NEUROBIOLOGY

Can Regeneration be Promoted Within the Spinal Cord?

DAMIEN KUFFLER, Ph D

Although regeneration in the peripheral nervous system (PNS) after injury is robust, regeneration in the central nervous system (CNS) is abortive. The results from differences in the balance of regeneration inhibiting and promoting factors, which in the CNS is skewed toward inhibition while in the PNS it is skewed towards promotion of nerve growth. In addition to lacking regeneration promoting factor the CNS has the ubiquitous distribution of factors that inhibit regeneration. PNS Schwann cells release a number of characterized and uncharacterized neurotrophic factors that exert powerful regeneration promoting influences on axons in the PNS. Thus it has been hypothesize that implantation of Schwann cells, or infusion of factors they release into the lesioned spinal cord should lead to CNS regeneration. However, Schwann cell implants alone are not very successful in promoting CNS regeneration Although still limited, improved regeneration takes place when there is the simultaneously inhibition of CNS regeneration blocking factors and the presence of Schwann cell-released factors. To further improve the extent of CNS regeneration we must determine the best combination of neurotrophic factors to infuse into the site of a CNS lesion, as well as be able to characterize and block all CNS regeneration inhibiting factors. This review examines what is known about promoting and inhibiting regeneration in both the PNS and CNS, and the approaches that may allow us to change the cellular environment of the CNS to one that is permissive to and promotes regeneration.

Key words: Central nervous system, Peripheral nervous system, Spinal cord regeneration, Inhibition of regeneration, Human nerve regeneration

During development and regeneration of the nervous system, axons must be promoted to regenerate and navigate to their specific targets through a complex molecular environment. Gradients of target-derived factors can both attract and repel regenerating axons (1-11). GTPases of the Ras family are involved in transducing extracellular signals into responses that lead to directed neurite outgrowth mediated by trkA receptors (12,13). Sprouting of axon collateral branches is important in the establishment and refinement of neuronal connections during both development and regeneration, and neurotrophins provide local cues to stimulate the formation of collateral axon branches (14).

Although the mammalian CNS becomes inhibitory to regeneration following birth and the completion of the formation of synaptic connections, the PNS remains permissive and promotes regeneration (14,15, 16, 17, 18). Embryonic neurons are much less responsive to CNS inhibitory factors than are adult neurons (20, 21). Until recently most experiments studying neurite outgrowth promoting and inhibiting factors have been carried out on embryonic neurons since they are easier to isolate and maintain *in vitro*. However, to demonstrate the effects of eliminating inhibitory factors such as chondroitin sulfate proteoglycans (CSPG), myelin associated glycoprotein (MAG), and other inhibitory factors, or disinhibiting the action of laminin in spinal cord tissues, while simultaneously promoting neurite outgrowth, it is essential to carry out experiments on adult neurons. A number of laboratories have now developed techniques to isolate and maintain adult rat (22, 23, 24) and human (25, 26) dorsal root ganglion neurons in vitro which are excellent models on which to develop techniques for promoting neurite outgrowth and inhibiting CNS outgrowth inhibiting factors, and that have important clinical relevance.

From the Institute of Neurobiology and the Department of Physiology, Medical Sciences Campus, University of Puerto Rico, San Juan, PR.

This study was supported by: ARO DAAL03-90-G-0189, ONR NB-91-12- 1 0 1, NIH 5 PO I NS07464-25, NSF-EPSCoR EHR9108775, DoD NOOO 14-93-1-13 80 and a Veterans Affairs grant.

Address correspondence to: Damien Kuffler, Ph D, Institute of Neurobiology, 201 Blvd. del Valle, San Juan, PR 00901 USA, tel/FAX: 787-721-1235 e-mail: d_kuffler@rcmaca.upr.clu.edu

Promoting PNS regeneration. Differential screening of cDNA libraries of crushed and non-injured rat sciatic nerve have allowed genes to be cloned and identified with specific and unknown functions during degeneration and regeneration of the nervous system. More than 60 genes and their products show a specific temporal pattern of up and down regulation following peripheral nerve lesion (27, 28, 29), including genes encoding for; transcription factors, growth factors and their receptors, cytokines, neuropeptides, myelin proteins, lipid carriers, and cytoskeletal proteins, as well as ECM, and cell adhesion molecules. Although initially postulated that neurotrophins act alone on various populations of neurons, it is now clear that factors up- and down-regulated at different times after nerve section serve different functions, and that more than one neurotrophin, some of which remain unidentified, act separately and simultaneously, at any time on specific neuronal population (30, 31).

