Molecular Neurobiology

ISSN0893-7648/00/21(3): 109–135/$16.75

Regulation of Neurotrophin Signaling in Aging Sensory and Motoneurons

Dissipation of Target Support?

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Abstract

A hallmark of senescence is sensorimotor impairment, involving locomotion and postural control as well as fine-tuned movements. Sensory and motoneurons are not lost to any significant degree with advancing age, but do show characteristic changes in gene-expression pattern, morphology, and connectivity. This review covers recent experimental findings corroborating that alterations in trophic signaling may induce several of the phenotypic changes seen in primary sensory and motoneurons during aging. Furthermore, the data suggests that target failure, and/or breakdown of neuron-target interaction, is a critical event in the aging process of sensory and motoneurons.

Index Entries: Aging; glial cell line-derived neurotrophic factor; nerve growth factor; brain-derived neurotrophic factor; neurotrophin-3; neurotrophin-4; trk receptor; $p75^{NTR}$; ret; GFR.

Sensorimotor Impairment During Aging

Aging is closely associated with sensorimotor impairment, which seriously compromises the daily behavioral activities of the elderly *(1–3).* Aberrations in sensory mechanism(s), changes in motor innervation, and muscle contraction speed and force are all important bits and pieces in the emergence of gait disturbances, deficits in postural control, and muscle weakness in senescence. Senile muscle atrophy is common, and changes in gait as well as balance control correlate with increased thresholds for exteroceptive and proprioceptive sensations in aged animals and humans *(1,4–13).*

Skin and skeletal muscles, targets of sensory and motoneurons, also show characteristic

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stigmata of aging. The skin becomes atrophic (epidermis) and the composition of dermis changes. Both sweat-gland function and skin perfusion decline with advancing age *(14–16).* In parallel there is a selective loss of epidermal and dermal innervation, involving both sensory and autonomic components *(15–24).* In senescence, impaired exteroception will compromise motor behavior, while decline in sweat-gland function and skin perfusion will have a negative impact on wound healing and body-temperature control *(14,25–27).*

Aging-related decrease of muscle strength and velocity has been attributed to "senile muscle atrophy" *(4,28,29).* Skeletal muscles of aged humans and rodents do almost invariably show a considerable wasting, caused by fiber loss and fiber atrophy. In parallel there is an increase in connective tissue and fat *(28,30–33).* The pattern of changes seen in aging muscles has led to the suggestion that senile muscle atrophy is of neurogenic origin. For a long time, loss of innervation was considered a consequence of an almost "facultative" loss of neurons with advancing age. A growing body of evidence clearly shows that this is not what is happening; neuron loss is small overall *(34–40).* Considering sensory and motoneurons, available data show that the loss is quite small even in advanced age (10–15%) *(41–47)* and, furthermore, show no clear-cut association with functional decline among the individuals. Instead, aging is manifested by loss of neuronal connections, axon dystrophy, myelin aberrations, phenotypic changes in gene-expression pattern, and neuron atrophy in certain cell populations. Thus, a key issue is: if the neurons are still there, why don't they remain connected? Is it due to processes intrinsic to the neurons? What is the role of the target cell(s) in this process? This review summarizes experimental data corroborating that changes in trophic signaling may trigger phenotypic changes occurring in senescent sensory and motoneurons, and that target failure, or a breakdown in nerve-target interaction, probably is a critical event in this process.

A range of molecules can act as growth factors, however, in this review we will focus on two families described in Fig. 1, namely, the nerve growth factor family of ligands (neurotrophins; NTs) and the glial cell line-derived neurotrophic factor family of ligands (GDNF family).

Aging Sensory Neurons

Loss of Primary Sensory Neurons Cannot Explain Sensory Deficits in Senescence

Primary sensory neurons (PSN) show a complex pattern of changes at the cellular level during aging. There is a small unselective loss of neurons, selective cell-body atrophy, abundant axon aberrations, and phenotypic alterations in the expression pattern of neuropeptides and neurotrophic receptors *(24,43,48–55).* The loss of sensory neurons during aging affects the populations of the small (giving rise to either C or Aδ axons) as much as the large (extending large myelinated Aα/β axons) sensory neurons (Fig. 2), and there is no apparent correlation between neuron loss and behavioral deficit among the individuals *(43).* Thus, cell loss itself cannot explain the sensory deficits seen in the elderly. Large PSN, however, show overt signs of axon and cell-body atrophy paralleled by a decreased expression of neurofilaments, indicating that they are more affected by aging than small PSN (Fig. 2) *(43,56–58).* It is well-established that aging is associated with axon aberrations, such as axon atrophy and dystrophy, de-/dysmyelination, and signs of degeneration in peripheral nerves and spinal roots (*59,60,* and references therein). Although axon aberrations are evident in unmyelinated as well as myelinated axons, the latter group is much more affected. The loss of axons is, however, small and compares well with the figures available for loss of PSN cell bodies in the dorsal root ganglia (DRG) *(43,53,60).* In contrast, the peripheral innervation and the central termination of sensory axons are markedly reduced *(16,24,53).* The attenuation of sensory innerva-

