNF and GDNF protein in rat spinal cord using light and electron microscopy immunocytochemistry. *Soc. Neurosci. Abstr.* **25,** 1272.
94. von Bartheld C. S. and Johnson J. E. (2001) Target-derived BDNF (brain-derived neurotrophic factor) is essential for the survival of developing neurons in the isthmo-optic nucleus. *J. Comp. Neurol.* **433,** 550–564.
95. Catsicas S. and Clarke P. G. H. (1987) Abrupt loss of dependence of retinopetal neurons on their target cells, as shown by intraocular injections of kainate in chick embryos. *J. Comp. Neurol.* **262,** 523–534.
96. Gunther E. C., von Bartheld C. S., Goodman L. J., Johnson J. E., and Bothwell M. (2000) The G-protein inhibitor, pertussis toxin, inhibits the release of brain-derived neurotrophic factor. *Neuroscience* **100,** 569–579.
97. Herzog K. H. and von Bartheld C. S. (1998) Contributions of the optic tectum and the retina as sources of brain-derived neurotrophic factor for retinal ganglion cells in the chick embryo. *J. Neurosci.* **18,** 2891–2906.
98. Suzuki H., Hase A., Miyata Y., Arahata K., and Akazawa C. (1998) Prominent expression of glial cell line-derived neurotrophic factor in human skeletal muscle. *J. Comp. Neurol.* **402,** 303–312.
99. Yan Q., Rosenfeld R. D., Matheson C. R., Hawkins N., Lopez O. T., Bennett L., et al. (1997) Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience* **78,** 431–448.
100. Acheson A., Conover J. C., Fandl J. P., DeChiara T. M., Russell M., Thadani A., et al. (1995) A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature* **374,** 450–453.
101. Ernfors P., Wetmore C., Olson L., and Persson H. (1990) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron* **5,** 511–526.
102. Smith M. A., Zhang L. X., Lyons W. E., and Mamounas L. A. (1997) Anterograde transport of endogenous brain-derived neurotrophic factor in hippocampal mossy fibers. *Neuroreport* **8,** 1829–1834.
103. Castrén E., Thoenen H., and Lindholm D. (1995) Brain-derived neurotrophic factor messenger RNA is expressed in the septum, hypothalamus and in adrenergic brain stem nuclei of adult rat brain and is increased by osmotic stimulation in the paraventricular nucleus. *Neuroscience* **64,** 71–80.
104. von Bartheld C. S. (1998) Neurotrophins in the developing and regenerating visual system. Invited review. *Histol. Histopathol.* **13,** 437–459.

105. Lindholm D., Castrén E., Tsoulfas P., Kolbeck R., Berzaghi M. da P., Leingartner A., et al. (1993) Neurotrophin-3 induced by tri-iodothyronine in cerebellar granule cells promotes Purkinje cell differentiation. *J. Cell Biol.* **122,** 443–450.
106. Ayer-le Lievre C., Stahlbom P. A., and Sara V. R. (1991) Expression of IGF-I and -II mRNA in the brain and craniofacial region of the rat fetus. *Development* **111,** 105–115.
107. Bondy C. A. (1991) Transient IGF-I gene expression during the maturation of functionally related central projection neurons. *J. Neurosci.* **11,** 3442–3455.
108. Villarroya H., Marie Y., Ouallet J. C., Le Saux F., Tchelingerian J. L., and Baumann N. (1997) Expression of TNF alpha in central neurons of Lewis rat spinal cord after EAE induction. *J. Neurosci. Res.* **49,** 592–599.
109. Simons M., Ikonen E., Tienari P. J., Cid-Arregui A., Monning U., Beyreuther K., et al. (1995) Intracellular routing of human amyloid protein precursor: axonal delivery followed by transport to the dendrites. *J. Neurosci. Res.* **41,** 121–128.
110. Parton R. G. and Dotti C. G. (1993) Cell biology of neuronal endocytosis. *J. Neurosci. Res.* **36,** 1–9.
111. Schwab M. and Thoenen H. (1977) Selective trans-synaptic migration of tetanus toxin after retrograde axonal transport in peripheral sympathetic nerves: a comparison with nerve growth factor. *Brain Res.* **122,** 459–474.
