Main Topic: Peripheral Nerve Surgery

EUROPEAN SURGERY
ACA Acta Chirurgica Austriaca
Printed in Austria

Eur Surg (2005) 37/4: 193–203
DOI 10.1007/s10353-005-0161-4

Experimental approaches to promote functional recovery after severe peripheral nerve injuries

T. Gordon[1], **J. G. Boyd**[2], and **O. A. R. Sulaiman**[3]

[1]Division of Physical Therapy and Rehabilitation, Center for Neuroscience, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada
[2]Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada
[3]Section of Neurosurgery, University of Manitoba, Winnipeg, Manitoba, Canada

Received May 11, 2005; accepted June 13, 2005

Experimentelle Zugänge zur Verbesserung des funktionellen Ergebnisses nach schweren Verletzungen peripherer Nerven

Zusammenfassung. *Hintergrund:* Die eingeschränkte Regenerationskapazität chronisch axotomierter Neurone und reduzierte Wachstumsunterstützung wegen atropher Schwann-Zellen sind die Hauptgründe für das schlechte Ergebnis nach proximalen Nervenverletzungen. In dieser Studie haben wir 2 Strategien untersucht, um diese Probleme zu umgehen: (1) Gabe von neurotrophen Faktoren auf chronisch axotomierte Motorneurone, um die Heilung zu stimulieren, (2) Anhebung des intrazellulären zyklischen AMPs (mittels Gabe von Forskolin und transformierendem Wachstumsfaktor β [TGF-β]), um Schwann-Zellen zur Proliferation zu stimulieren und dazu, den nichtmyelinierten, das Wachstum unterstützenden Phänotypen einzunehmen.

Methoden: (1) Tibiale Motorneurone der Ratte wurden chronisch für 2 Monate axotomiert und mit den neurotrophen Faktoren BDNF (vom Gehirn stammend) und/oder GDNF (von der Glia stammend) vor Auszählung retrograd markierter sich regenerierender Neurone versorgt. (2) Schwann-Zellen vom Nervus ischiadicus wurden für 24 Wochen vor Präparation denerviert und 48 Stunden in vitro in Kochsalzlösung oder Forskolin/TGF-β inkubiert und in einer 1 cm langen Plastikhülle zwischen abgetrennten Nervus-tibialis-Stümpfen in vivo reinseriert. Nach 6 Monaten wurden die regenerierten Motorneuronen mittels retrograder Markierung ausgezählt

Ergebnisse: (1) Niedrig dosierter, nicht aber hoch dosierter BDNF und/oder GDNF haben die Zahl der tibialen Motorneurone signifikant vermehrt, welche Axone nach chronischer, nicht aber nach akuter Axotomie regenerierten. Der BDNF-Effekt wurde durch trkB-Rezeptoren mediiert, die Hemmung durch hoch dosierten BDNF mittels p75-blockierenden Antikörpers gehemmt. (2) Behandlung chronisch denervierter Schwann-Zellen mit Forskolin/TGF-β verdoppelte die Zahl der Motorneurone, die ihre Axone durch die behandelten Schwann-Zellen regenerierten. Die regenerierten Axone waren gut myelinisiert.

Schlussfolgerungen: Die schädlichen Folgen chronischer Nervendurchtrennung und Schwann-Zell-Denervation können möglicherweise durch Applikation neurotropher Faktoren und Zytokine behandelt werden. Die angeführten experimentellen Strategien könnten neue therapeutische Konzepte zur Regeneration verletzter peripherer Nerven darstellen.

Schlüsselwörter: Nervenregeneration, transformierender Wachstumsfaktor β, neurotropher Faktor BDNF, neurotropher Faktor GDNF, Schwann-Zelle.

Summary. *Introduction:* Reduced regenerative capacity of chronically axotomized neurons and reduced growth support by atrophic Schwann cells are key factors that account for the poor functional outcomes after proximal nerve injuries. In this study we examine two strategies aimed to circumvent deleterious effects of chronic axotomy and chronic denervation on axonal regeneration: (1) exogenous application of neurotrophic factors to chronically axotomized motoneurons to reverse time-related decline in regenerative capacity and (2) intracellular elevations of cyclic AMP (via exogenous application of forskolin and transforming growth factor β [TGF-β]) to induce atrophic dormant Schwann cells to proliferate and re-enter the nonmyelinating growth-supportive phenotype that normally supports axonal regeneration.

Methods: (1) Rat tibial motoneurons were chronically axotomized for 2 months and brain-derived neurotrophic factor (BDNF) and/or glia-derived neurotrophic factor (GDNF) supplied exogenously prior to enumeration of retrogradely labeled neurons that regenerate their axons. (2)

Correspondence: T. Gordon, Ph.D., Division of Neuroscience, 523, Alberta Heritage Research Centre, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2S2.
Fax: +1-780-7921617
E-mail: tessa.gordon@ualberta.ca

Sciatic nerve Schwann cells were chronically denervated for 24 weeks prior to preparation and 48 h in vitro incubation of the nerve explants with saline or forskolin/TGF-β and reinsertion in a 1 cm long silastic sheath between cut tibial nerve stumps in vivo. Motoneurons that regenerated axons were enumerated 6 months later by retrograde labeling.

Results: (1) Low-dose but not high-dose BDNF and/or GDNF significantly increased the numbers of TIB motoneurons that regenerated axons after chronic but not immediate axotomy. The BDNF positive effect was mediated via trkB receptors, the inhibitory effects of high-dose BDNF being blocked by a p75-blocking antibody. (2) Treatment of chronically denervated Schwann cells with forskolin/TGF-β more than doubled the number of motoneurons that regenerated their axons through the treated Schwann cells. Regenerated axons were also well myelinated.

Discussion: Deleterious effects of chronic axotomy and Schwann cell denervation may be reversed by exogenous application of neurotrophic factors and by cytokines, respectively. Such experimental approaches may translate into strategies to promote functional recovery after nerve repair of the injured peripheral nervous system.

Key words: nerve regeneration, transforming growth factor β, brain-derived neurotrophic factor, glia-derived neurotrophic factor, Schwann cells.

Introduction

The Schwann cells of the peripheral nervous system (PNS) and the oligodendrocytes of the central nervous system (CNS) form the myelin sheaths around axons that are the basis for saltatory conduction from one node of Ranvier to another [1–3]. Both glial cells sub serve this common function despite differing in several ways. These include first the one-to-one relationship between Schwann cells and axons as opposed to the myelination of several axons by the oligodendrocytes [4]. Second, Schwann cells support axonal regeneration after nerve injury but oligodendrocytes do not [5, 6]. This contrast has been generalized to the current view that axonal regeneration restores functional recovery after PNS nerve injuries as apposed to failure of CNS axons to regenerate and restore function [4, 5, 7]. These generalized views are inaccurate because PNS axonal regeneration seldom restores complete functional recovery and there may be some recovery that occurs after incomplete CNS nerve injuries [8–11]. The inaccuracies stem primarily from the outcome measures commonly used to judge recovery. For example, open field locomotor scores such as the BBB score, used to assess behavioral recovery in spinal injured rats, fail to discriminate functional recovery that follows axonal sprouting and synaptic changes that occur above and below the spinal cord lesions [7, 11–15].