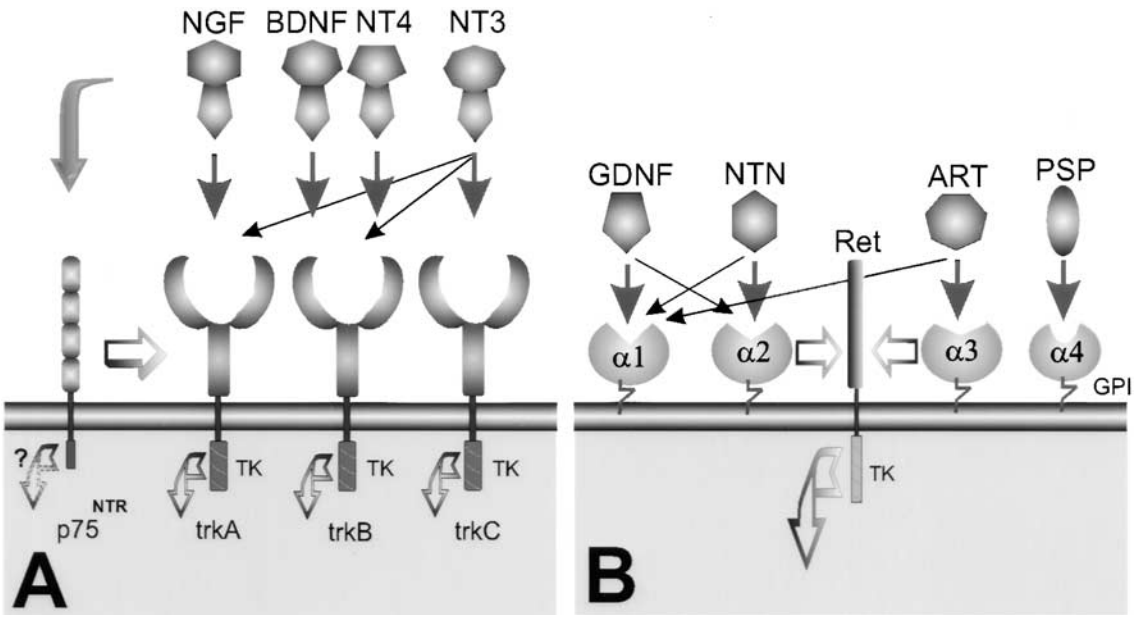

Fig. 1. **(A)** The neurotrophins (NTs) are a family of structurally and functionally related proteins that consists of nerve growth factor (NGF; *199*), brain-derived neurotrophic factor (BDNF; *200,201*), neurotrophin-3 (NT3; *202–204*), and neurotrophin-4 (NT4; *163,205,206*). The neurotrophins exert their effects by binding to specific receptors (reviewed in *131,207*). All neurotrophins bind to the p75 neurotrophin receptor (p75NTR; *208–210*). In addition, each neurotrophin binds to members of the tropomyosin receptor kinase (trk) family of tyrosine kinase receptors; trk A is the preferred receptor for NGF *(211,212),* while both BDNF and NT4 bind to trkB *(213,214).* NT3 interacts primarily with trkC *(215)* but also with trkA and trkB *(213,214,216).* The specificity of neurotrophin effects and the vast majority of biological responses are mediated through trk-receptor signaling *(131,207,217,218).* Noncatalytic isoforms, lacking the intracellular tyrosine kinase domain, are also expressed by neurons and glia cells (trkB; *219,220*). Truncated trk receptors may act as negative regulators of neurotrophin-receptor function *(221)* or to restrict the diffusion of cognate ligand from sites of release *(222).* **(B)** The GDNF family of ligands, member of the transforming growth factor-β (TGF-β) superfamily, contains the glial cell line-derived neurotrophic factor (GDNF; *223*), neurturin (NTN; *224*), artemin *(225),* and persephin *(226).* GDNF was identified as a very potent trophic factor for dopaminergic and motoneurons, but has also been shown to promote survival and differentiation of sensory and autonomic neurons *(136,223,227–232).* The GDNF family of ligands signal through a multicomponent-receptor system, with the transmembrane tyrosine kinase receptor RET as the common signal-transducing component *(233,234).* Ligand preference and binding of RET is accomplished by high-affinity binding of GDNF family ligands to a family of glycosyl-phosphatidyl inositol- (GPI-) anchored proteins (GFRα1-4) *(235–242).* Similar to the neurotrophin receptors, GDNF family ligand receptors seem to be positively regulated by the amounts of accessible ligand *(243,244).*

tion more extensively involves terminal branches of myelinated proprioceptive fibers than unmyelinated nociceptive fibers (Fig. 3) (17, and references cited above). Consistent with this pattern of changes, aging individuals are more affected by disturbed proprioception than by changes in nociception (reviewed by e.g., *1*). From a morphological viewpoint, these data also imply that aging affects neurons in a distal-to-proximal process, being most marked and, perhaps initiated, in the axon domains of the peripheral and central target regions.

Pattern of Neuropeptide Expression Changes During Aging

PSN synthesize neuromodulatory peptides *(61–63)* such as calcitonin gene-related peptide

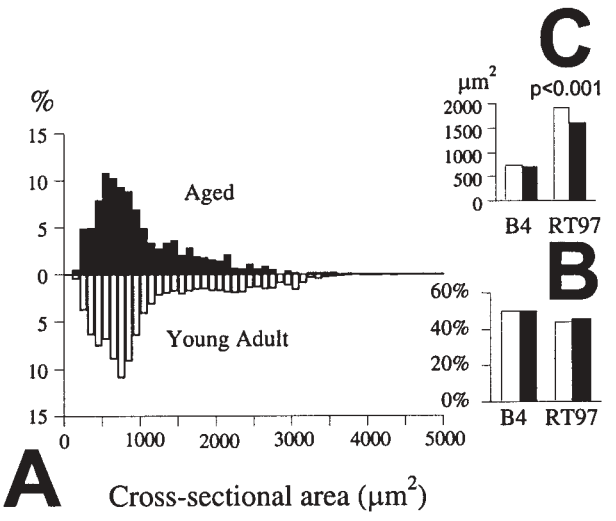

Fig. 2. **(A)** The number of PSN decrease by about 10–15% in senescence. PSN cell-size distribution shows a shift towards smaller cell categories of aged rats (data replotted from *43*). This may be interpreted either as a selective loss of large PSN or that cell loss is unselective while large PSN become atrophic. **(B)** By examining the distribution of B4 *(245)* and RT97 *(246),* as selective markers for small unmyelinated and large myelinated PSN, respectively, no change in the relative frequencies of B4- or RT97-positive PSN were evident with advancing age. **(C)** In contrast, RT97-, but not B4-positive, PSN were found to have a significantly smaller cross-sectional area in aged rats. Combined, the data indicates that cell loss is unselective while cell atrophy is selectively affecting large myelinated PSN.

(CGRP), substance P (SP) and galanin. Upon release, neuropeptides subserve neuromodulatory functions both at the central and the peripheral terminations (reviewed by *61, 64, 67–71*) and the expression of neuropeptides in sensory neurons is influenced by several factors. PSN axon crush or transection, induces a downregulation of CGRP and SP, whereas galanin and neuropeptide tyrosine (NPY) are upregulated (reviewed in *65*). This pattern of neuropeptide regulation has been considered of importance for adaptation to the deficits induced by lesioning PSN in adult rats.

PSN of aged rats disclosed a dramatic decrease in CGRP expression affecting mainly small (B4 binding) PSN *(49,52).* In the same subpopulation of sensory neurons there is also a downregulation of SP, although less marked. In large myelinated (RT97 positive) PSN of the same individuals, both a novel expression of galanin and a robust upregulation of NPY are evident *(49,52).* The downregulation of, in particular, CGRP but also SP in small nociceptive PSN may have relevance for the observed increase in nociceptive threshold in senescence *(43),* because NGF-induced hyperalgesia in young animals has been associated with upregulation of CGRP and SP *(66).* It is well established that stimulation of primary afferents can induce cutaneous vasodilatation and plasma extravasation, mediated by SP and CGRP in the cutaneous axon reflex *(67–70).* In this context, the findings of a decrease in CGRP in perivascular axons, as well as a decrease in CGRP and SP, in primary sensory neurons of aged rats, fit well with previous descriptions of a weakened axon reflex in senescence *(14,24,49,51,71).* A diminished axon reflex will have a negative impact on inflammatory responses and wound healing, i.e., well-established traits of aging (for references, *see* above). Large myelinated PSN in the aged animal and axotomized adults show similarities, with an increase of galanin and NPY, possibly relating to the emergence of axon lesions during aging.