112. Schwab M. E., Suda K., and Thoenen H. (1979) Selective retrograde transsynaptic transfer of a protein, tetanus toxin, subsequent to its retrograde axonal transport. *J. Cell Biol.* **82,** 798–810.
113. Fawcett J. P., Aloyz R., McLean J. H., Pareek S., Miller F. D., McPherson P. S., et al. (1997) Detection of brain-derived neurotrophic factor in a vesicular fraction of brain synaptosomes. *J. Biol. Chem.* **272,** 8837–8840.
114. Luo X., Rush R. A., and Zhou X. (2001) Ultrastructural localization of brain-derived neurotrophic factor in rat primary sensory neurons. *Neurosci. Res.* **39,** 377–384.
115. Michael G. J., Averill S., Nitkunan A., Rattray M., Bennett D. L., Yan Q., et al. (1997) Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *J. Neurosci.* **17,** 8476–8490.
116. Kohara K,. Kitamura A., Morishima M., and Tsumoto T. (2001) Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. *Science* **291,** 2419–2423.
117. Barker P. A. and Murphy R. A. (1992) The nerve growth factor receptor: a multicomponent system that mediates the actions of the neurotrophin family of proteins. *Mol. Cell. Biochem.* **110,** 1–15.
118. Bothwell M. (1995) Functional interactions of neurotrophins and neurotrophin receptors. *Annu. Rev. Neurosci.* **18,** 223–253.
119. Garner A. S., Menegay H. J., Boeshore K. L., Xie X.-Y., Voci J. M., Johnson J. E., et al. (1996) Expression of trkB receptor isoforms in the developing avian visual system. *J. Neurosci.* **16,** 1740–1752.
120. Lewin G. R. and Barde Y. A. (1996) Physiology of the neurotrophins. *Annu. Rev. Neurosci.* **19,** 289–317.
121. Middlemas D. S., Lindberg R. A., and Hunter T. (1991) trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. *Mol. Cell. Biol.* **11,** 143–153.
122. Grimes M. L., Zhou J., Beattie E. C., Yuen E. C., Hall D. E., Valletta J. S., et al. (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. *J. Neurosci.* **16,** 7950–7964.
123. Grimes M. L., Beattie E., and Mobley W. C. (1997) A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. *Proc. Natl. Acad. Sci. USA* **94,** 9909–9914.
124. Kahle P. and Hertel C. (1992) Nerve growth factor (NGF) receptor on rat glial cell lines. Evidence for NGF internalization via p75NGFR. *J. Biol. Chem.* **267,** 13,917–13,923.
125. Curtis R., Adryan K. M., Stark J. L., Park J. S., Compton D. L., Weskamp G., et al. (1995) Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron* **14,** 1201–1211.
126. Gargano N., Levi A., and Alema S. (1997) Modulation of nerve growth factor internalization by direct interaction between p75 and TrkA receptors. *J. Neurosci. Res.* **50,** 1–12.
127. Kahle P., Barker P. A., Shooter E. M., and Hertel C. (1994) p75 nerve growth factor receptor modulates p140trkA kinase activity, but not ligand internalization, in PC12 cells. *J. Neurosci. Res.* **38,** 599–606.

128. Eveleth D. D. and Bradshaw R. A. (1992) Nerve growth factor nonresponsive pheochromocytoma cells: altered internalization results in signaling dysfunction. *J. Cell Biol.* **117,** 291–299.
129. Saragovi H. U., Zheng W., Maliartchouk S., DiGugliemo G. M., Mawal Y. R., Kamen A., et al. (1998) A TrkA-selective, fast internalizing nerve growth factor-antibody complex induces trophic but not neuritogenic signals. *J. Biol. Chem.* **273,** 34,933–34,940.
130. Zhang Y. z., Moheban D. B., Conway B. R., Bhattacharyya A., and Segal R. A. (2000) Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. *J. Neurosci.* **20,** 5671–5678.
131. Kryl D., Yacoubian T., Haapasalo A., Castrén E., Lo D., and Barker P. A. (1999) Subcellular localization of full-length and truncated Trk receptor isoforms in polarized neurons and epithelial cells. *J. Neurosci.* **19,** 5823–5833.