Within 3-12 hours of nerve lesion, interleukin-1 (IL-1) bioactivity, as well as mRNA levels of the growth factor and cytokine genes encoding nerve growth factor (NGF) (32, 33, 34, 35), IL-6, and granulocyte-macrophage colony stimulating factor (MG-CSF), growth-associated-protein (GAP) 43/B50, and peripherin (36, 37) are up-regulated dramatically in the distal portion of the nerve. BDNF mRNA expression begins to rise slowly 7 days post nerve lesion (38, 39). In contrast, the mRNA level of neurotrophin NT-3 rapidly declines following nerve lesion (40, 41, 34, 35, 42, 43). While NGF has a potent neurotrophic influence on embryonic and adult sensory and sympathetic neurons, other factors, such as BDNF (22) and CNTF (44) also exert an influence on adult sensory neurons. The neurite outgrowth promoting influence of NT-3 is additive to that of BDNF, although the influence of NT-3 is substantially less than that of BDNF (45). Further, novel PNS neurotrophic factors continue to be found, such as galectin-1 (46) and vascular endothelial growth factor (VEGF) which has neurotrophic actions on cultured adult mouse superior cervical ganglia (SCG) and dorsal root ganglia (DRG) (47).

Neuropoetic factors such as leukemia inhibitory factor (LIF), IL-6 and ciliary neurotrophic factor (CNTF) share signaling pathways, including the IL-6 signal transduction receptor component gp130 (48, 49). LIF is a neurotrophic factor for sensory and motoneurons (49, 50) and within 2 hours of nerve section, its mRNA level increases in Schwann cells adjacent to the lesion site and remains high for about 1 week (51). In contrast, the quantity of mRNA for CNTF, present in large quantities in Schwann cells of non-injured nerves (43), decreases to very low levels in the distal portion of the nerve 4 days after nerve section, remains almost unchanged for approximately 3 weeks, and then begins to rise again (52). Other factors are regulated within various time frames, such as transforming growth factor (TGF)-α1 (maximum expression 4 days post section), TGF-α3 mRNA (expression decreases markedly immediately upon section (53), and insulin-like factors (expression increases significantly within 3 days of section, peaks between day 4 to day 6, and declines during the following 2 weeks) (54,55).

Inhibiting PNS regeneration. Although long accepted that the PNS lacks regeneration inhibiting factors and only possesses regeneration promoting factors, the distal nerve is inhibitory to regeneration for about 1 week, until Wallerian degeneration is complete, and only then does it promote regeneration (56). The inhibition results in part from Schwann cells upregulating their release of neurite outgrowth inhibitors (57) such as CSPG (58, 59, 60). To examine the influences of cell surface, extracellular matrix (ECM), and diffusible factors, in inhibiting and promoting regeneration cryosections of innervated and denervated peripheral nerves have been used as substrates for neurons. Neurons extend neurites of equal lengths on both types of sections, surprising since denervated Schwann cells increase their synthesis of the neurite outgrowth promoter laminin (60). However, this is attributed to the influence of the laminin being inhibited by its association with Schwann cell-derived CSPG (57, 60, 61), since application of exogenous laminin to cultures of neurons on peripheral nerve sections overrides the inhibitory influence of peripheral nerve MAG (62). Although another series of experiments showed that neurite outgrowth on denervated peripheral nerve was twice as great as on non denervated nerve, this growth resulted from the neurites avoiding MAG and CSPG expressing membranes and extending on endoneurial cells of the denervated sections (63). Even though CSPG synthesis remains high during the regeneration process it would appear that axons eventually regenerate once the Schwann cells synthesize and release neurotrophic factors in sufficient amounts to overwhelm the inhibitory influences of the CSPG. PNS regeneration rates can be increased by digesting CSPG (57, 56), or blocking its synthesis with ß-D-Xylosides (56). It has been shown that in spite of the regeneration inhibitory influence of peripheral nerve during the first week post nerve section, its cells actually secrete a sufficient concentration of neurotrophic factors within 1-2 days of sectioning to induce extensive neurite outgrowth in vitro (24, 62, 65).

Inhibiting CNS regeneration. Although axons within the CNS do not regenerate, the portion of these same axons within the PNS regenerate well, as do CNS axons provided a PNS environment (66, 67, 68, 69, 70). Thus, the ability of axons to regenerate depends on their local cellular

environment (70,72,73). CNS regeneration is inhibited by the nearly ubiquitous presence of regeneration inhibiting factors associated with astrocytes, oligodendrocytes, and oligodendrocyte/type 2 astrocyte progenitors (O2A cells) (15, 16, 74, 75).