Decreased Neurotrophin-trk Signaling in Senescence

Accumulating evidence indicates disturbed neurotrophin-trk signaling as a mechanism in peripheral neuropathies and aging-related aberrations (reviewed by *72,73–76; see* also Fig. 1). In the aged rat, the characteristic size distribution of trk-expressing primary sensory neurons is maintained, with a selective expression of trkA and trkC in small and large PSN neurons, respectively, and trkB expressed by neurons of all size categories *(49,51).* However, the level of trk expression (protein and mRNA) in aged sensory neurons is downregulated and, furthermore, a covariation between the

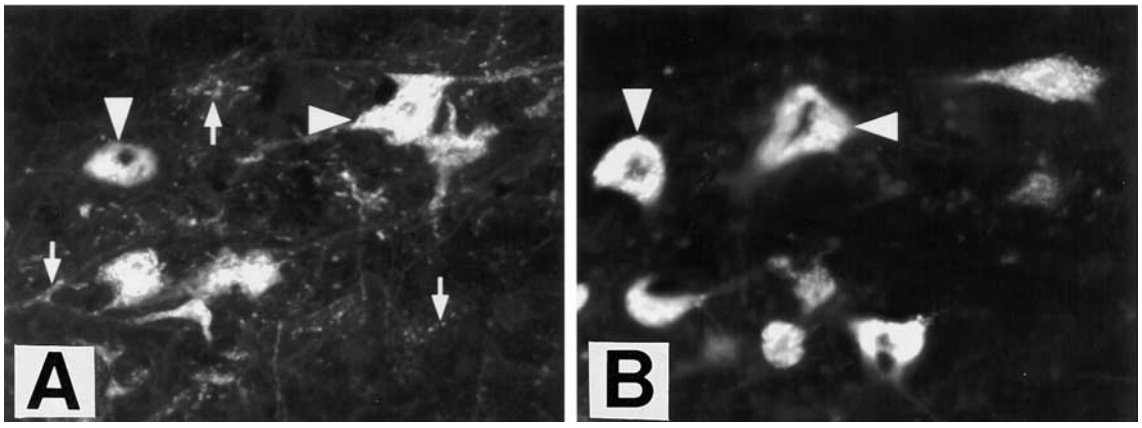

Fig. 3. Injection of the lectin cholera toxin B subunit (CTB), that binds and is transported in large mechanoreceptive PSN *(246),* into peripheral nerves of aged rats revealed a profound decrease of large PSN fibers and terminals in the dorsal horn and motor nuclei of the spinal cord (Bergman et al., unpublished). **(A)** and **(B)** show images from the lumbar motor nucleus from adult (A) and aged (B) rats. Note the similar labeling of large, presumably, α-motoneurons in (A) and (B) (arrowheads), while the network of muscle spindle (Ia) afferent fibers and terminals (arrows in A) is markedly attenuated in B.

distribution of symptoms and trk-downregulation is evident with the sensory neurons innervating the hind limbs being more affected than those of the face (Fig. 4)*(51).*

Lesion experiments and transgenic animals have shown that target-derived neurotrophins can regulate the cognate trk receptor expression in primary sensory neurons both during development and in adulthood ("feed forward regulation," reviewed by *77–86*). Thus, because trk downregulation in aging PSN may reflect a failure of targets to synthesize neurotrophic factors (*see* also *57,87–89*), we examined the expression of neurotrophins in peripheral target tissues of aged rats. In target muscles, a downregulation of all neurotrophin mRNAs was observed (Fig. 5) and, notably, the decrease co-varied with the extent of behavioral sensorimotor disturbances among the individuals *(55).* These findings imply an attenuated neurotrophin signaling between target tissues and PSN in senescence. In order to resolve if sensory neurons remain dependent on the target for their expression of trk receptors in senescence, we transected peripheral nerves

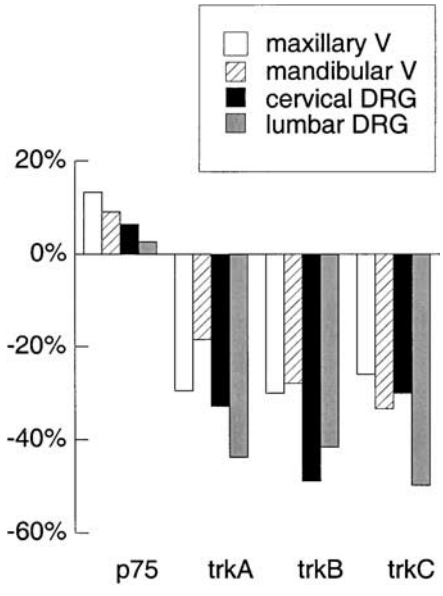

Fig. 4. Changes in expression levels of mRNA for neurotrophin receptors in PSN of aged rats as revealed by *in situ* hybridization (data replotted from *51*). Column height indicates the mean labeling density ratio of aged over young adult rats. Key to the different locations is present in framed area.

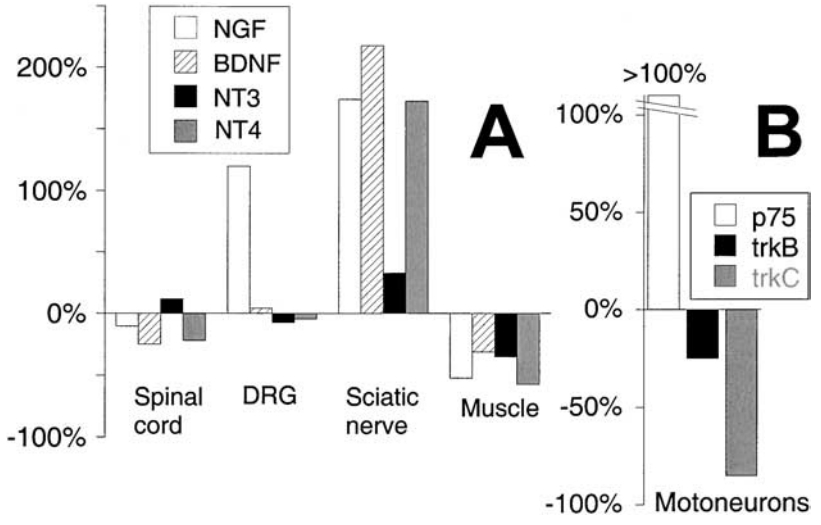

Fig. 5. **(A)** Expression of NGF, BDNF, NT3, and NT4 mRNAs in different tissue regions of aged rats (RT-PCR data replotted from *55*). The data are presented as the percentage difference between aged and adult rats. **(B)** Changes in expression of neurotrophin receptor mRNAs in lumbar motoneurons of aged rats as revealed by *in situ* hybridization. (Data replotted from *186.*) Column height indicates labeling ratio of aged over young adult rats.

and examined the expression level of trk receptors in PSN *(51).* It is well-established that disruption of the target contacts in adulthood induces typical phenotypic changes in PSN including a downregulation of trk receptors and an upregulation of neurotrophins in the target tissues *(84,85,90–92).* In aged PSN, axonal severance was found to induce a further downregulation of trk receptors and, furthermore, the expression level of neurotrophin receptors was found to be very similar in adult and aged rat PSN following axotomy *(51).* One interpretation of the latter (observation) is that the residual expression of trks following target-tissue disconnection depends on other signals possibly provided through auto/paracrine neurotrophin loops *(93)* or via the central connections of PSN. If this is the case, then we may conclude that this nontarget tissue-dependent expression of trks is unchanged in senescence. With the exception of an upregulation of NGF in DRGs, this notion is consistent with the observed lack of significant changes in neurotrophin expression in both DRGs and the spinal cord in senescence (Fig. 5).