132. Drake C. T., Milner T. A., and Patterson S. L. (1999) Ultrastructural localization of full-length trkB immunoreactivity in rat hippocampus suggests multiple roles in modulating activity-dependent synaptic plasticity. *J. Neurosci.* **19,** 8009–8026.
133. Claude P., Hawrot E., and Parada I. (1982) Ultrastructural studies on the intracellular fate of ^{125}I-nerve growth factor in cultured rat sympathetic neurons. *J. Cell. Biochem.* **20,** 1–13.
134. Sandow S. L., Heydon K., Weible M. W. 2nd, Reynolds A. J., Bartlett S. E., and Hendry I. A. (2000) Signalling organelle for retrograde axonal transport of internalized neurotrophins from the nerve terminal. *Immunol. Cell Biol.* **78,** 430–435.
135. Sommerfeld M. T., Schweigreiter R., Barde Y. A., and Hoppe E. (2000) Down-regulation of the neurotrophin receptor TrkB following ligand binding. Evidence for an involvement of the proteasome and differential regulation of TrkA and TrkB. *J. Biol. Chem.* **275,** 8982–8990.
136. Seidah N. G., Benjannet S., Pareek S., Savaria D., Hamelin J., Goulet B., et al. (1996) Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. *Biochem. J.* **314,** 951–960.
137. Mowla S. J., Farhadi H. F., Pareek S., Atwal J. K., Morris S. J., Seidah N. G., et al. (2001) Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J. Biol. Chem.* **276,** 12,660–12,666.
138. Hammerschlag R., Stone G. C., Bolen F. A., Lindsey J. D., and Ellisman M. H. (1982) Evidence that all newly synthesized proteins destined for axonal transport pass through the Golgi apparatus. *J. Cell Biol.* **93,** 568–575.
139. Bernd P. and Greene L. A. (1983) Electron microscopic radioautographic localization of iodinated nerve growth factor bound to and internalized by PC12 cells. *J. Neurosci.* **3,** 631–643.
140. Claude P., Hawrot E., Dunis D. A., and Campenot R. B. (1982) Binding, internalization, and retrograde transport of ^{125}I-nerve growth factor in cultured rat sympathetic neurons. *J. Neurosci.* **2,** 431–442.
141. Hogue-Angeletti R., Stieber A., and Gonatas N. K. (1982) Endocytosis of nerve growth factor by PC12 cells studied by quantitative ultrastructural autoradiography. *Brain Res.* **241,** 145–156.
141a. Rohrer H., Schafer T., Korsching S., and Thoenen H. (1982) Internalization of nerve growth factor by pheochromocytoma PC12 cells: absence of transfer to the nucleus. *J. Neurosci.* **2,** 687–697.
142. Stieber A., Hickey W. F., Hogue-Angeletti R., and Gonatas N. K. (1984) Endocytosis of nerve growth factor by `differentiated' PC12 cells studied by quantitative ultrastructural autoradiography. *Brain Res.* **310,** 223–234.
143. Bos K., Wraight C., and Stanley K. K. (1993) TGN38 is maintained in the trans-Golgi network by a tyrosine-containing motif in the cytoplasmic domain. *EMBO J.* **12,** 2219–2228.
144. Felder S., Miller K., Moehren G., Ullrich A., Schlessinger J., and Hopkins C. R. (1990) Kinase activity controls the sorting of the epidermal growth factor receptor within the multivesicular body. *Cell* **61,** 623–634.
145. Pavelka M., Ellinger A., Debbage P., Loewe C., Vetterlein M., and Roth J. (1998) Endocytic routes to the Golgi apparatus. *Histochem. Cell Biol.* **109,** 555–570.
146. Mallet W. G. and Maxfield F. R. (1999) Chimeric forms of furin and TGN38 are transported with the plasma membrane in the trans-Golgi network via distinct endosomal pathways. *J. Cell Biol.* **146,** 345–359.
147. Chapman R. E. and Munro S. (1994) Retrieval of TGN proteins from the cell surface requires endosomal acidification. *EMBO J.* **13,** 2305–2312.