Oligodendrocytes and their myelin induce contact mediated inhibition of neurite growth from sympathetic and sensory neurons (18, 20). This inhibition is due to 2 minor membrane proteins (MW 35 and 250 kDa) (bNI-22 and NI-35/250) (15, 16), as well as MAG, tenascin-R, and NG-2. The active protein has a mass of 220 kDa and an isoelectric point between 5.9 and 6.2, its inhibitory activity is sensitive to protease treatment, resists harsh treatments like 9 M urea or short (18). Neutralizing these factors in vitro with monoclonal antibodies IN-1 and IN-2 allows neurites to overgrow oligodendrocytes and elongate on a substrate of CNS myelin (16, 20, 18, 76, 77, 78), while in vivo rats with complete bilateral lesions of the cortico-spinal tract treated with IN-1 have massive sprouting through the lesion site (74). Although MAG is another myelin inhibitory component (79, 80, 81, 82, 84, 21), its physiological importance is controversial, since one study reported an enhancement of the rate of axon regeneration in MAG -/- mice (84, 85), while another showed no improvements (86). More recently DRG neurons transplanted into degenerating CNS white matter undergoing Wallerian degeneration extended axons despite contact with the myelin, indicating that degenerating white matter beyond a glial scar has a much greater ability to support axon regeneration than previously thought (87). In addition to blocking neurite growth the myelin-associated protein Nogo-A, an antigen for the monoclonal antibody IN- I (88) raised against CNS NI-35/250 (98) appears to play a role in regulating axon plasticity to maintain the proper targeting of terminal arbors within specific gray matter regions (90).

Although it is generally considered that astrocytes inhibit CNS regeneration there is increasing evidence to indicate that they possess both axon-growth promoting and axon-growth inhibitory properties (91). Astrocytes express known neurite-outgrowth promoting molecules such as laminin, fibronectin and N-cadherin as well as the growth inhibitory molecules tenascin and chondroitin sulphate proteoglycan, plasminogen activator, plasminogen activator inhibitor activity, and growth cone collapsing activity. These findings suggest that the functional differences between the permissive and the inhibitory astrocyte cell lines resides largely within their ECM. The dorsal root entry zone (DREZ) forms the junction between the dorsal roots of the PNS and the spinal cord (CNS). In rats older than 1 week, reactive astrocytes of the DREZ stop lesioned primary sensory axons from elongating (92, 93, 94, 95, 96, 97 , 98, 99, 100, 101) due to an impenetrable membrane bound molecular barrier of CSPG, composed of cytotactin/tenascin (CT) and chondroitin 6-sulfate-containing proteoglycans (C-6S-PG) (96), which also inhibit Schwann cell invasion of the CNS (96). CNS injury also induces reactive astrocytes to form regeneration inhibitory astrocytic scars (96, 97, 87), where neurites that fail to regenerate are enclosed by CSPG (95, 97). Reactive astrocytes in vitro express high levels of CSPG and are inhibitory to neurite growth, but become permissive when treated with glycosaminoglycan (GAG)-degrading enzymes (94), suggesting that CSPG is a primary regeneration inhibitory molecule (95, 97). In adult rats a lesion of the entorhinal cortex causes reactive astrocytes to express neuronal CSPG neurocan. However, they to not prevent regeneration but serve as boundaries that determine the path along which the axon regenerate (100, 102). Neurons grafted atraumatically into adult rat white matter tracts regenerate their axons, while grafts associated with trauma do not (97, 87). Thus astrocytes can inhibit axon growth (103, 104) once trauma makes them reactive (97). Reactive astrocytes are phenotypically diverse and vary in their ability to support neurite outgrowth (105, 106, 91, 107). The Neu7 astrocyte cell line is the most inhibitory, apparently due to its expression of CSPGs (91) among which are NG2, versican, and the proteoglycan(s) recognized by the CS-56 antibody, which are at higher levels than the other cell lines (108). Versican, which in vitro appears on astrocytes in patches, is not avoided by extending neurites (108). In some astrocytes and O2A cells NG2 synthesis is rapidly upregulated after injury and forms a dense NG2-rich network (108). The inhibitory influence of membrane bound and diffusible NG2 is eliminated by digesting the core protein, or removing the CS chains (109, 108). Disrupting proteoglycan sulfation with sodium chlorate increases axon growth through three-dimensional cultures of primary astrocytes (109). The inhibitory influence of astrocytes can also be eliminated by inhibiting the synthesis or proteoglycan with ß-D-Xylosides (109). This also reduces the ability of astrocytes to form boundaries that repel axons (107). Thus, although several CSPGs are differentially expressed by astrocytes, NG2 and its side chains appear to be the most important obstacle to axon regeneration (110, 111, 112).