Expression of Neurotrophins and trks Increases in Peripheral Nerves During Aging

In the peripheral nerve, there is a significant upregulation of all neurotrophin mRNAs except NT3 during aging (Fig. 5). This pattern of regulation bears strong resemblance to that observed in lesioned nerves of adult rats, where the upregulation of neurotrophins has been suggested to take place mainly in Schwann cells and to play a role in the regenerative processes of sensory neurons *(90–92,94).* However, the increased levels of neurotrophins in the nerve do not appear to affect the typical phenotypic switches occurring in PSN following axotomy or during aging *(55,83,85).* Thus, it seems that target-derived, but not Schwann cell-derived, neurotrophins can regulate trk-receptor expression in PSN. Of relevance in

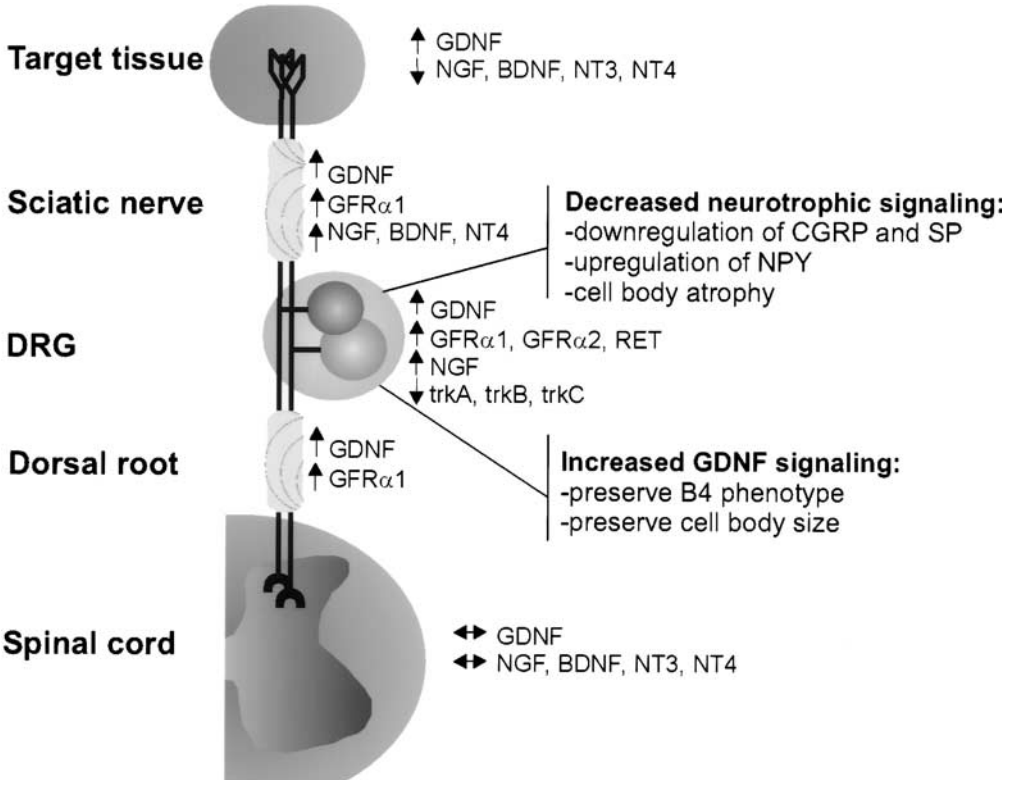

Fig. 6. Schematic drawing summarizing the regulation of neurotrophic (neurotrophins and GDNF family ligands) factors and their receptor compliments in PSN, supportive and target tissues. The changes are indicated by arrows as follows: upregulation (↑), downregulation (↓), and no change (↔). (Modified from *54*.)

this context is that Schwann cells also increase their expression of neurotrophin receptors in response to nerve insult in adulthood *(91,95)*, and in accordance with this we have observed increased levels of trkA and trkB, but not trkC, in Schwann cells of aged rats (*48*). This may indicate that Schwann cell-derived neurotrophins have a predominantly local effect *(96,97)*.

What May Be the Consequences of a Decreased Neurotrophin-trk Signaling in Aging Sensory Neurons?

First of all, decreased neurotrophin-trk signaling may explain the changes in neuropeptide expression during aging. As mentioned previously, decreased NGF-trkA signaling seems to lower the expression of CGRP and SP in small nociceptive PSN (*85*). The changed levels of these peptides may not only contribute to the increase in nociceptive threshold during aging but may also be mechanistic in the weakening of the axon reflex (*25,27*). NGF signaling also appears to be able to suppress galanin and NPY expression *(98)*, and NPY expression can, furthermore, be restrained by NT3 *(99)*. Thus, the regulatory changes in neuropeptide expression pattern during aging are fully consistent with a parallel decrease of neurotrophin-trk signaling (Fig. 6).

NPY upregulation in large myelinated PSN following axon lesioning in adult, and in non-manipulated aged rats, may share a common

mechanism in a decreased NT3-trkC signaling. During aging there is a slowing of axon conduction velocity of fast-conducting Aα/Aβ PSN *(100,101).* This fits well with the abundant morphological aberrations among large myelinated sensory axons in senescence. In adulthood, NT3 has been shown to play a role in the maintenance of muscle-spindle afferents and exogenous NT3 can protect axon conduction of large myelinated axons *(102,103).* Furthermore, exogenous NT3 ameliorates the damaging effects of cisplatin and high-dose pyridoxin on proprioception *(104–107).* Recent evidence for NT3 impact on large proprioceptive sensory neurons in adulthood comes from data showing that NT3, but not BDNF or GDNF, can promote regenerative growth of such fibers in the dorsal column of the spinal cord *(108).* Although the cellular mechanism(s) by which NT3-trkC signaling protects large myelinated sensory neurons in the wide range of situations described here remains elusive, a common link may be that neurotrophins can regulate expression of cytoskeletal proteins in adult neurons *(57,109–112).* A reduced NT3-trkC signaling in senescence may therefore be of relevance for the axon/cell-body atrophy and decrease in neurofilament expression in large sensory neurons with advancing age *(43,51,57).*

Does Preserved p75^{NTR} Expression and Increased GDNF Signaling Protect Nociception in Senescence?