In the PNS, measurements that include muscle weight, EMG, contractile forces and walking track analysis often fail to detect considerable reductions in the number of motoneurons that regenerate their axons after injury. This is because regenerated motor axons compensate for the reduced number of reinnervated motor units via their capacity to reinnervate more denervated muscle fibers at a ratio greater than is seen in intact muscles [16]. Thus, for example, when motoneurons are axotomized and prevented from regenerating for up to one year prior to surgical repair of the cut nerve (chronic axotomy), only a third of the motoneurons regenerate their axons to reinnervate muscles [17]. The capacity of regenerating motoneurons to form enlarged reinnervated motor units fully compensated for the reduction in their number [17] so long as the remaining number of motor units did not fall below the normal by 15–20% [17–23]. On the other hand, prevention of regeneration into distal nerve stumps for periods of up to one year (chronic denervation) prior to reinnervation by regenerating axons reduced the success of axonal regeneration so severely [18, 24, 25] that formation of enlarged motor units could not compensate for the ultimate reductions in contractile forces and muscle weights [24].

The severe effects of chronic axotomy and chronic denervation of reducing the regenerative capacity of motoneurons are likely to account for the very poor functional outcomes after proximal nerve injuries (i.e., brachial or lumbar plexus) that require surgical apposition of the severed nerves [8–10]. Under these conditions, many neurons remain chronically axotomized for lengthy periods of time as their axons regenerate at a rate of 1–3 mm/day over distances that may exceed meters. Likewise, the Schwann cells in the distal nerve stumps that remain denervated for periods of months and years as axons regenerate sluggishly over the required distan-

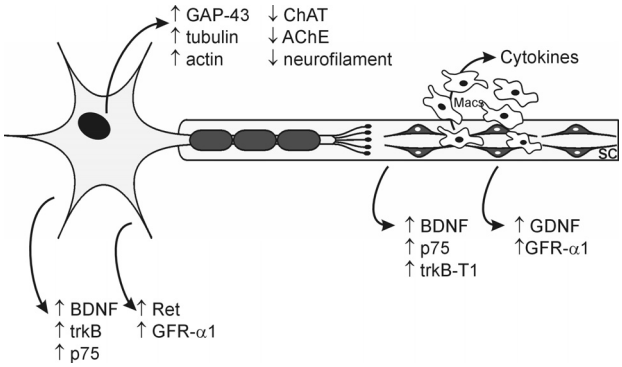

Fig. 1. Characteristic morphological and molecular changes in injured neurons and Schwann cells (SC) of the distal nerve stumps are illustrated figuratively after complete axonal transection. Chromatolytic changes in the axotomized neuron include morphological changes such as an eccentric shift of the nucleus in the cell body and dissolution of the Nissl substance (NS) that reflect underlying changes in gene transcription and protein translation. In general terms, the shift in gene expression in both Schwann cells and the cell bodies reflect the change in the functional status of these cells with regards to neurotransmission, myelination and regeneration. Regenerative associated genes such as GAP-43, tubulin, actin and neurotrophins/receptors are upregulated and neurotransmission-associated genes (ChAT, AChE) are downregulated. Macrophages which infiltrate the distal nerve stumps secrete many cytokines/growth factors that constitute part of the active interaction between the Schwann cells and macrophages during the process of Wallerian degeneration

ces, atrophy and progressively fail to support axonal regeneration [5, 20, 24, 26–31].

Moreover, regeneration of axons across a surgical suture site is critical both with respect to a timely regeneration across the suture site and regeneration into appropriate distal nerve stumps and target organs. Although, there is evidence that regenerating axons preferentially reinnervate motor endoneurial tubes leading to appropriate muscle targets [32–34], inability of motor axons to discriminate between their original and inappropriate denervated muscles severely compromises recovery of function [35–38]. Regeneration of axons across the surgical site is slow and occurs in a "staggered" fashion requiring up to 2 weeks to regenerate across the surgical site [39]. These substantial delays prior to regeneration within the Schwann cells of the distal nerve stumps are likely to compound the problems of progressive chronic axotomy and chronic Schwann cell denervation as mentioned above.

The onset of the progressive declining capacity of chronically denervated Schwann cells to support axonal regeneration coincides with the completion of Wallerian degeneration in the denervated distal nerve stumps [40–43]. During the first month of denervation, Schwann cells convert from myelinating to the growth-supportive nonmyelinating phenotype that expresses markers that include the p75 neurotrophic factor receptor, erbB receptors for neuregulin and neurotrophic factors [44–47] (Fig. 1). It is during this time that Schwann cells interact actively with infiltrating macrophages that play a critical role in the process of Wallerian degeneration by removing myelin/axonal debris [40–43] and stimulating (via the release of cytokines) the production of neurotrophic factors by all the nonneuronal cells of the distal nerve stumps [45]. Transforming growth factor β (TGF-β), a cytokine released both from invading macrophages and from the Schwann cells themselves, has been implicated in sustaining the nonmyelinating growth-permissive phe-

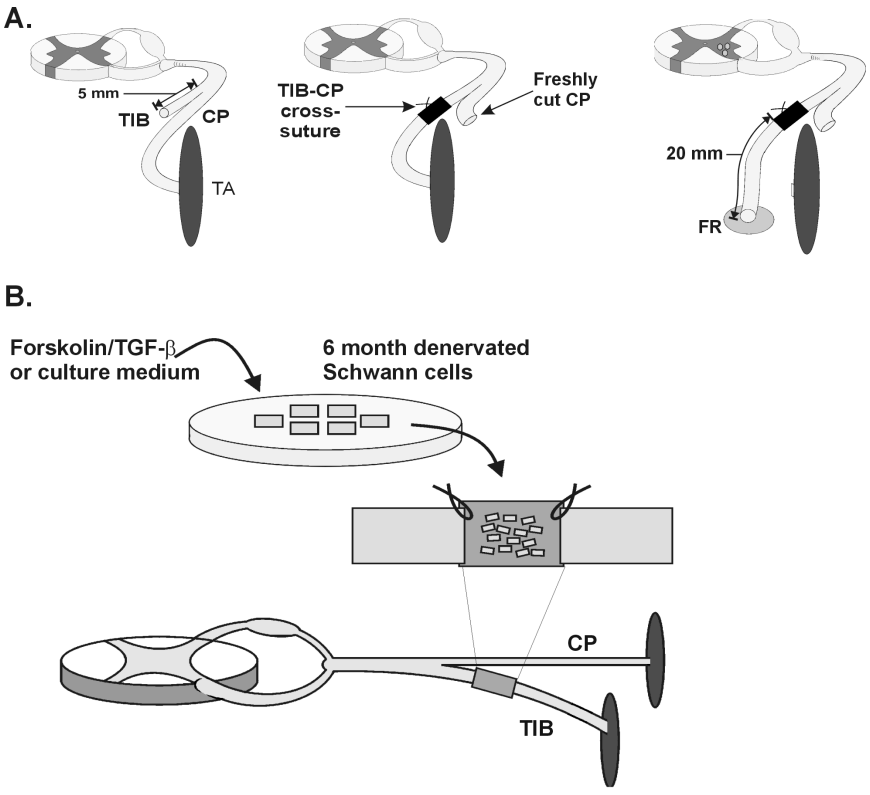

Fig. 2. A Figurative illustration of the tibial (*TIB*) nerve transection 5 mm from its branching from the sciatic nerve and the cross-suture of either the freshly axotomized or 2-month chronically axotomized TIB nerve stump to the distal nerve stump of the freshly cut common peroneal (*CP*) nerve. Neurotrophic factors were delivered at a continual flow rate of 2.5 μl/h for 28 days via a 5 mm long silastic cuff around the cross-suture site from a subcutaneous Alzet mini-osmotic pump. Thereafter, fluorogold (FG) or fluororuby (FR) was applied to the distal nerve stump 20 mm from the cross-suture site to backlabel TIB motoneurons that regenerated their axons (see Methods). **B** Six-month chronically denervated sciatic nerve stumps devoid of epineurium and blood vessels were sliced into 3–4 mm^2 segments in vitro and incubated with culture medium alone or culture medium containing TGF-β and forskolin for 48 h. Thereafter, the nerve explants were placed in 1 cm long silastic cuffs that bridged between the proximal and distal nerve stumps of freshly cut tibial (*TIB*) nerves to encourage axonal regeneration by the freshly axotomized motoneurons through the chronically denervated and treated Schwann cells and into the distal TIB nerve stump. Six months later, the regenerating axons were exposed to fluorescent dyes (FG or FR) to backlabel the motoneurons whose axons had regenerated through the chronically denervated Schwann cells that had been exposed to TGF-β/forskolin or culture medium alone and 20 mm into the distal TIB nerve stump