148. Mellman I. (1996) Endocytosis and molecular sorting. *Annu. Rev. Cell. Dev. Biol.* **12,** 575–625.

149. Opresko L. K., Chang C. P., Will B. H., Burke P. M., Gill G. N., and Wiley H. S. (1995) Endocytosis and lysosomal targeting of epidermal growth factor receptors are mediated by distinct sequences independent of the tyrosine kinase domain. *J. Biol. Chem.* **270,** 4325–4333.
150. Overly C. C. and Hollenbeck P. J. (1996) Dynamic organization of endocytic pathways in axons of cultured sympathetic neurons. *J. Neurosci.* **16,** 6056–6064.
151. Strous G. J. and Govers R. (1999) The ubiquitin-proteasome system and endocytosis. *J. Cell Sci.* **112,** 1417–1423.
152. von Bartheld C. S. and Butowt R. (2000) Expression of neurotrophin-3 (NT-3) and anterograde axonal transport of endogenous NT-3 by retinal ganglion cells in chick embryos. *J. Neurosci.* **20,** 736–748.
153. Griesbeck O., Canosa M., Campana G., Gärtner A., Hoener M. C., Nawa H., et al. (1999) Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. *Microsc. Res. Tech.* **45,** 262–275.
154. Haubensak W., Narz F., Heumann R., and Lessmann V. (1998) BDNF-GFP containing secretory granules are localized in the vicinity of synaptic junctions of cultured cortical neurons. *J. Cell Sci.* **111,** 1483–1493.
155. Möller J. C., Krüttgen A., Heymach J. V. Jr., Ghori N., and Shooter E. M. (1998) Subcellular localization of epitope-tagged neurotrophins in neuroendocrine cells. *J. Neurosci. Res.* **51,** 463–472.
156. Gärtner A., Shostak Y., Hackel N., Ethell I. M., and Thoenen H. (2000) Ultrastructural identification of storage compartments and localization of activity-dependent secretion of neurotrophin 6 in hippocampal neurons. *Mol. Cell. Neurosci.* **15,** 215–234.
157. Goldstein L. S. and Yang Z. (2000) Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annu. Rev. Neurosci.* **23,** 39–71.
158. Yano H., Lee F. S., Kong H., Chuang J. Z., Arevalo J. C., Perez P., et al. (2001) Association of trk neurotrophin receptors with components of the cytoplasmic dynein motor. *J. Neurosci.* **21,** RC125:1–7.
159. Vallee R. B. and Bloom G. S. (1991) Mechanisms of fast and slow axonal transport. *Annu. Rev. Neurosci.* **14,** 59–92.
160. von Bartheld C. S., Williams R., Lefcort F., Clary D. O., Reichardt L. F., and Bothwell M. (1996) Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75^{NTR} and trkB receptors. *J. Neurosci.* **16,** 2995–3008.
161. Blöchl A. and Thoenen H. (1996) Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. *Mol. Cell. Neurosci.* **7,** 173–190.
162. Goodman L. J., Valverde J., Lim F., Geschwind M. D., Federoff H. J., Geller A. I., et al. (1996) Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. *Mol. Cell. Neurosci.* **7,** 222–238.
163. Nieto-Bona M. P., Busiguina S., and Torres-Aleman I. (1995) Insulin-like growth factor I is an afferent trophic signal that modulates calbindin-28kD in adult Purkinje cells. *J. Neurosci. Res.* **42,** 371–376.
164. Nieto-Bona M. P., Garcia-Segura L. M., and Torres-Aleman I. (1997) Transynaptic modulation by insulin-like growth factor I of dendritic spines in Purkinje cells. *J. Dev. Neurosci.* **15,** 749–754.
165. Burgess T. L. and Kelly R. B. (1987) Constitutive and regulated secretion of proteins. *Annu. Rev. Cell Biol.* **3,** 243–293.
166. Bartfai T., Iverfeldt K., Fisone G., and Serfözö P. (1988) Regulation of the release of coexisting neurotransmitters. *Annu. Rev. Pharmacol. Toxicol.* **28,** 285–310.
167. Blöchl A. and Thoenen H. (1995) Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. *Eur. J. Neurosci.* **7,** 1220–1228.
168. Hoener M. C. (2000) Role played by sodium in activity-dependent secretion of neurotrophins – revisited. *Eur. J. Neurosci.* **12,** 3096–3106.