In contrast to trk receptors, the expression of p75^{NTR} (mRNA and protein) is slightly increased in senescence (Fig. 4). The functional consequence of an increased/sustained p75^{NTR} expression is far from straightforward considering the multitude of possible functions ascribed this receptor. p75^{NTR} is not required for neurotrophin signaling *(113)* and, moreover, whereas the p75^{NTR} null mutant mice may have a normal life span *(114),* trk null mutations are lethal (for references, *see* above). However, experimental evidence has implicated p75^{NTR} in a number of functions related to neurotrophin-trk signaling *(115–120).* Moreover, p75^{NTR} null mutated mice appear equipped with a reduced set of primary sensory and motoneurons *(114,121,122),* corroborating that p75^{NTR} plays a significant role for neuron survival in vivo. A number of studies have shown that p75^{NTR} may signal independently of trk receptors, possibly mediating responses ranging from apoptosis to cell migration and differentiation *(96,123–130).* Although p75^{NTR} may activate cell-death pathways also in PSN under certain circumstances, the net effect in vivo of p75^{NTR} expression appears to be promotion of survival in PSN (*see* above). An increased p75^{NTR}:trk ratio could possibly represent a compensatory mechanism for the decreased availability of target-derived neurotrophins in aged rats, by means of a positive effect of p75^{NTR} on formation of high-affinity binding sites, internalization and retrograde transport of neurotrophins, or possibly by increasing the selectivity of trk receptors for their preferred ligand(s) *(131–133).* That p75^{NTR} knockout mice have an altered response to NGF, but not to other neurotrophins, suggests an effect of p75^{NTR} primarily on nociceptive trkA expressing sensory neurons *(121,134).* In these neurons then, the effects of a decrease in trkA may partly be compensated for by a preserved/increased p75^{NTR} expression, whereas no such effect would be anticipated for trkB or trkC expressing PSN. Thus, if this mechanism is in operation it could help to explain the more severe effect of aging on myelinated than unmyelinated PSN.

Still, the more successful aging of nociceptive than of proprioceptive PSN may be due to signaling of other neurotrophic factors in adulthood. It was recently shown by several groups that small PSN appear to acquire a dependence on GDNF-GFR/RET (Fig. 1) signaling during postnatal development and in adulthood *(135–138).* In aged rats, there is a significant upregulation of GFRα1 and RET (Fig. 7), while the characteristic distribution of these receptors in distinct populations of DRG neurons appears preserved *(50).* In parallel, GDNF expression is dramatically upregulated

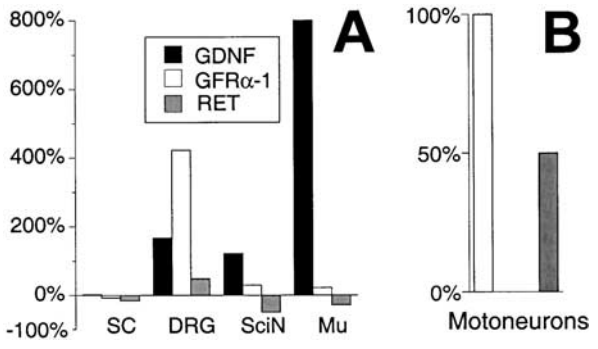

Fig. 7. **(A)** Expression of GDNF, GFRα-1 and RET mRNAs in different tissue regions of aged rats (RT-PCR data replotted from *55*). The data are presented as the percentage difference between aged and adult rats. **(B)** Changes in expression of GFRα-1 and RET mRNAs in lumbar motoneurons of aged rats as revealed by in situ hybridization. (Data replotted from *186*.) Column height indicates labeling ratio of aged over young adult rats.

in target muscles, and a smaller yet significant increase is evident in peripheral nerves, spinal-nerve roots, and DRGs of aged rats (Fig. 7) *(54)*. An increased GDNF-GFR/RET signaling during aging is of considerable interest, because GDNF can protect the B4 phenotype and the axon conduction velocity of small nociceptive PSN, whereas it has a negligible effect on CGRP expression or the slowing of axon conduction velocity in large myelinated fibers following axon lesioning *(103,135,136)*. Taken together, an increased GDNF-GFR/RET signaling and a decreased neurotrophin signaling combined may clarify many of the phenotypic changes characterizing aging PSN, and furthermore shed light on why myelinated are more severely affected than unmyelinated sensory neurons (Fig. 6).

The Complex Pattern of Trophic Signaling Between Target and Different Sets of Sensory Innervation Shows Distinct Changes in Senescence

From what has been discussed earlier, it may appear as changes in sensory neurons during aging follow a fairly simple mechanistic scheme. In contrast, studies of follicle-sinus complexes (FSCs) in mutated mice lacking either neurotrophins or neurotrophin receptors have revealed that each set of sensory nerve endings terminating in the skin requires a spatial and temporal unique combination of neurotrophin-trk receptor signaling to develop normally *(139–141)*. We therefore turned to the whisker FSCs of the mystacial pad to examine sensory nerve-target interaction during aging in closer detail.

In senescence, the mystacial-pad innervation shows pronounced aberrations, with an extensive but selective attenuation of, in particular, large-caliber mechanoreceptors innervating the FSC, the epidermis, and blood vessels *(24)*. The most detrimentally affected population of large-caliber mechanoreceptors is the grid of Merkel cell-neurite complexes (MCNC) located at the level of ring sinus in the FSC (Fig. 8). Interestingly, the MCNC located at the rete ridge collar of the FSC showed no sign of degeneration in the aged animal. The difference in susceptibility during aging may be explained by the fact that the MCNC located at the level of ring sinus, as apposed to those located at the rete ridge collar, are believed to have a high turnover owing to mechanical wear-and-tear. Furthermore, these endings also show a more complex ligand-receptor expression phenotype in the adult rat than those located at the rete ridge collar *(48)*. In senescence, there is a dramatic decline in the expression of NT3 in the Merkel cells located at the level of ring sinus with a concomitant downregulation of trkC in the large-caliber axons terminating as Merkel endings at this level. In contrast, the expression of trkA in the same axons is unaltered in the aged animal. Thus, the loss of MCNC at the level of ring sinus in the aged animal can spatially and temporally be correlated with the loss of Merkel cell-derived NT3 and the reduction of trkC in Merkel axons. To develop normally, MCNC depend on NT3 signaling through trkC *(140,142)* but also through trkA to promote terminal plasticity (Fig. 8; *48,140*). Thus, the failure in supporting the Merkel terminals results in

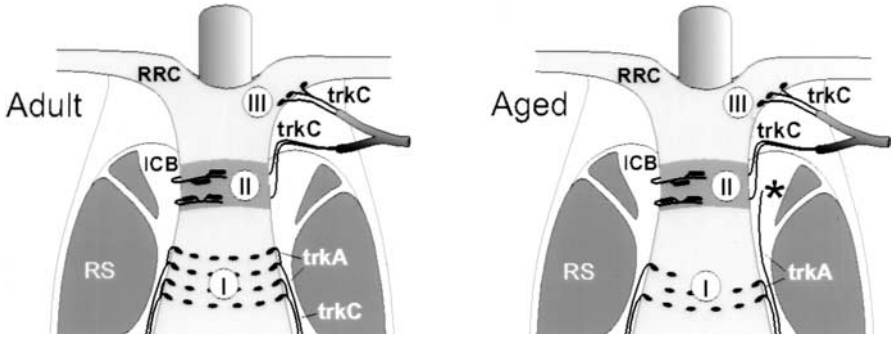

Fig. 8. Schematic illustration depicting the suggested mechanisms underlying the selective loss of Merkel cell-neurite complexes (MCNC) at the level of ring sinus and the concomitant growth of Merkel axons to adjacent targets. (Adapted from ref. 48.) **I.** The substantial reduction of NT3 in Merkel cells at the level of ring sinus (RS) and the loss of trkC in Merkel axons result in degeneration of MCNC (NT3/trkC) and a decreased Merkel terminal plasticity (NT3/trkA). **II.** The maintained NT3 expression at the adjacent target, inner conical body (ICB), attracts detached Merkel axons (*) from the level of ring sinus. **III.** The maintained expression of NT3 in Merkel cells located at the rete ridge collar (RRC) supports the MCNC through trkC.