notype of the Schwann cells [46, 48–53]. It has also been shown to be essential for the effect of many neurotrophic factors including glia-derived neurotrophic factor (GDNF) on neurons, especially motoneurons [54–56].

The progressive decline in the capacities of axotomized motoneurons to regenerate axons and denervated Schwann cells to support regeneration is likely related to losses of neurotrophic support of the injured neurons and active Schwann cell/macrophage interactions in the distal nerve stumps, respectively. In this study, we examine two possible strategies aimed to prevent the deleterious effects of chronic axotomy and chronic denervation. These include (1) exogenous application of neurotrophic factors to chronically axotomized motoneurons to reverse the time-related decline in regenerative capacity and (2) intracellular elevations of cyclic AMP (cAMP) (via exogenous application of forskolin and TGF-β) to induce atrophic dormant Schwann cells to proliferate and re-enter the nonmyelinating growth-supportive phenotype that normally supports axonal regeneration.

Methods

Chronic neuron axotomy and exposure to exogenous neurotrophic factors

All animal procedures were conducted in accordance with the Canadian guidelines for animal experimentation and with a local animal welfare committee. As previously described [57, 58], under deep sodium pentobarbital (30 mg/kg i.p.) anesthesia and aseptic conditions, tibial (TIB) nerves in adult female (200–225 g) Sprague-Dawley rats were cut 5 mm distal to its bifurcation from the common peroneal (CP) nerve. Either the proximal TIB nerve stump was cross-sutured to the freshly denervated CP nerve stump to encourage axonal regeneration, or axonal regeneration was prevented for 2 months by ligation of the proximal TIB nerve stump to the surrounding musculature (chronic TIB axotomy). After the 2 month period of chronic axotomy, the proximal TIB nerve stump was cross-sutured to the freshly denervated CP distal nerve stump with an 8-0 suture (Fig. 2A). The cross-suture was performed within a loosely fitting 5 mm long silastic cuff (Dow Corning, 0.64 mm i.d., 1.19 mm o.d.) that was connected to an Alzet 2ML4 mini-osmotic pump (Alza Corp., Palo Alto, California) to deliver either saline (control) or exogenous BDNF diluted in saline in doses of between 0.2 μg to 20 μg/day or GDNF in 7 doses from 0.1 to 10 μg/day or both (2 μg/day BDNF and 0.1 μg/day GDNF) to the cross-suture site at a continual flow rate of 2.5 μl/h (Fig. 2a). To examine the role of the p75 receptors in mediating the effects of exogenous BDNF, the p75 function-blocking antibody REX [59, 60] was included in the pump that delivered the BDNF. One month later, the number of axotomized TIB motoneurons that regenerated their axons 20 mm into the distal nerve stump was counted on longitudinal 50 μm frozen sections of 4% paraformaldehyde-fixed spinal cords 6 days after application of retrograde dyes. Either fluorogold (4% in cacodylic acid; Fluorochrome Inc) was applied to the cut nerve in a Vaseline well for 1 h, or fluororuby crystals (dextran tetramethylrodamine; Molecular Probes, D-1817) were applied directly to the cut nerve for 2 h under deep surgical anesthesia. Sections were viewed under a Leitz-Diaplan fluorescence microscope using appropriate filters (Fig. 3B).

BDNF and GDNF were kindly provided by Regeneron and by Amgen Pharmaceuticals, respectively. REX was a kind gift of Dr. L. Reichardt, University of California, San Francisco, Calif., USA.

Chronic nerve denervation and exposure to TGF-β

Using the same protocol of deep anesthesia and aseptic conditions, the sciatic nerve was cut and ligated unilaterally in 6 adult Sprague-Dawley rats in order to chronically denervate Schwann cells for 24 weeks. After the 24 w period of chronic denervation, the sciatic distal nerve stumps were dissected and cleaned of their epineurium, associated blood vessels and connective tissue prior to being sectioned into 2–3 mm^2 explants in Liebovitz's L-15 medium (Gibco) that was supplemented with antibiotics (50 U/ml penicillin and 0.05 mg/ml streptomycin). The nerve explants were transferred and incubated for 48 h in a 35 mm culture dish that contained Dulbecco's modified Eagle's medium (Gibco) supplemented with 15% fetal calf serum alone (control D-15 untreated explants) or medium supplemented with 15% fetal calf serum (D-15) and both 1 ng/ml TGF-β and 0.5 M forskolin (experimental D-15/TGF-β–forskolin-treated explants) (Fig. 2B).

In a second set of Sprague-Dawley rats, 5–6 of either the control or experimental explants were placed in the center of a 1 cm long silastic tubing that bridged the gap between the proximal and distal nerve stumps of a freshly cut TIB nerve anchored to the silastic tube via a 10-0 suture. At least 3 mm long gaps existed between the explants and the TIB nerve stumps to minimize the influence of Schwann cell migration from the freshly cut nerve stumps on axonal regeneration. Regeneration through the explants and into the distal TIB nerve stump was assessed 6 months after repair by application of retrograde dyes to sectioned distal TIB nerve stumps 25 mm from the silastic tube. Application of fluorescent dyes and enumeration of retrogradely labeled neurons in the spinal cord have been previously described and restated above.

Nerve histology

Five days after application of retrogradely transported fluorescent dyes, all rats were sacrificed and underwent transcardiac perfusion with paraformaldehyde. Then, 3–5 mm long pieces of reinnervated distal nerve stumps in both experiments and a piece of reinnervated explants in the second experiments were excised and processed for light and electron microscopy. Briefly, the nerves were fixed by immersion in glutaraldehyde (3% in 0.1 M phosphate buffer), stained with osmium tetroxide (3% solution in 0.1 M phosphate buffer), dehydrated in ascending alcohols and embedded in araldite. Semithin sections (0.5 um) were stained with toluidine blue.

Results

Chronic axotomy and application of neurotrophic factors

TIB motoneurons either regenerated their axons into the freshly cut distal nerve stump of the CP nerve immediately after axotomy or 2 months after cutting and ligating the proximal TIB nerve stump (chronic axotomy). One month after the cross-suture of the TIB and CP nerves, TIB motoneurons that had regenerated their axons were backlabelled and identified by the fluorescent dye retrogradely transported to their cell bodies (Fig. 3). One month after immediate nerve suture, about 400 motoneurons regenerated their axons into the distal CP nerve stump. Two months of chronic axotomy of the TIB motoneurons significantly reduced this number. However, application of either low doses of BDNF or GDNF daily to the regenerating axons elevated this number back to the number that regenerated after immediate axotomy. Combination of both low-dose BDNF and GNDF more than doubled the number of motoneurons that regenerated their axons.