Specific similarities and differences between oligodendrocytes and astrocytes. Oligodendrocytes and astrocytes both inhibit CNS regeneration via proteoglycans, particularly those with CS side chains (113, 114, 110), cell surface factors, and constituents of the ECM (111, 112, 114, 115, 116, 117, 118). However the specific inhibitory factors differ, with astrocyte versican

not being inhibitory (59, 108), while oligodendrocyte versican is a potent inhibitor (119), likely due to the two populations expressing different isoforms of versican (V1 for astrocytes and the V2 splice variant for oligodendrocytes). Bovine oligodendrocyte myelin also contains a neurite growth-inhibitory activity associated with CSPG that is ascribed to another CSPG, brevican (119). Oligodendrocytes and astrocytes also differ in that for oligodendrocytes the CSPG core protein is a potent inhibitor (120, 110), although its associated CS side chains are not, since their elimination does not reduce inhibition (119). However, digestion of astrocyte CS side chains eliminates inhibition (107). Thus any attempt to change the environment of the CNS from regeneration inhibitory to permissive/promoting requires blocking or inactivating a number of different factors on a variety of cell types.

Pro-inflammatory factors improve CNS recovery following trauma. Trauma-induced lesions in the brain and spinal cord induce a complex cascade of cellular reactions at the local lesion site including leukocyte infiltration, blood-brain barrier (BBB) breakdown, secondary tissue death, inflammatory reactions, as well as scar and cavity formation (121, 122). These phenomena are more pronounced in the spinal cord than in the praenchyma of the brain (121, 122). Transcription of pro-inflammatory cytokines TNF alpha and IL-1, as well as MIP-1 alpha and MIP-l beta is upregulated within the first hour following injury (123). Resident CNS cells, probably microglial cells and not peripheral inflammatory cells, are the primary source of cytokines and chemokine mRNA (123). Animals receiving a cytokine mixture of interleukin-1 beta (IL-l beta), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF alpha) within 1 day of CNS trauma showed a smaller amount of tissue loss 7 days after the trauma than animals receiving Ringer solution (124). The inflammatory reaction of the spinal cord has serious detrimental consequences and blocking the formation of inflammation, or reducing it is critical for functional recovery. Infusion of the glucocorticosteroid, methylprednisolone (MP), within 8 h of a spinal cord transection in the adult rat gives rise to improved neurological recovery as well as reducing both spinal tissue loss and dieback of vestibulospinal fibers, and causing a transient sprouting of vestibulospinal fibers near the lesion at 1 and 2 weeks post-lesion (125). However the relationships between the inflammatory changes, spinal tissue sparing, and axonal survival and sprouting are complex and not well understood.

Vascularization improves CNS. Following CNS trauma increased vascularization appears to improve CNS regeneration, but such increased vascularization is limited. Although 4 days following spinal cord trauma there is a significant increase in the number of blood vessels present at the lesion site, after 1 week the number of vessels declines (126). Thus, although physiologically there are significant initial attempts to repair disrupted vasculature following trauma it is not fully completed and does not lead to the restoration of a compact tissue and it does not prevent the subsequent formation of caverns (126). The expression of VEGF mRNA, which is required for vascular formation, is restricted to fibre tracts precisely in the areas where the changes in the vasculature are observed later on (123). These results suggest that experimental attempts to induce sustained vascularization in the region of spinal cord trauma may improve the success of CNS regeneration following trauma.

Alternative Approaches for Promoting CNS Regeneration and Their Relative Benefits

1. Inactivating growth inhibitory factors in the CNS

A. Infusion of antibodies that neutralize growth inhibitory molecule bioactivity. Infusion into lesioned adult rat spinal cords of the IN-1 antibody that neutralizes the biological activity of the growth inhibitory Nogo protein induces limited behavioral recovery in motor and sensory tests (77, 74, 78, 127, 88). If and when additional inhibitory factors are found infusion of antibodies against, them plus the IN-1 antibody, should allow additional regeneration to take place.