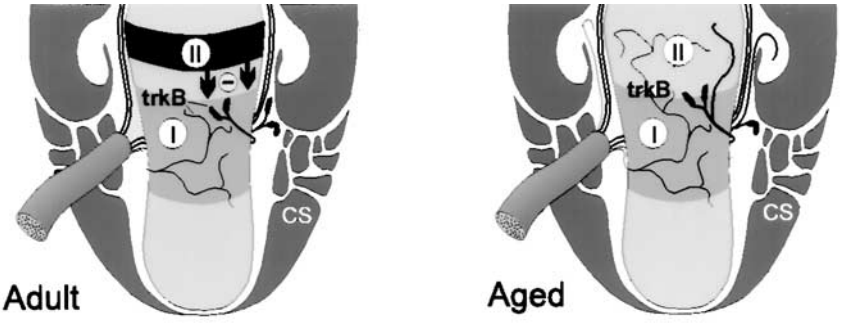

Fig. 9. Schematic illustration showing the suggested mechanisms underlying aberrant growth of trkB-IR axons in aged rats. (Adapted from ref. *48*.) **I.** A maintained expression of BDNF (light gray) at the level of upper cavernous sinus supports trkB expressing Ruffini endings and small-caliber axons. **II.** In the absence of NT4 (dark gray) in the aged rat, trkB-IR axons from the cavernous sinus sprouts into adjacent regions, which is normally devoid of innervation. In the adult rat, the expression of NT4 (dark gray) at the level of ringwulst might suppress the innervation terminating at the level of the upper cavernous sinus. NT4 has previously been shown to elicit suppressive/repellent effects on selective sets of epidermal innervation during development (*140,141*).

denervation, whereas the target-detached trkA expressing Merkel axons disclose a preserved regenerative capacity by growing towards the level of the inner conical body, which has a preserved NT3 expression *(24,48,140).* In the FSC several other populations of sensory nerve endings also show signs of maintained plasticity in senescence. For example, sensory nerve endings emanating from both large-and small-caliber axons "sprout" into new target fields, which in adulthood are devoid of innervation. (Fig. 9; *48*). Thus, available data indicate that many populations of sensory nerve endings remain intact and capable of responding to neurotrophic changes in their environment during aging.

Although observations are still limited, changes in sensory innervation during aging seem to be not only highly type- but also site-specific. Furthermore, the alterations appear closely associated with, and are perhaps instigated by, aberrations in target-tissue trophic signaling. Consistent with this, aging-related deficiency in vascular innervation can be restored by NGF *(88)* and sprouting can be induced in aged nonmanipulated sympathetic neurons by NGF *(143).* Combined, available data suggest that target and/or target interaction, rather than purely neuronal mechanisms undermine the maintenance of peripheral sensory and autonomic innervation in senescence *(87,144–146).* It is noteworthy that similar mechanisms may operate in disease-associated neuropathies as well. For example in streptatocin-induced diabetic rats, NGF can ameliorate the nociceptive deficits typical for the early phase of diabetic neuropathies *(72,89,133,147,148).* In both streptatocin-induced diabetic rats as well as diabetic humans, target-tissue expression of NGF is decreased. Furthermore, in the animal model trkA is downregulated in PSN and the retrograde transport of NGF is slowed in parallel with a decrease of $p75^{NTR}$ content in the PSNs. The later observation supports the notion that a preserved/increased level of $p75^{NTR}$ in aged PSN may work to maintain nociception through enhancing retrograde transport of NGF from the target.

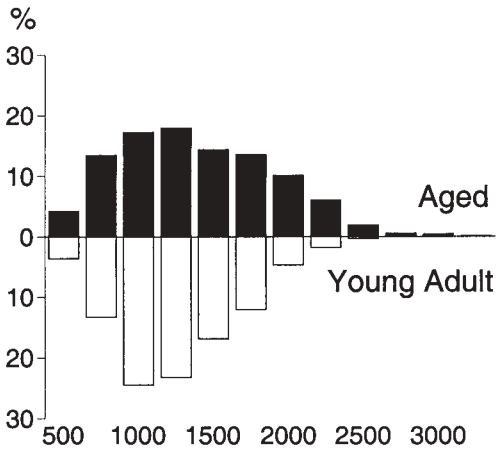

Fig. 10. Size-frequency histograms showing the distribution of cell profiles in the lateral part of the lower lumbar spinal-cord motor nucleus in young adult (unfilled columns) and aged animals (black columns). (Data replotted from *186.*) Motoneurons cross-sectional area is, on average, 9% larger in aged than young adult rats.

Aging Motoneurons

Aging motoneurons show a distinct pattern of phenotypic changes, while loss of motoneurons is small (~15% at 30 mo of age) despite frequent (~75%) and overt signs of behavioral motor deficits (*44,* and literature cited above). Several studies have reported on a loss of large myelinated axons in aged peripheral nerves but these results are questioned (*see* 60, and references therein) and, moreover, do not evidence that motor axons, in fact, are gone. The reason for this is that aged motor axons frequently show extensive signs of axon dystrophy, atrophy, and aberrations in their myelination (*60,* and references therein) and may, therefore, not have been included in the quantitative estimates. Thus, even in the very old individual with profound behavioral impairment, the vast majority of motoneurons are still present but apparently not connected to an intact set of target muscle cells. Consistent with the distribution of behavioral motor deficits, aging-related axon lesions are more prevalent in ventral roots and peripheral nerves of the lumbar than the cervical spinal cord *(149,150).* Furthermore, the distal part of a nerve is more severely affected than the proximal part *(151,152),* suggesting that this is progressing in a distal-to-proximal direction. A successive dropout of motoneurons would fit with the pattern of changes seen in aging skeletal muscle (*see* above). Particularly, in the initial phase of muscle denervation, motoneurons still connected to the muscle may try to compensate through collateral re-innervation *(4,29,153),* a process where terminal Schwann cells may play a key role (Fig. 11) (reviewed by