In contrast to the dose-dependent effect of GDNF of significantly elevating the number of motoneurons that regenerated after chronic axotomy, elevation of BDNF doses had a striking inhibitory effect on regenerative success. Application of p75-blocking antibodies negated the inhibitory effects of high-dose exogenous BDNF (Fig. 3B). These findings provide strong evidence that BDNF exerts its positive effects on axonal regeneration at low doses but may halt axonal regeneration at high [20, 46].

Normally each myelinating Schwann cell is associated with a single intact axon (Fig. 4A). Following nerve transection and cross-suture, each Schwann cell is associated typically with 3–5 axon profiles (Fig. 4B), consistent with previous descriptions [45, 61]. Administration of low-dose BDNF and GDNF increased the number of axons in a regenerating unit (Fig. 4C) to an average of 10 (Fig. 4D), significantly higher than the numbers observed for either GDNF or BDNF alone [58].

Chronic denervation and TGF-β

Chronic denervation of the distal nerve stump severely restricted the number of motoneurons that regen-

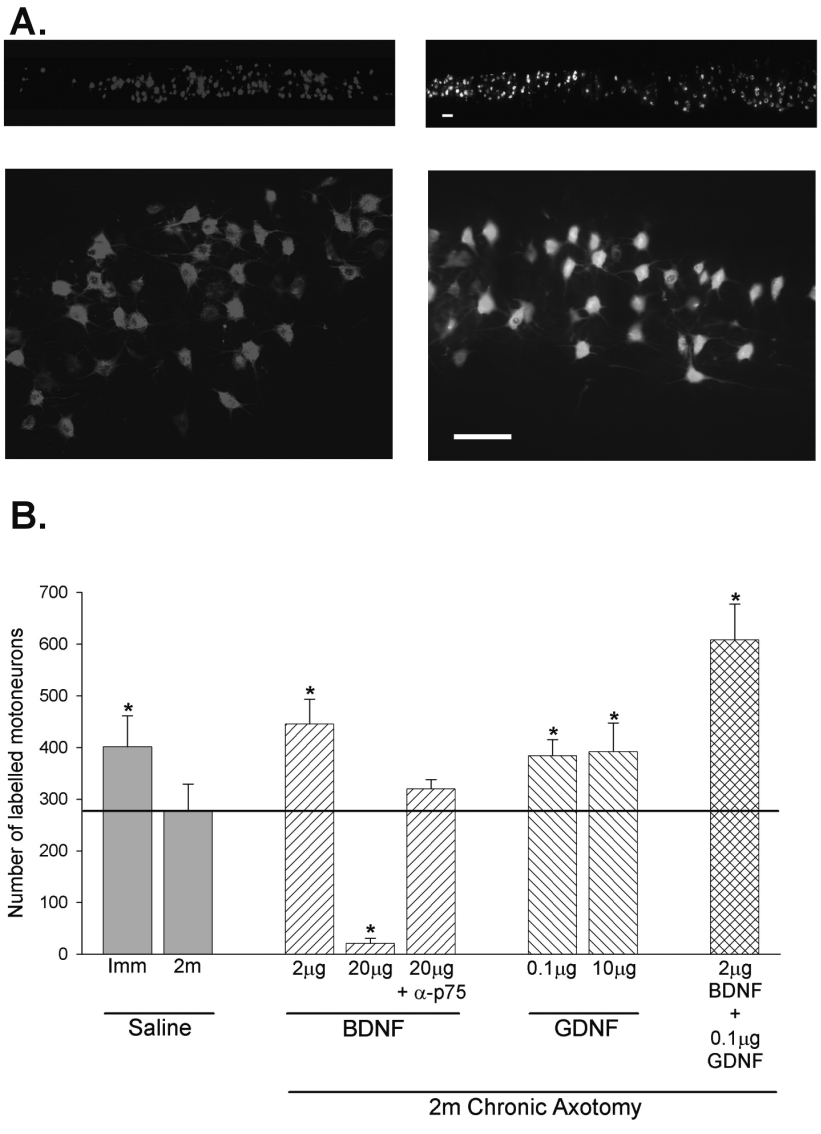

Fig. 3. A Low- and high-magnification photomicrographs of fluororuby- and fluorogold-labeled intact TIB motoneurons in 50 μm longitudinal sections through the ventral horn of the T11-L1 spinal segments. Motoneurons identified in serial sections were enumerated and the numbers corrected to correct for split nuclei by the method previously described by Abercrombie [92]. **B** Chronic axotomy of TIB motoneurons prior to cross-suture to the freshly denervated CP distal nerve stump significantly reduces the number of motoneurons that regenerate their axons in 28 days as compared to motoneurons that regenerate their axons after immediate cross-suture. Low-dose BDNF significantly increases the number of motoneurons that regenerate their axons after 2 months of chronic axotomy, but high-dose BDNF has a strong inhibitory effect on axonal regeneration, which is reversed by including a function-blocking antibody against p75 receptors, α-p75. GDNF, in contrast to BDNF, has a facilitatory effect on the number of motoneurons that regenerate their axons in concentrations that range over a 100-fold. Low-dose BDNF and GDNF have a very pronounced effect of promoting the regeneration of chronically axotomized motoneurons but not of freshly axotomized motoneurons (not shown, but see text)

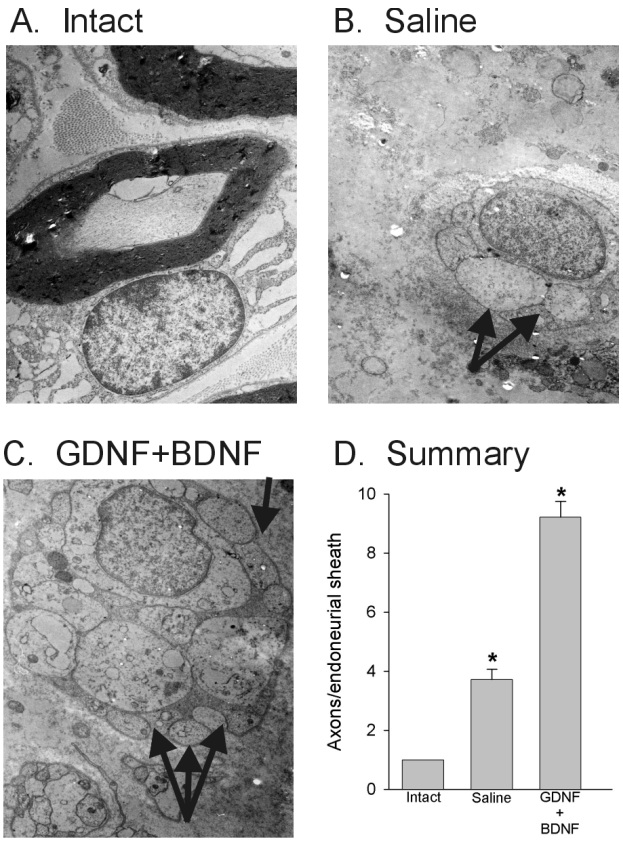

Fig. 4. A In intact nerves, a single Schwann cell is normally associated with only one axon. **B** After nerve injury, a Schwann cell and 3–5 daughter axons sprouted from single axons in the proximal nerve stump comprise a regenerating unit. **C** In GDNF- and BDNF-treated regenerating nerves, there are many more daughter axons in the regenerating unit, the number more than double the number after saline treatment (**D**). This number also exceeded the number of daughter axons per regenerating unit observed after either BDNF or GDNF alone (not shown)