B. Degrading regeneration inhibiting factors or down regulating their synthesis.

i. Neurons synthesize and transport metallo-proteinase-2 (MMP-2) to their growth cones where it is released and degrades the neurite growth inhibiting of CSPG (56). The membrane-bound MMP-2 activity (C6-MP) shares several biochemical and pharmacological characteristics with MT-1 -MMP (128), and enables rat and human glioblastoma cells to migrate into CNS white matter which they would otherwise do not do (129). Thus proteolytic inactivation of CSPG and myelin associated growth inhibitory proteins by growth cone secreted MMP-2 would expose ECM laminin, that is otherwise masked by CSPG, making the substrate neurite outgrowth promoting (113). In a synthetic ECM NGF induces metalloproteinase-dependent neurite growth (131). These results suggest that induction of an increased synthesis and release of metalloproteinase activity from growth cones of CNS neurons would therefore

degrade growth inhibitory factors and lead to CNS regeneration.

ii. Exposure of isolated lengths of PNS nerve to chondroitinase ABC (C-ABC) degrades CSPG and, enhances neurite growth on these nerves in vitro (56). Exposure of DRG neuron-associated Schwann cells with their CSPG rich membranes to C-ABC eliminates the CSPG inhibition of neurite growth (132). Thus infusion of dorsal roots, the DREZ, and the CNS with C-ABC should allow the regeneration of DRG axons into and within the CNS (132).

C. Down-regulating the synthesis of CNS growth inhibiting factors. ß-D-Xylosides blocks the synthesis of CSPG and systemic injection of ß-D-Xylosides into adult rats enhances the rate of PNS nerve regeneration (56). Exposure of oligodendrocytes in vitro to ß-D-Xylosides blocks their surface presentation of brevican and versican V2 resulting in the elimination of growth cone collapse following contact with these oligodendrocytes (119). Therefore systemic injection of ß-D-Xylosides, or its infusion into the site of a CNS lesion should allow regeneration through the lesion.

2. Eliminating growth cone sensitivity to CNS growth inhibitory factors.

A. Elevating a neuron's intracellular cAMP concentration in vitro by neurotrophins or dbAMP before they encounter neurite growth inhibitory factors eliminates their sensitivity to the growth inhibitors MAG and myelin (21). Thus elevating the concentration of neuronal cAMP in the region of a CNS lesion should allow the injured axons to regenerate within the CNS.

B. For adult dorsal root ganglion neurons, even though they remain in contact with the growth inhibitory CSPG of Schwann cells, the inhibition of the CSPG can be reduced by exposing the neurons to several neurotrophins presented singly (NGF, BDNF, NT-3) and further reduced when these neurotrophins are present simultaneously (132). However, exposure to a complex combination of neurotrophic and other factors completely abolishes the CSPG inhibitory influence while simultaneously inducing massive neurite growth (132). These are exciting results since CSPG appears to be a major growth inhibitor in the dorsal roots, DREZ and CNS. Thus infusion of the appropriate combination of neurotrophic factors should completely eliminate the growth inhibition of CSPG and both allow and induce significant regeneration into and within the CNS.

3. Grafting regeneration supporting cells into the CNS

Schwann cells appear to release most of the neurotrophic and substrate bound factors necessary to permit, promote, and direct axon outgrowth in the PNS (133, 24, 14, 9, 65, 11). Therefore they have been considered ideal candidates for implanting into the CNS, or as bridges between lesioned CNS regions. These implants induce neuron survival, promote sprouting, and myelinate CNS processes and can lead to functional recovery via the release of diffusible neurotrophic factors and/or growth promoting substrates (32, 134, 135, 136, 137, 138, 139, 81, 140, 141, 142,143, 144). Other cells such as ensheathing cells cultured from adult rat olfactory bulb have also been successful in promoting axons to regenerate through the implants which then continue to regenerate into the denervated caudal spinal cord tract (140). Such bridges are successful especially when spinal cord lesion causes a gap that must be bridged by the regenerating axons. Cells genetically modified to release a variety of factors are an additional means of providing the lesioned CNS with a local regeneration promoting environment. Implantation of genetically modified Schwann cells bring about improved neuron survival, axon growth and myelination (145, 146). Transplants of primary autologous cells genetically modified to produce NGF prevent injury-induced degeneration of cholinergic neurons (147) while genetically modified fibroblasts engineered to produce NGF and BDNF accelerate recovery from traumatic spinal cord injury in the adult rat (148, 49, 150). However, it has also been reported that the grafted cells alone neither support regeneration of injured cortical spinal tract fibers or prevent die-back of CNS neurites after injury (151). However when these Schwann cell grafts are accompanied by the IN-1 antibody some CNS neuron sprouting occurs, although neurite die-back continues (151). If Schwann cell grafts are accompanied by acidic fibroblast growth factor (aFGF) nerve regeneration into the grafts is supported and neurite die-back is reduced (151). Although these results are encouraging because they indicate that Schwann cells can synthesize and release all the factors necessary to promote CNS regeneration they suggest that once implanted into the CNS they are prevented from doing so. Most likely factors released from astrocyte and other cells that are known to change the number, type, and ratio of Schwann cell-released factors prevent the Schwann cells from synthesizing and releasing all the factors required to promote CNS regeneration (152). Therefore to promote CNS regeneration Schwann cell grafts must be accompanied by the simultaneous infusion of additional growth promoting factors.