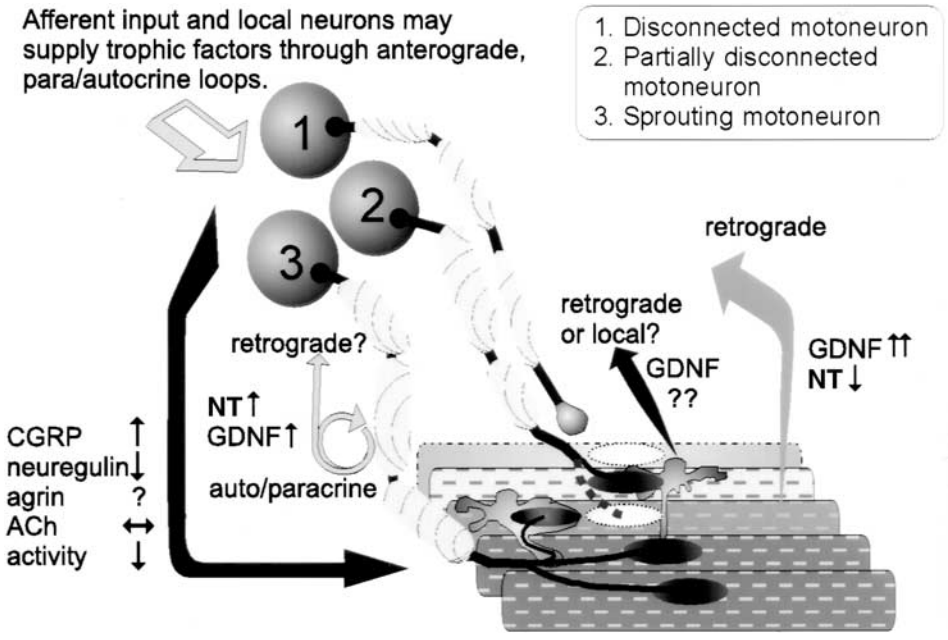

Fig. 11. Schematic drawing summarizing some of the trophic signaling loops among motoneurons, supportive cells (en passent and terminal Schwann cells) and muscle fibers. Changes in expression occurring in senescence have been indicated with up-, down-, or horizontal (no change) arrows.

154). However, if senile muscle atrophy would be caused purely by a neurogenic mechanism, it is difficult to explain why aging motoneurons are not lost, why they have a preserved cholinergic phenotype, and why they display no signs of cell-body atrophy (Fig. 10). Both CGRP and growth-associated protein 43 (GAP43) are markedly upregulated in aged motoneurons, a regulatory pattern typical of growth and regeneration (Fig. 11) *(44).* It has also been shown that aged motoneurons appear to have a preserved capacity to reinnervate target muscle fibers following experimental axon damage *(155,156).* Thus, evidence indicates that the process underlying senile muscle atrophy is more complex than just a dropout of parent motoneurons. In this context it should also be considered that muscle fibers can be replenished from satellite cells *(157),* but this capacity is low in adulthood and may decay even further in senescence *(158–160).* Thus, number of muscle fibers may decrease during aging due to a restrained regenerative capacity of the muscle tissue itself.

In addition to the upregulation of CGRP and GAP43, aged motoneurons disclose a highly specific pattern of neurotrophin and GDNF family ligand-receptor regulation (Figs. 5 and 6). This pattern shares some similarity but also distinct differences from that expressed by adult motoneurons disconnected from their target.

Target Muscles Fail to Maintain Adult Levels of Neurotrophins in Senescence

In adulthood, target muscles express all members of the neurotrophins (Fig. 1) *(55,92, 161–164),* however, adult motoneurons only express trkB and trkC and, consistent with this, NGF is not internalized and transported in motor axons *(132,165–169).* Whereas BDNF is retrogradely transported by intact as well as

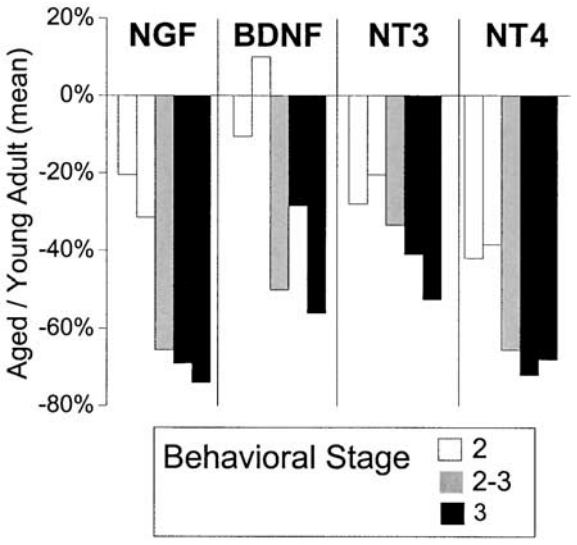

Fig. 12. Expression of neurotrophin mRNAs in muscle tissue. Relative expression of NGF, BDNF, NT3, and NT4 mRNAs in hind-limb muscles of individual aged rats. (Data replotted from *55.*) The data are presented as the percentage difference of aged and young adult rats. The behavioral stage of the individual aged rats is coded (see key in framed area).

axotomized motoneurons, NT3 seems transported only in small, presumably, γ-motoneuron in uninjured animals *(132,170,171).* Peripherally derived trkB and trkC ligands appear to be important for maintaining motoneuron-axon conduction velocity in adulthood *(172).* BDNF, NT3, and NT4 can protect the cholinergic phenotype of adult motoneurons, while BDNF and NT3 can also promote survival and regeneration *(171–179).* BDNF and NT3 may both play a role in the maturation process of the neuromuscular junction (NMJ) *(180,181)* and the levels of expression decrease to adult levels during late development *(162).* Disconnection of motoneurons from muscle fibers in adulthood induces a slow upregulation of BDNF and a downregulation of NT4, but has no robust effect on the NT3 level in the target *(92,162).* This pattern of regulation seems, at large, to be in line with the proposed function of the neurotrophins in adult motoneurons (*see* also below).

In aged rats, skeletal muscles show a downregulation of all neurotrophin mRNAs (Fig. 5) and the degree of decrease correlate with the extent of behavioral sensorimotor impairment among the individuals (Fig. 12) *(55).* Thus, animals with minor symptoms show a preserved expression of BDNF and only NT4 of the neurotrophins discloses a robust downregulation (>40%) also in behaviorally fairly intact aged animals. The expression level of muscle NT4 increases postnatally and several lines of evidence indicate that NT4 is positively regulated by NMJ impulse activity in adulthood *(91,92,162).* Some evidence points at the intriguing possibility that muscle-derived NT4 may have a strictly local signal-enhancing effect on the NMJ *(182),* and that retrograde transport of NT4 is a $p75^{NTR}$-dependent process taking place in lesioned but not intact motor axons *(120,132,183).* This is consistent with the marked upregulation, from very low levels, of $p75^{NTR}$ in adult motoneurons subjected to axon lesion *(184,185).* It seems quite plausible that the regulation of muscle NT4 in senescence reflects the decreased motor activity typical of aged rats. A decreased motor activity does not necessarily reflect a decreased innervation of the muscle. The concomitant lowering of NGF, NT3, and BDNF in the target would not be expected if neuromuscular aging was exclusively due to a dropout of motoneurons. Rather it suggests a possible incapacitation of the target to maintain adult expression levels of neurotrophins.

Do Changes in trk Receptor Expression in Aging Motoneurons Reflect Target Muscle Neurotrophin Deficiency?

Aged motoneurons show a robust downregulation of trkC mRNA (Fig. 5), evident also in individuals with only minor behavioral deficits *(186).* The downregulation probably reflects the lowered levels of NT3 in the target *(186,187),* because other potential sources of the trkC ligand NT3 such as PSN in DRGs, the spinal cord, or its motor nuclei, express NT3

at unaltered levels in senescence *(55,186).* In contrast to adult motoneurons disconnected from their target, trkB mRNA expression is also decreased in aged motoneurons *(166,167,186,188).* This downregulation is less marked in animals with mild symptoms of impairment, while animals with severe symptoms show a more robust decrease of trkB *(186).* The changed pattern of trkB mRNA expression in aged motoneurons closely mirrors the changes seen for BDNF mRNA in the target muscles (Fig. 12). Furthermore, the levels of trkB ligand mRNAs in DRGs and spinal cord appear largely unchanged with advancing age, arguing against that altered access to alternative sources of ligands is mechanistic in the downregulation of trkB (Fig. 5) *(55,186).* Thus it seems reasonable to infer that trkB is downregulated in aged motoneurons due to a decreased access to target-derived ligands.