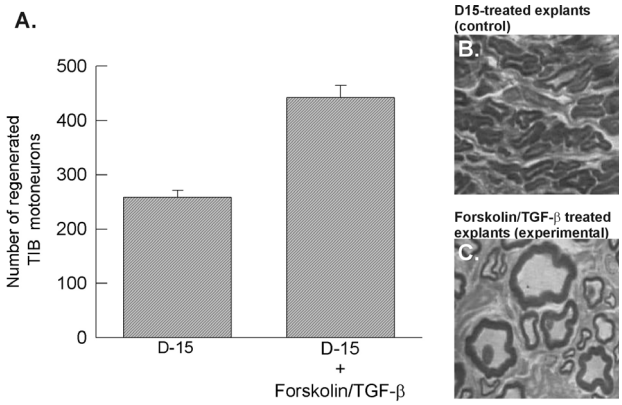

Fig. 5. A Histograms illustrating the dramatic effect of incubating 6 month chronically denervated Schwann cells in vitro with D-15 or D15 plus forskolin/TGF-β on their capacity to support regeneration of TIB axons in vivo. Forskolin/TGF-β-treated Schwann cells supported the regeneration of twice the number of TIB motoneurons that regenerated when Schwann cells were treated with D-15 alone. **B** and **C** Axons regenerating through the forskolin/TGF-β-treated Schwann cells are less atrophic and had better myelination (**C**) compared with axons regenerating through the D-15-treated Schwann cells (**B**)

erated their axons [24, 25]. When 6-month chronically denervated Schwann cells were untreated and simply incubated in D15 culture medium for 48 h in vitro, implantation of the explants into a 1 cm silastic tube that bridged between freshly cut TIB nerve stumps supported the regeneration of only about 25% of all TIB motoneurons (Fig. 5A). In contrast, treatment of these chronically denervated Schwann cells in 2–3 mm explants with both forskolin and TGF-β more than doubled the number of motoneurons that regenerated their axons through chronically denervated and forskolin/TFG-β-treated Schwann cells (Fig. 5A). These findings indicate that the promotion of mitosis and expression of the nonmyelinating growth-permissive phenotype of Schwann cells by forskolin and TGF-β, respectively, significantly improved the growth support of regenerating axons. Moreover, the relatively few axons that regenerated through the chronically denervated Schwann cells whicht were incubated in culture medium alone, were atrophic but well myelinated. This was in contrast to the higher number of large and well myelinated axons in the silastic cuff after treatment with forskolin and TGF-β (compare Fig. 5 C and B). Thus, not only did more motoneurons regenerate their axons but the axons that did regenerate through the forskolin/TFG-β-treated Schwann cells matured more rapidly, increasing the size of their axons and the thickness of the myelin sheath surrounding the axons (Fig. 5).

Discussion

The findings of this study demonstrate that (1) exogenous application of the neurotrophic factors to axotomized motoneurons and (2) stimulating chronically denervated Schwann cells with the cytokine TGF-β can both reverse the time-dependent decline in the regenerative capacity of the PNS. These, in turn, may translate into improved functional outcome, although this was not assessed in these experiments.

Low-dose BDNF and GDNF promote axonal regeneration after chronic axotomy

Indirect measures of axonal regeneration that include the sciatic function index or gait analysis indicated small but positive effects of neurotrophic factors on peripheral nerve regeneration [62–64]. However, this claim was not completely substantiated by our quantitative evaluation of the number of motoneurons that regenerated their axons. Exogenous application of either low-dose BDNF or GDNF had no effect on the number of neurons that regenerated their axons after immediate nerve transection and surgical repair. High-dose BDNF, however, had a strong inhibitory effect on axonal regeneration. After a period of 2 months of chronic axotomy, the daily administration of low-dose BDNF and GDNF had, in contrast to the lack of effect of axon regeneration of freshly axotomized motoneurons, a significant effect of increasing

the number of motoneurons that regenerated their axons. This effect negated the negative effects of chronic axotomy on regenerative capacity. Nonetheless the negative effects of high-dose BDNF persisted after chronic axotomy of the motoneurons.

The facilitatory effect of low-dose BDNF on axon regeneration after chronic axotomy and not after nerve section and immediate nerve repair are consistent with findings that a function-blocking anti-BDNF antibody significantly reduced the number, density and length of regenerating axons [65] and that adenoviral transfection of axotomized motoneurons with the serine/threonine kinase, Akt, increased motoneuronal regeneration [66]. BDNF is upregulated in motoneurons immediately after axotomy and its expression in denervated Schwann cells increases within a week of nerve injury [44, 67–69]. Motoneurons that are regenerating their axons therefore express the neurotrophic factor and are likely to be exposed to the factor when the regenerating axons make contact with Schwann cells. The functional anti-BDNF antibody, by reducing access to the BDNF, reduces axonal regeneration after immediate nerve repair. After delayed nerve repair when axotomized motoneurons are not exposed to BDNF on the denervated Schwann cells, a situation which would occur as motoneurons regenerate their axons in progressively deteriorating pathways of chronically denervated Schwann cells, exposure to exogenous BDNF appears to compensate for the reduced exposure to the neurotrophin in the growth pathway. High-affinity trkB receptors upregulated on axotomized motoneurons [67, 68, 70] appear to mediate these positive effects of the neurotrophin on axon regeneration because motor axonal regeneration is significantly reduced in trkB heterozygous knockout mice [71]. That exogenous BDNF completely reversed the negative effects of chronic axotomy on the regeneration of motor axons is consistent with the failure of ~70% trkB (+/–) motoneurons to regenerate their axons at all over an 8 week period of time [71].

The co-expression of both trkB receptors and p75 receptors in motoneurons accounts for the bimodal effects of BDNF on motor axonal regeneration [44, 72]. Low doses of BDNF that act via the trkB receptors mediate facilitatory effects of axon regeneration, while progressively higher doses of exogenous BDNF are likely to act via both receptors. Using the function-blocking p75-antibody REX we observed that the strong inhibitory effects of high-dose BDNF on axon regeneration after both immediate and chronic axotomy were blocked with REX. This exposed the underlying facilitatory effect mediated via the trkB receptor. Not only do these findings argue against a methodological basis for the strong inhibitory effect of high-dose BNDF, they provide strong evidence that p75 mediates the inhibitory effects of high-dose BDNF. Similarly Kohn et al. [60] demonstrated that p75 mediates the inhibitory effects of BDNF on NGF-induced neurite outgrowth from sympathetic neurons in vitro. Findings that significantly more motoneurons regenerated their axons in p75 knockout mice [71] provide further evidence to support the contention that p75 receptors mediate the inhibitory actions of high-dose BDNF.