169. Edwards R. H., Selby M. J., Mobley W. C., Weinrich S. L., Hruby D. E., and Rutter W. J. (1998) Processing and secretion of nerve growth factor: expression in mammalian cells with a vaccinia virus vector. *Mol. Cell. Biol.* **8,** 2456–2464.
170. Heymach J. V. Jr., Krüttgen A., Suter U., and Shooter E. M. (1996) The regulated secretion and vectorial targeting of neurotrophins in neuroendocrine and epithelial cells. *J. Biol. Chem.* **271,** 25,430–25,437.

171. Nomoto H., Tomotoshi K., Ito H., and Furukawa S. (2000) Balance of two secretion pathways of nerve growth factor in PC12 cells changes during the progression of their differentiation, with a decrease in constitutive secretion in more differentiated cells. *J. Neurosci. Res.* **59,** 632–642.
172. Morris S. J., Laliberté J., Mowla S. W., and Murphy R. A. (2000) The molecular determinants of neurotrophin sorting. *Soc. Neurosci. Abstr.* **26,** 1900.
173. Mannion R. J., Costigan M., Decosterd I., Amaya F., Ma Q. P., Holstege J. C., et al. (1999) Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity. *Proc. Natl. Acad. Sci. USA* **96,** 9385–9390.
174. Hille B., Billiard J., Babcock D. F., Nguyen T., and Koh D. S. (1999) Stimulation of exocytosis without a calcium signal. *J. Physiol.* **520,** 23–31.
175. Kasai H. (1999) Comparative biology of Ca^{2+}-dependent exocytosis: implications of kinetic diversity for secretory function. *Trends Neurosci.* **22,** 88–93.
176. Balkowiec A. and Katz D. M. (2000) Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. *J. Neurosci.* **20,** 7417–7423.
177. Schinder A. J. and Poo M. M. (2000) The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci.* **23,** 639–645.
178. Androutsellis-Theotokis A., McCormack W. J., Bradford H. F., Stern G. M., and Pliego-Rivero F. B. (1996) The depolarisation-induced release of [^{125}I]BDNF from brain tissue. *Brain Res.* **743,** 40–48.
179. Kafitz K. W., Rose C. R., Thoenen H., and Konnerth A. (1999) Neurotrophin-evoked rapid excitation through TrkB receptors. *Nature* **401,** 918–921.
180. Aoki C., Wu K., Elste A., Len G. W., Lin S. Y., McAuliffe G., et al. (2000) Localization of brain-derived neurotrophic factor and TrkB receptors to postsynaptic densities of adult rat cerebral cortex. *J. Neurosci. Res.* **59,** 454–463.
181. Fryer R. H., Kaplan D. R., Feinstein S. C., Radeke M. J., Grayson D. R., and Kromer L. F. (1996) Developmental and mature expression of full-length and truncated TrkB receptors in the rat forebrain. *J. Comp. Neurol.* **374,** 21–40.
182. Garcia-Segura L. M., Rodriguez J. R., and Torres-Aleman I. (1997) Localization of the insulin-like growth factor I receptor in the cerebellum and hypothalamus of adult rats: an electron microscopic study. *J. Neurocytol.* **26,** 479–490.
183. Lin S. Y., Wu K., Levine E. S., Mount H. T., Suen P. C., and Black I. B. (1998) BDNF acutely increases tyrosine phosphorylation of the NMDA receptor subunit 2B in cortical and hippocampal postsynaptic densities. *Mol. Brain Res.* **55,** 20–27.
184. Wu K., Xu J.-l., Suen P. C., Levine E., Huang Y. y., Mount H. T. J., et al. (1996) Functional trkB neurotrophin receptors are intrinsic components of the adult brain postsynaptic density. *Mol. Brain Res.* **43,** 286–290.
185. Snider W. D. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77,** 627–638.
186. Black I. B. (1999) Trophic regulation of synaptic plasticity. *J. Neurobiol.* **41,** 108–118.
187. Lohof A. M., Ip N. Y., and Poo M. M. (1993) Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363,** 350–353.
188. Lu B. and Figurov A. (1997) Role of neurotrophins in synapse development and plasticity. *Rev. Neurosci.* **8,** 1–12.