4. Infusion of neurotrophic factors

Receptors to BDNF, NT-3 and CNTF have been localized in the CNS (153, 1543, 155, 156, 157), neurotrophin mRNA is present in the brain of adult mice (45), and small amounts of the neurotrophins BDNF and

NT-3 (158) are present in adult brain. These findings suggest that one challenge for the CNS in its ability to regenerate is the lack of sufficient growth promoting factors. Although the introduction of either NGF (135, 158, 159, 160, 161), and NT-3 (162, 163) into the CNS induce axon regeneration, the simultaneous introduction of multiple exogenous neurotrophins (such as combinations of NGF, BDNF, NT-3, CNTF, and NT-4) (141, 155, 159, 161, 162, 164, 166, 167, 168, 169, 170), induces more extensive regeneration within the spinal cord than any neurotrophic factor alone.

5. Infusion of neurotrophic factors combined with Schwann cell grafts.

Induction of CNS regeneration by Schwann cell grafts accompanied by aFGF (151) indicates that additional factors are required for more successful regeneration. Infusion of BDNF and NT-3 into the CNS simultaneously with implantation of a Schwann cell graft induces more regeneration than the Schwann cells alone or when they are combined with a single neurotrophic factor (141, 142, 171, 172). Thus the use of even larger numbers of neurotrophins would most likely bring about an even greater amount of CNS regeneration.

6. Infusion of Schwann cell-released neurotrophic factors combined with factors that block CNS growth inhibiting factors.

The success of infusing the CNS with the IN-1 antibody or PNS neurotrophic factors in promoting CNS regeneration suggests that combining the two approaches would further improve CNS regeneration. The simultaneous infusion of the IN-1 antibody with the infusion of NT-3 yields significantly better CNS regeneration than either alone (173). Further experiments are required to test the regeneration promoting influence of combinations of IN-1 and other factors that block CNS growth inhibiting factors, such as C-ABC, and ß-D-Xylosides, together with neurotrophic factors known to promote CNS regeneration alone: NGF (135, 159, 81, 161,) , NT-3 (163), or in combinations, such as or BDNF, NT-3, CNTF, and NT-4 (141, 142, 158, 159, 161, 162, 164, 166, 167, 168, 169, 170).

7. Genetically modified cells as implants to promote CNS regeneration.

As mentioned, a limitation of implanting Schwann cells into the CNS it that the release of their entire complement of neurotrophic and other factors is modified by factors in the CNS environment. To avoid this difficulty attempts have been made to engineer cells that continuously secrete the factors considered important to promote CNS regeneration such as NGF (150, 174, 164, 171, 175,), BDNF and NT-4/5 (176), and NGF, BDNF, NT-3, or basic FGF (177). Although these cells enhance CNS regeneration their influence is limited. However this in not surprising since one week after Schwann cells have been denervation they release a minimum of 7 identified neurotrophic factors, all of which must be present simultaneously to achieve optimal growth promotion (178). Further, following their denervation, more than 60 genes cells are up and down regulated in denervated Schwann and each has its own temporal pattern or regulation (27, 28, 29). Thus for genetically modified cells to be maximally effective they must be engineered to release these same factors, in the appropriate ratios, and time sequence.

8. An optimal approach?

Infusion of Schwann cell-released factors. Each of the approaches discussed induces some, although often limited, regeneration of the lesioned CNS, and when several techniques are combined they appear to further improve CNS regeneration over what is induced by any single technique. Therefore optimal regeneration should be induced by combining a number of the discussed approaches. One combination that appears most promising is infusing a mixture of factors known to block the different CNS growth inhibiting factors while simultaneously infusing the CNS with a cocktail of physiologically released Schwann cell factors the composition of which changes over time. To produce this exact and ever changing cocktail of Schwann cell-released factors would be extremely difficult task. However an alternative and initial approach would be to continuously harvest the Schwann cell released factors from Schwann cell conditioned medium (CM), from host-derived cultured Schwann cells, and infuse this cocktail into the lesioned spinal cord.