GDNF had no effect on freshly axotomized motoneurons and only a positive effect of promoting axonal regeneration from chronically axotomized motoneurons. This contrasts with the biphasic facilitatory and inhibitory effects of low- and high-dose BDNF, respectively. GDNF is the most potent neurotrophic factor on motoneurons [73–75], acting at concentrations that are 100-fold lower than BDNF (Fig. 3). GDNF acts via a single receptor complex to induce downstream phosphorylation of MEK and Akt, as does BDNF via the trkB receptor [44]. The GDNF receptor subunits and the trkB receptor are upregulated in axotomized motoneurons [76–78] and mediate the positive effects of exogenous GDNF on axonal regeneration of the chronically axotomized motoneurons. Presumably, sufficient GDNF is available on denervated Schwann cells [79] to support axonal regeneration such that exogenous GDNF does not affect axonal regeneration after immediate nerve repair of cut TIB nerves. After chronic axotomy, however, exogenous GDNF appears to supplement available endogenous GDNF to significantly increase the number of motoneurons that regenerate their axons. BDNF binding to trkB receptors in the membrane also induces phosphorylation of the receptor and downstream phosphorylation of MEK and Akt. The molecular overlap of signaling pathways is illustrated by the blockade of the survival-promoting effects of both GDNF [80] and BDNF [81] by pharmacological blockade of PI3 kinase. Hence GDNF cooperates with low-dose BDNF to promote axonal regeneration via their binding to the membrane receptors that act downstream via PI3 kinase. Together, the neurotrophic factors promoted regeneration of axons from about 600 motoneurons which comprises at least 60% of the motoneurons, a doubling of the number of motoneurons that regenerate their axons after 2 months of chronic axotomy (Fig. 3). In light of the staggered regeneration of axons across a suture site demonstrated in experiments on cut and sutured femoral nerve [39], regeneration of all motoneurons would be expected to occur after periods of regeneration of 2 months or more. Importantly, exogenous administration of the growth factors was required throughout the 4 week period of regeneration because reduced periods of administration reduced the effects [44]. Interestingly, both neurotrophic factors promote axonal outgrowth as evidenced by the increased number of axons that regenerate into the distal nerve stump (Fig. 4).

Our results indicate therefore that therapeutic application of exogenous neurotrophic factors would require continuous infusion over the entire time-course of axonal regeneration. Alternative methods of administration of neurotrophic factors would be more efficacious. One possible approach would be to electrically stimulate the motoneurons to upregulate the expression of the neurotrophic factors in the motoneurons [34]. Alternatively, Schwann cells may be genetically modified to produce these neurotrophic factors as shown for olfactory ensheathing cells for example [82]. Other possibilities are discussed in a recent review by Fenrich and Gordon [5].

TGF-β counteracts the atrophy of chronically denervated Schwann cells and enhances their capacity to support axonal regeneration

Chronically denervated Schwann cells progressively atrophy and many, although not all, die [24, 83]. Adult Schwann cells in contrast to immature Schwann cells can survive in the absence of axon-derived neuregulin-1, due in large part to the existence of other mitotic autocrine Schwann cell loops that include insulin-like growth factor-2 (IGF-2), platelet-derived growth factor-BB, neurotrophin-3 (NT-3), leukemia inhibitory factor (LIF) and lysophosphatidic acid (LPA)-like activity [53, 84]. Laminin also acts with these factors to promote survival [84]. However, the endoneurial pathways that contain laminin gradually fragment as the Schwann cells lose their capacity to sustain the synthesis of extracellular matrix components that include collagen type IV, laminin and heparin proteoglycans that form the basal lamina [85–88]. Their progressive failure to proliferate in response to regenerating axons concurs with their loss of erb receptors that normally respond to neuregulin released from regenerating axons [29, 30]. Nonetheless, those Schwann cells that remain and support the regeneration of a few axons maintain their capacity to differentiate into the myelinating phenotype and in turn, remyelinate regenerating axons [24]. Approximately 10% of the motoneurons regenerate axons into distal nerve stumps that have been chronically denervated for a period of 6 months and the Schwann cells remyelinate the axons [18, 24, 25].

Proliferation of Schwann cells after denervation and their conversion from the myelinating to the nonmyelinating growth-supportive phenotype involves the cytokines released by the macrophages as well as by the dedifferentiating Schwann cells during Wallerian degeneration [41, 42, 46, 52]. TGF-β is known to be involved in the regulation of Schwann cell population during development via induction of apoptosis, especially after injury [89]. This action of TGF-β has been implicated in the matching of the number of Schwann cells and axons [53]. In contrast, adult Schwann cells in tissue culture media containing serum and cAMP-elevating agents, respond to TGF-β by proliferation, expression of the nonmyelinating phenotype and suppression of myelin-related genes [53, 84]. TGF-β enhances the formation of extracellular matrix, upregulates the expression of collagen type IV mRNA in Schwann cells and prevents disintegration of basal lamina [90, 91]. The important finding of our experiments that forskolin and TGF-β, both of which promote the nonmyelinating growth-supportive phenotype of Schwann cell, "refresh" the nonmyelinating growth-supportive phenotype of the atrophic chronically denervated Schwann cells and dramatically improved their capacity to support axonal regeneration. Many more motoneurons regenerated through the forskolin/TGF-β-treated Schwann cells in vivo than Schwann cells that were exposed only to serum-containing L-15 culture medium in vitro (Fig. 5). We found that a component of the effects of forskolin/TGF-β in converting the chronically denervated Schwann cells to the growth-supportive phenotype include re-expression of the receptors for neuregulin, including erbB2 and erbB3 (Sulaiman and Gordon, unpubl. data). This way, these Schwann cells become responsive to axon-derived neuregulin by proliferation and probably remodeling of the endoneurial pathways. Schwann cell proliferation after nerve injury and reinnervation is associated with upregulation of neurotrophic factors [79]. Hence their proliferative response may also be associated with upregulation of neurotrophic factors such as BDNF and GDNF. In addition, there is evidence that the neurotrophic effect of GDNF is attenuated in the absence of TGF-β [54, 55], hence TGF-β may be exerting its positive effect via augmentation of survival effect of GDNF on injured motoneurons.

Conclusions

Important components of the growth-permissive nonmyelinating phenotype of the Schwann cell are their proliferation and expression of growth factors that include BDNF and GDNF [77]. Our experiments demonstrate that the deleterious effects of (1) chronic axotomy can be reversed via exogenous application of BDNF and GDNF and (2) chronic denervation can be reversed by reactivation of chronically denervated Schwann cells with forskolin/TGF-β, which induces their growth-supportive phenotype, mechanisms of which may be related to their proliferation and/or neurotrophic support via GDNF. Understanding the mechanisms of the effects of forskolin/TGF-β as well as functional outcomes are the objectives of ongoing experiments in our laboratory.

Nonetheless, these experiments demonstrate that the deleterious effects of chronic axotomy and denervation are reversible, which may in turn translate into strategies that can be utilized to promote functional recovery after peripheral nerve injuries.

Acknowledgements. We are grateful to the Canadian Institute of Health Research for their financial support of this work. The experiments formed part of the thesis work carried out by Drs Sulaiman and Boyd toward their PhD degrees. Dr. Gordon is an Alberta Heritage Foundation for Medical Research Senior Scientist.