Taken together, the pattern of regulation of neurotrophin and trk-receptor transcripts in senescence suggests that spinal motoneurons compete for a decreasing amount of target-derived trk ligands. The loss of muscle fibers and the atrophy of the remaining fibers will combined impose a reduced target size where the remaining muscle fibers express neurotrophins at a decreased level.

Possible Impact of Decreased Neurotrophin-trk Signaling on Aging Motoneurons

The functional implications of a decreased trkB and trkC signaling in aged motoneurons are not resolved. However, as mentioned earlier, BDNF and NT3 can protect motoneuron axon properties *(172)* and, thus, a decreased BDNF/NT3 signaling may contribute to the processes underlying the axon aberrations and slowing of the motor-axon conduction velocity, evident in senescence (*189*). As mentioned previously, neurotrophins can regulate the expression of cytoskeletal proteins, and some evidence points at a trophic function of these ligands on myelinating cells *(96,97);* both mechanisms are highly relevant for the changes observed in aging motor axons. Furthermore, motoneurons produce and release several factors that regulate muscle acetylcholin receptor expression (AChR). One such factor, neuregulin (NRG/ARIA) *(190,191)* increases AChR transcription through activation of muscle-expressed erbB receptors *(192).* NRG is expressed by motoneurons during development and in adulthood, but becomes downregulated in senescence (Edström et al., unpublished observations). Loeb and Fishbach *(193)* showed that, in particular, BDNF and NT3 can upregulate NRG in motoneurons. Interestingly, the isoform of NRG most likely to bind to the basal lamina of the NMJ was only upregulated by BDNF. Although direct evidence is lacking, the downregulation of NRG in motoneurons may represent yet another consequence of a decreased neurotrophin signaling in senescence.

$p75^{NTR}$ is Upregulated in Aged Motoneurons

In contrast to the trks, $p75^{NTR}$ (mRNA and protein) is markedly upregulated in aged motoneurons (Fig. 5). An increased expression is evident in animals with minor as well as severe symptoms *(186).* Motoneurons express high levels of $p75^{NTR}$ during development (when programmed cell death occurs) and in response to target disconnection in adulthood *(184,185).* The upregulation $p75^{NTR}$ in lesioned adult motoneurons is triggered by a yet-undiscovered retrogradely transported signal *(194)* and $p75^{NTR}$ levels become normalized upon target re-innervation *(185).* In aged rats, axonal severance induces a further upregulation of $p75^{NTR}$, indicating that the retrograde triggering mechanism present in adult animals is maintained in senescence *(186).* Although the mechanism(s) regulating $p75^{NTR}$ expression in aged nonmanipulated as well as in axotomized motoneurons remains obscure, increased levels of $p75^{NTR}$ may work as an enhancer of neurotrophin-trk signaling in a situation of dimin-

ished access to target-derived neurotrophins. Evidence supporting this view comes from experiments on null mutated $p75^{NTR}$ mice showing a decreased capacity among motoneurons to withstand insult *(122).* As mentioned previously, $p75^{NTR}$ may signal independently of trk receptors and an intriguing possibility is that $p75^{NTR}$ through inducing ceramide release have a non-neurotrophin/trk-associated protective function on motoneurons *(195).*

Does Increased GDNF Signaling Protect Cell-Body Size and the Cholinergic Phenotype of Aging Motoneurons?

GDNF is one of the most potent neurotrophic factors found so far for motoneurons. In adulthood it is present in glial cells and in peripheral target tissues, and the preferred receptor complement is expressed by motoneurons (Fig. 1). In aged rats there is an upregulation in spinal motoneurons of both the binding protein GFRα-1 and the signal-transducing RET components of the GDNF receptor (Fig. 7) *(50).* The target muscles show a marked upregulation of GDNF mRNA (Fig. 7) *(54).* A more modest upregulation of GDNF transcripts is also evident in the peripheral nerves but not in the spinal cord of aged rats. Combined this set of data imply an increased GDNF signaling between, in particular, the target muscles and the motoneurons. This may serve as a compensatory mechanism in the context of decreased neurotrophin-trk signaling in aging motoneurons, promoting axon regeneration *(137,138).* sprouting, and muscle-fiber re-innervation *(196);* and may be mechanistic in the protection of cell-body size and cholinergic phenotype. In support of the latter, Sagot and coworkers *(197)* reported that in $^{pmn/pmn}$ mice, suffering a lethal mutation causing severe muscle dystrophy and progressive caudal-cranial motoneuron degeneration, exogenous GDNF could prevent cell-body atrophy and protect the cholinergic phenotype of the motoneurons, but not impede the progressive damage of their axons. It has also been shown that GDNF has inhibitory effects on the generation of radical oxygen species *(195)* and, thus, may work in orchestra with $p75^{NTR}$ (*see* above) to protect aging motoneurons from oxidative stress.

A crucial point, yet unresolved due to the method used in the study by Ming et al. *(54),* is if the cellular source of GDNF is the muscle fibers or the terminal Schwann cells located in the muscle tissue *(54,198).* If the increased levels of GDNF mRNA derive from terminal Schwann cells, it could serve as a compensatory mechanism sustaining motoneurons when target muscle fibers become incapacitated. However, if the upregulation takes place in muscle fibers, we need to decode a highly specific and complex regulation of different neurotrophic factors in the target muscle cells during aging.

Conclusions

The incidence of sensorimotor disturbances increases with advancing age, and among the most elderly, will compromise the quality of daily life. In this review, we have argued against the notion that loss of neurons is mechanistic in this process. Several lines of evidence demonstrate that sensory, autonomic, and somatic motoneurons can maintain their adult phenotype, including target innervation pattern, growth, and target-reinnervation capacity, given that the appropriate stimuli are present. The most compelling evidence supporting the view that target failure is mechanistic is that a range of phenotypic changes observed in the aged neurons match the aging-related changes in target-tissue expression of neurotrophic factors. The interactions between neurons, supportive cells, and target to maintain, as well as adapt, the innervation is highly site- and type-specific. Similarly, sensorimotor deficits emerging in senescence are both type- and site-selective. Efforts designed to elucidate the "fine print" of the cross-talk among neurons, supportive cells, and target tissues may eventually provide a more definite

answer to these issues. The importance of the target tissues in sensorimotor aging, originally put forward by Cowen *(87),* also suggests that more global regulatory systems influenced by aging, such as hormones, should be taken into account *(3).*

Acknowledgments

This work was sponsored by grants from The Swedish Medical Research Council (project 10820), Karolinska Institutet, L. and H. Ostermans fond, M. och A. Wallenbergs Minnesfond, Konung Gustaf V:s och Drottning Victorias Stiftelse, and G. and B. Stohnes Stiftelse, Karolinska Institutets fonder.

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