Conclusion

Our increasing understanding of the factors that inhibit CNS regeneration and those that promote PNS regeneration, is allowing us to design new approaches by which to change the CNS environment from one that it inhibitory to one that is both permissive to and promotes regeneration. Thus we can block the action of factors that inhibit CNS regeneration while simultaneously introducing factors that promote CNS regeneration. This has lead to exciting experiments in which regeneration can be induced in adult rats following spinal lesions allowing them to walk. To improve recovery further requires additional research to determine whether the CNS contains yet unknown factors regeneration inhibiting factors that must be blocked, and whether additional regeneration promoting factors can be found. Thus we have finally come to the point where there is real promise

that in the not too distant future those suffering from neurological deficits from spinal cord and brain injuries will be able to recover some if not many of their lost functions.

Resumen

A pesar de que la regeneración del sistema nervioso periférico (SNP) luego de una lesión es sólida, la regeneración de sistema nervioso central (SNC) no se completa. Esto se debe a las diferencias en el balance de los factores que inhiben y promueven la regeneración, que en el SNC están dirigidos hacia la inhibición, mientras que en el SNP se desvian hacia la promoción del crecimiento del nervio. Además de carecer del factor para promover la regeneración, el SNC tiene una distribución ubicua de factores de distribución que inhiben la regeneración. Las células de Schwann del SNP liberan un número de factores neurotróficos caracterizados y no caracterizados que ejercen una poderosa influencia en inducir la regeneración en los axones del SNP. Por lo tanto, se han elaborado hipótesis sobre la implantación de células de Schwann o de factores de infusión que estos liberan hacia la médula espinal lesionada, los que deberían conducir a la regeneración de SNC. Aunque aún está limitado, ocurre una mejor regeneración cuando ocurre inhibición simultánea de los factores de bloqueo de la regeneración y la presencia de factores liberadores células de Schwann. Para mejorar más el alcance de la regeneración del SNC, debemos determinar la mejor combinación de los factores neurotróficos para infundir en el lugar de la lesión en SNC. En este artículo se revisa lo que se conoce sobre la inhibición y la promoción de la regeneración, tanto en el SNP y SNC así como los acercamientos que nos permitirán cambiar el ambiente celular del SNC a uno que permita y promueva la regeneración.