References

1. Edgar JM, Garbern J (2004) The myelinated axon is dependent on the myelinating cell for support and maintenance: molecules involved. J Neurosci Res 76: 593–598
2. Salzer JL (2003) Polarized domains of myelinated axons. Neuron 40: 297–318
3. Scherer SS (1999) Nodes, paranodes, and incisures: from form to function. Ann NY Acad Sci 883: 131–142
4. Selzer ME (2003) Promotion of axonal regeneration in the injured CNS. Lancet Neurol 2: 157–166
5. Fenrich K, Gordon T (2004) Canadian Association of Neuroscience Review: axonal regeneration in the peripheral and central nervous systems – current issues and advances. Can J Neurol Sci 31: 142–156
6. Schwab ME (2004) Nogo and axon regeneration. Curr Opin Neurobiol 14: 118–124
7. Steward O, Zheng B, Tessier-Lavigne M (2003) False resurrections: distinguishing regenerated from spared axons in the injured central nervous system. J Comp Neurol 459: 1–8

8. Kline DG, Hudson AR (1995) Nerve injuries: operative results for major nerve injuries, entrapments and tumors. Saunders, Philadelphia
9. Sunderland S (1878) Nerve and nerve injuries. Livingstone, Edinburgh
10. Lundborg G (1988) Nerve injury and repair. Churchill Livingstone, New York
11. Fouad K, Pearson K (2004) Restoring walking after spinal cord injury. Prog Neurobiol 73: 107–126
12. Bregman BS, Coumans JV, Dai HN, Kuhn PL, Lynskey J, McAtee M, Sandhu F (2002) Transplants and neurotrophic factors increase regeneration and recovery of function after spinal cord injury. Prog Brain Res 137: 257–273
13. Li Y, Field PM, Raisman G (1997) Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 277: 2000–2002
14. Keyvan-Fouladi N, Li Y, Raisman G (2002) How do transplanted olfactory ensheathing cells restore function? Brain Res Brain Res Rev 40: 325–327
15. Weidner N, Ner A, Salimi N, Tuszynski MH (2001) Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. Proc Natl Acad Sci USA 98: 3513–3518
16. Rafuse VF, Gordon T (1996) Self-reinnervated cat medial gastrocnemius muscles. 1. Comparisons of the capacity for regenerating nerves to form enlarged motor units after extensive peripheral nerve injuries. J Neurophysiol 75: 268–281
17. Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. J Neurosci 15: 3876–3885
18. Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci 15: 3886–3895
19. Gordon T, Hegedus J, Tam SL (2004) Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. Neurol Res 26: 174–185
20. Gordon T, Sulaiman OAR, Boyd JG (2003) Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst 8: 236–250
21. Brown WF, Doherty TJ, Chan M, Andres A, Provost SM (2000) Human motor units in health and disease. Muscle Nerve Suppl 9: S7–18
22. Gordon T, Yang JF, Ayer K, Stein RB, Tyreman N (1993) Recovery potential of muscle after partial denervation: a comparison between rats and humans. Brain Res Bull 30: 477–482
23. Rafuse VF, Gordon T, Orozco R (1992) Proportional enlargement of motor units after partial denervation of cat triceps surae muscles. J Neurophysiol 68: 1261–1276
24. Sulaiman OAR, Gordon T (2000) Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia 32: 234–246
25. Sulaiman OAR, Gordon T (2002) Transforming growth factor-β and forskolin attenuate the adverse effects of long-term Schwann cell denervation on peripheral nerve regeneration in vivo. Glia 37: 206–218
26. Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM (2002) Survival of Schwann cells in chronically denervated skeletal muscles. Acta Neuropathol (Berl) 103: 565–574
27. Holmes WYJZ (1942) Nerve regeneration after immediate and delayed suture. J Anat 77: 63–108
28. Guttmann EYJZ (1944) The re-innervation of muscle after various periods of atrophy. J Anat 78: 15–44
29. Li H, Terenghi G, Hall SM (1997) Effects of delayed reinnervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. Glia 20: 333–347
30. Li H, Wigley C, Hall SM (1998) Chronically denervated rat Schwann cells respond to GGF in vitro. Glia 24: 290–303
31. You S, Petrov T, Chung PH, Gordon T (1997) The expression of the low affinity nerve growth factor receptor in long-term denervated Schwann cells. Glia 20: 87–100
32. Brushart TM (1993) Motor axons preferentially reinnervate motor pathways. J Neurosci 13: 2730–2738
33. Brushart TM (1988) Preferential reinnervation of motor nerves by regenerating motor axons. J Neurosci 8: 1026–1031
34. Al-Majed AA, Neumann CM, Brushart TM, Gordon T (2000) Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. J Neurosci 20: 2602–2608
35. Brushart TM, Mesulam MM (1980) Alteration in connections between muscle and anterior horn motoneurons after peripheral nerve repair. Science 208: 603– 605
36. Thomas CK, Stein RB, Gordon T, Lee RG, Elleker MG (1987) Patterns of reinnervation and motor unit recruitment in human hand muscles after complete ulnar and median nerve section and resuture. J Neurol Neurosurg Psychiatry 50: 259–268
37. Gillespie MJ, Gordon T, Murphy PR (1986) Reinnervation of the lateral gastrocnemius and soleus muscles in the rat by their common nerve. J Physiol London 372: 485–500
38. Dohm S, Streppel M, Guntinas-Lichius O, Pesheva P, Probstmeier R, Walther M, Neiss WF, Stennert E, Angelov DN (2000) Local application of extracellular matrix proteins fails to reduce the number of axonal branches after varying reconstructive surgery on rat facial nerve. Restor Neurol Neurosci 16: 117–126
39. Brushart TM, Hoffman PN, Royall RM, Murinson BB, Witzel C, Gordon T (2002) Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. J Neurosci 22: 6631–6638
40. Avellino AM, Hart D, Dailey AT, MacKinnon M, Ellegala D, Kliot M (1995) Differential macrophage responses in the peripheral and central nervous system during wallerian degeneration of axons. Exp Neurol 136: 183–198
41. Kury P, Stoll G, Muller HW (2001) Molecular mechanisms of cellular interactions in peripheral nerve regeneration. Curr Opin Neurol 14: 635–639
42. Stoll G, Muller HW (1999) Nerve injury, axonal degeneration and neural regeneration: basic insights. Brain Pathol 9: 313–325
43. Dailey AT, Avellino AM, Benthem L, Silver J, Kliot M (1998) Complement depletion reduces macrophage infiltration and activation during Wallerian degeneration and axonal regeneration. J Neurosci 18: 6713–6722
44. Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Mol Neurobiol 27: 277–324
45. Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 14: 67–116
46. Sulaiman OAR, Boyd JG, Gordon T (2005) Axonal regeneration in the peripheral nervous system of mammals. In:

Kettenman H, Ransom BR (eds) Neuroglia, 2nd edn. Oxford University Press Oxford pp 454–466
47. Scherer SS, Salzer JL (2001) Axon-Schwann cell interactions during peripheral nerve degeneration and regeneration. In: Jessen K, Richardson W (eds) Glial cell development: basic principles and clinical relevance. Oxford University Press, Oxford, pp. 299–330
48. Eccleston PA, Jessen KR, Mirsky R (1989) Transforming growth factor-β and γ-interferon have dual effects on growth of peripheral glia. J Neurosci Res 24: 524–530
49. Einheber S, Hannocks MJ, Metz CN, Rifkin DB, Salzer JL (1995) Transforming growth factor-β 1 regulates axon/Schwann cell interactions. J Cell Biol 129: 443–458
50. Stewart HJ, Curtis R, Jessen KR, Mirsky R (1995) TGF-βs and cAMP regulate GAP-43 expression in Schwann cells and reveal the association of this protein with the trans-Golgi network. Eur J Neurosci 7: 1761–1772
51. Stewart HJ, Rougon G, Dong Z, Dean C, Jessen KR, Mirsky R (1995) TGF-βs upregulate NCAM and L1 expression in cultured Schwann cells, suppress cyclic AMP-induced expression of O4 and galactocerebroside, and are widely expressed in cells of the Schwann cell lineage in vivo. Glia 15: 419–436
52. Unsicker K, Strelau J (2000) Functions of transforming growth factor isoforms in the nervous system. Eur J Biochem 267: 6972–6975
53. Jessen KR, Mirsky R (2005) The Schwann cell lineage. Neuroglia 85–100
54. Schober A, Hertel R, Arumae U, Farkas L, Jaszai J, Krieglstein K, Saarma M, Unsicker K (1999) Glial cell line-derived neurotrophic factor rescues target-deprived sympathetic spinal cord neurons but requires transforming growth factor-β as cofactor in vivo. J Neurosci 19: 2008–2015
55. Krieglstein K, Henheik P, Farkas L, Jaszai J, Galter D, Krohn K, Unsicker K (1998) Glial cell line-derived neurotrophic factor requires transforming growth factor-β for exerting its full neurotrophic potential on peripheral and CNS neurons. J Neurosci 18: 9822–9834
56. Krieglstein K, Unsicker K (1996) Distinct modulatory actions of TGF-β and LIF on neurotrophin-mediated survival of developing sensory neurons. Neurochem Res 21: 843–850
57. Boyd JG, Gordon T (2002) A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. Eur J Neurosci 15: 613–626
58. Boyd JG, Gordon T (2003) Glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor sustain the axonal regeneration of chronically axotomized motoneurons in vivo. Exp Neurol 183: 610–619
59. Weskamp G, Reichardt LF (1991) Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. Neuron 6: 649–663
60. Kohn J, Aloyz RS, Toma JG, Haak-Frendscho M, Miller FD (1999) Functionally antagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation. J Neurosci 19: 5393–5408
61. Morris JH, Hudson AR, Weddell G (1972) A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. II. The development of the "regenerating unit". Z Zellforsch Mikrosk Anat 124: 103–130
62. Moir MS, Wang MZ, To M, Lum J, Terris DJ (2000) Delayed repair of transected nerves: effect of brain-derived neurotrophic factor. Arch Otolaryngol Head Neck Surg 126: 501–505
63. Lewin SL, Utley DS, Cheng ET, Verity AN, Terris DJ (1997) Simultaneous treatment with BDNF and CNTF after peripheral nerve transection and repair enhances rate of functional recovery compared with BDNF treatment alone. Laryngoscope 107: 992–999
64. Utley DS, Lewin SL, Cheng ET, Verity AN, Sierra D, Terris DJ (1996) Brain-derived neurotrophic factor and collagen tubulization enhance functional recovery after peripheral nerve transection and repair. Arch Otolaryngol Head Neck Surg 122: 407–413
65. Zhang JY, Luo XG, Xian CJ, Liu ZH, Zhou XF (2000) Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. Eur J Neurosci 12: 4171–4180
66. Namikawa K, Honma M, Abe K, Takeda M, Mansur K, Obata T, Miwa A, Okado H, Kiyama H (2000) Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration. J Neurosci 20: 2875–2886
67. Al-Majed AA, Brushart TM, Gordon T (2000) Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. Eur J Neurosci 12: 4381–4390
68. Kobayashi NR, Bedard AM, Hincke MT, Tetzlaff W (1996) Increased expression of BDNF and trkB mRNA in rat facial motoneurons after axotomy. Eur J Neurosci 8: 1018–1029
69. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H (1992) Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol 119: 45–54
70. Hammarberg H, Piehl F, Risling M, Cullheim S (2000) Differential regulation of trophic factor receptor mRNAs in spinal motoneurons after sciatic nerve transection and ventral root avulsion in the rat. J Comp Neurol 426: 587–601
71. Boyd JG, Gordon T (2001) The neurotrophin receptors, trkB and p75, differentially regulate motor axonal regeneration. J Neurobiol 49: 314–325
72. Sendtner M, Holtmann B, Hughes RA (1996) The response of motoneurons to neurotrophins. Neurochem Res 21: 831–841
73. Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C, Armanini M, Simmons L, Moffet B, Vandlen RA, Simpson LC [corrected to Simmons] (1994) GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. Science 266: 1062–1064
74. Vejsada R, Tseng JL, Lindsay RM, Acheson A, Aebischer P, Kato AC (1998) Synergistic but transient rescue effects of BDNF and GDNF on axotomized neonatal motoneurons. Neurosci 84: 129–139
75. Yuan Q, Wu W, So KF, Cheung AL, Prevette DM, Oppenheim RW (2000) Effects of neurotrophic factors on motoneuron survival following axonal injury in newborn rats. NeuroReport 11: 2237–2241
76. Tsujino H, Mansur K, Kiryu-Seo S, Namikawa K, Kitahara T, Tanabe K, Ochi T, Kiyama H (1999) Discordant expression of c-Ret and glial cell line-derived neurotrophic factor receptor alpha-1 mRNAs in response to motor nerve injury in neonate rats. Brain Res Mol Brain Res 70: 298–303

77. Naveilhan P, ElShamy WM, 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
78. Burazin TC, Gundlach AL (1998) Up-regulation of GDNFR-alpha and c-ret mRNA in facial motor neurons following facial nerve injury in the rat. Brain Res Mol Brain Res 55: 331–336
79. Hoke A, Gordon T, Zochodne DW, Sulaiman OAR (2002) A decline in glial cell line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. Exp Neurol 173: 77–85
80. Dolcet X, Egea J, Soler RM, Martin-Zanca D, Comella JX (1999) Activation of phosphatidylinositol 3-kinase, but not extracellular-regulated kinases, is necessary to mediate brain-derived neurotrophic factor-induced motoneuron survival. J Neurochem 73: 521–531
81. Soler RM, Dolcet X, Encinas M, Egea J, Bayascas JR, Comella JX (1999) Receptors of the glial cell line-derived neurotrophic factor family of neurotrophic factors signal cell survival through the phosphatidylinositol 3-kinase pathway in spinal cord motoneurons. J Neurosci 19: 9160–9169
82. Cao L, Liu L, Chen ZY, Wang LM, Ye JL, Qiu HY, Lu CL, He C (2003) Olfactory ensheathing cells genetically modified to secrete GDNF to promote spinal cord repair. Brain 127: 1–15
83. Wood PM, Cuervo EF, Bunge RP, Gordon T (1998) Functional capacities of longterm denervated Schwann cells. Society for Neuroscience Annual Meeting Abstracts 28: 690.8
84. Jessen KR, Mirsky R (2003) Embryonic Schwann cell development and the initiation of myelination. In: Lazzarini RA (ed) Biology and disorders. Elsevier, San Diego, pp 329–370
85. Martin GR, Timpl R (1987) Laminin and other basement membrane components. Ann Rev Cell Biol 3: 57–85
86. Chernousov MA, Carey DJ (2000) Schwann cell extracellular matrix molecules and their receptors. Histol Histopathol 15: 593–601
87. Chernousov MA, Stahl RC, Carey DJ (2001) Schwann cell type V collagen inhibits axonal outgrowth and promotes Schwann cell migration via distinct adhesive activities of the collagen and noncollagen domains. J Neurosci 21: 6125–6135
88. Giannini C, Dyck PJ (1990) The fate of Schwann cell basement membranes in permanently transected nerves. J Neuropathol Exp Neurol 49: 550–563
89. Parkinson DB, Dong Z, Bunting H, Whitfield J, Meier C, Marie H, Mirsky R, Jessen KR (2001) Transforming growth factor β (TGF-β) mediates Schwann cell death in vitro and in vivo: examination of c-Jun activation, interactions with survival signals, and the relationship of TGF-β-mediated death to Schwann cell differentiation. J Neurosci 21: 8572–8585
90. Ignotz RA, Massague J (1986) Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261: 4337–4345
91. Ignotz RA (1991) TGF-β and extracellular matrix related influences on gene expression and phenotype. Crit Rev Eukaryot Gene Expr 1: 75–84
92. Abercrombie M (1946) Estimation of nuclear population from microtome sections. Anat Rec 94: 239–247