189. Poo M. M. (2001) Neurotrophins as synaptic modulators. *Nature Rev. Neurosci.* **2,** 24–32.
190. Schuman E. M. (1999) Neurotrophin regulation of synaptic transmission. *Curr. Opin. Neurobiol.* **9,** 105–109.
191. Thoenen H. (1995) Neurotrophins and synaptic plasticity. *Science* **270,** 593–598.
192. Torres-Aleman I. (1999) Insulin-like growth factors as mediators of functional plasticity in the adult brain. *Horm. Metab. Res.* **31,** 114–119.
193. Snider W. D. and Lichtman J. W. (1996) Are neurotrophins synaptotrophins? *Mol. Cell. Neurosci.* **7,** 433–442.
194. Wang X. H. and Poo M. M. (1997) Potentiation of developing synapses by postsynaptic release of neurotrophin-4. *Neuron* **19,** 825–835.
195. Haymon S. M., Tartaglia N., Du J., Tyler W. J., Neale E., Lu B., et al. (2000) BDNF increases the number of docked vesicles and the expression levels of synaptic vesicle proteins in hippocampal slices. *Soc. Neurosci. Abstr.* **26,** 880.
196. Wang X. X., Butowt R., and von Bartheld C. S. (2000) Effects of NT-3 on the number, size and vesicle density of retinotectal synapses in chick embryos. *Soc. Neurosci. Abstr.* **26,** 843.
197. Canossa M., Griesbeck O., Berninger B., Campana G., Kolbeck R., and Thoenen H. (1997)

Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. *Proc. Natl. Acad. Sci. USA* **94,** 13,279–13,286.
198. Krüttgen A., Möller J. C., Heymach J. V. Jr., and Shooter E. M. (1998) Neurotrophins induce release of neurotrophins by the regulated secretory pathway. *Proc. Natl. Acad. Sci. USA* **95,** 9614–9619.
199. Vaughn J. E. (1989) Fine structure of synaptogenesis in the vertebrate central nervous system. *Synapse* **3,** 255–285.
200. McAllister A. K., Katz L. C., and Lo D. C. (1999) Neurotrophins and synaptic plasticity. *Annu. Rev. Neurosci.* **22,** 295–318.
201. Clarke P. G. H. (1991) The roles of trophic communication in biological neural networks. *Concepts Neurosci.* **2,** 201–219.
202. Galli-Resta L. and Resta G. (1992) A quantitative model for the regulation of naturally occurring cell death in the developing vertebrate nervous system. *J. Neurosci.* **12,** 4586–4594.
203. Pollock G. S., Vernon E., Forbes M. E., Yan Q., Ma Y.-T., Hsieh T., et al. (2001) Effects of early visual experience and diurnal rhythms on BDNF mRNA and protein levels in the visual system, hippocampus and cerebellum. *J. Neurosci.* **21,** 3923–3931.
204. Saper C. B., Wainer B. H., and German D. C. (1987) Axonal and transneuronal transport in the transmission of neurological disease: potential role in system degenerations, including Alzheimer's disease. *Neuroscience* **23,** 389–398.
205. Korsching S. (1993) The neurotrophic factor concept: a reexamination. *J. Neurosci.* **13,** 2739–2748.
206. Nawa H., Carnahan J., and Gall C. (1995) BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. *Eur. J. Neurosci.* **7,** 1527–1535.
207. Nixon R. A. and Cataldo A. M. (1995) The endosomal-lysosomal system of neurons: new roles. *Trends Neurosci.* **18,** 489–496.
208. Levy J. R. and Olefsky J. M. (1987) The trafficking and processing of insulin and insulin receptors in cultured rat hepatocytes. *Endocrinology* **121,** 2075–2086.
209. Schäfers M., Geis C., Brors D., Yaksh T.L., and Sommer C. (2001) Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve. *J. Neurosci.* (in press).
210. Schwartz P. M., Borghesani P. R., Levy R. L., Pomeroy S. L., and Segal R. A. (1997) Abnormal cerebellar development and foliation in BDNF$^{-/-}$mice reveals a role for neurotrophins in CNS patterning. *Neuron* **19,** 269–281.