References

1. Baier H, Bonhoeffer F. Axon guidance by gradients of a target-derived component. Science 1992;255:472-475.
2. Colamarino S A, Tessier-Lavigne M. The axonal chemoattractant netrin-1 is also a chemorepellant for trochlear motor axons. Cell 1995;8:621-629.
3. Kennedy T E, Serafini T, de La Torre J R, Tessier-Lavigne M. Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. Cell 1994;78: 425-436.
4. Kuffler D P. Chemoattraction of sensory neuron growth cones by diffusible concentration gradients of acetylcholine. Mole Chem Neuropathy 1996;28:199-208.
5. Messersmith E K, Leonardo E D, Shatz C J, Tessier-Lavigne M, Goodman CS, Kolodkin AL. Semaphorin III can function as a selective chemorepellant to pattern sensory projections in the spinal cord. Neuron 1995;14:949-959.
6. Sato M, Lopez-Mascaraque L, Heffner C D, O'Leary D D M. Action of a diffusible target-derived chemoattractant on cortical axon branch induction and directed growth. Neuron 1994;13:791-803.
7. Song H, Ming G, Poo M. cAMP-induced switching in turning direction of nerve growth cones. Nature 1997;388:277-279.
8. Song H, Ming G, He l, Lehman M, McKerracher L, Tessier-Lavigne M, Poo M. Conversion of neuronal growth cone response from repulsion to attraction by cyclic nucleotides. Science 1998;281:1515-1518.
9. Pérez N L, Sosa M S, Kuffler D P. Growth cones turn up concentration gradients of diffusible peripheral target-derived factors. Exp Neurol 1997;145:196-202.
10. Hill E, Latalladi G, Kuffler D P. Diffusible concentration gradients of target-derived factors direct motor axon regeneration in vitro. Neurosci Lett 2000;277:87-90.
11. Zheng M, Kuffler D P. Regenerating axons in vivo grow up concentration gradients of target-derived factors. J Neurobiol 2000;42:212-219.
12. Gallo G, Letourneau P C. Localized sources of neurotrophins initiate axon collateral sprouting. J Neurosci 1998a;18:5403-5414.
13. Gallo G, Letourneau P C. Axon guidance: GTPases help axons reach their targets. Curr Biol 1998b;8:R80-82.
14. Gallo G, Lefcort F B, Letourneau P C. The trkA receptor mediates growth cone turning toward a localized source of nerve growth factor. J Neurosci 1997;17:5445-5454.
15. Caroni P, Schwab M E. Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. Neuron 1988a;1:85-96.
16. Caroni P, Schwab M E. Two membrane fractions from rat central myelin with inhibitory properties of neurite growth and fibroblast spreading. J Cell Biol 1988b;106:1281-1288.
17. Caroni P, Savio T, Schwab M E. Central nervous system regeneration: oligodendrocytes and myelin as non-permissive substrates for neurite growth. Prog Brain Res 1988;78:363-370.
18. Schwab M E, Kapfhammer J P, Bandtlow C E. Inhibitors of neurite growth. Ann Rev Neurosci 1993;16:565-595.
19. Spillmann A A, Bandtlow C E, Lottspeich F, Keller F, Schwab M E. Identification and characterization of a bovine neurite growth inhibitor (bNI-220). J Biol Chem 1998;273:19283-19293.
20. Bandtlow C, Zachleder T, Schwab M E. Oligodendrocytes arrest neurite growth by contact inhibition. J Neurosci 1990;10: 3837-3848.
21. Cai D, Shen Y, DeBellard M, Tang S, Filbin M T. Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. Neuron 1999;22:89-101.
22. Lindsay R M. Nerve growth factors (NGF, BDNF) enhance axonal regeneration but are not required for survival of adult sensory neurons. Neuroscience 1988;8:2394-2405.
23. Lankford K R, Rand M B, Waxman S G, Caccias J D. Blocking Cao+ mobilization with thapsigargin reduces neurite initiation in cultured adult rat DRG neurons. Brain Res Dev Brain Res 1995;84:151-163.
24. Dobretsov M, Dobretsov A, Kuffler D P. Influence of factors released from sciatic nerve on adult dorsal root ganglion neurons. J Neurobiol 1994;25:1249-1266.
25. Valeyev A Y, Hackman J C, Wood P M, Davidoff R A. Pharmacologically novel GABA receptor in human dorsal root ganglion neurons. J Neurophysiol 1996;76:3555-3558.
26. Sosa I J, Reyes O, Inserni J, Kuffler D P. Isolation and long-term survival of adult human sensory neurons in vitro. Neurosurg 1998;42:681-686.
27. Gillin C, Gleichmann M, Spreyer P, Muller H W. Differentially

expressed genes after peripheral nerve injury. J Neurosci Res 1995;42:159-171.
28. Gillen C, Korfhage C, Muller H W. Gene expression in nerve regeneration. Neuroscientist 1997;3:112-122.
29. Zigmond R E. LIF, NGF, and the cell body response to axotomy. Neuroscientist 1997;3:176-185.
30. Sorenson E J, Windebank A J. Relative importance of basement membrane and soluble growth factors in delayed and immediate regeneration of rat sciatic nerve. J Neuropath Exp Neurol 1993;52:216-222.
31. Gu X, Thomas P K, King R H M. Chemotropism in nerve regeneration studied in tissue culture. J Anat 1995;186:153-163.
32. Richardson P M, Ebendal T. Nerve growth activities in rat peripheral nerve. Brain Res 1982;246:57-64.
33. Skene J H P, Shooter E M. Denervated sheath cells secrete a new protein after nerve injury. Proc Natl Acad Sci USA 1983;80:4169-4173.
34. Heumann R, Lindholm D, Bandtlow C, Meyer M, Radeke M J, Misko T P, Shooter E, Thoenen H. Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. Proc Natl Acad Sci (USA) 1987;84:8735-8739.
35. Heumann R, Korshung S, Bandtlow C, Thoenen H. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. J Cell Biol 1987;104:1623-1631.
36. Van der Zee C E, Nielander H B, Vos J P, Lopes da Silva S, Verhaagen J, Oestreicher A B, Schrama L H, Schotman P, Gispen W H. Expression of growth associated protein B-SO (GAP43) in dorsal root ganglia and sciatic nerve during regenerative sprouting. J Neurosci 1989;9:3505-3512.1.
37. Wong J, Oblinger M M. Differential regulation of peripherin and neurofilament gene expression in regenerating rat DRG neurons. J Neurosci Res 1990;27:332-341.
38. Funakoshi H, Frisen J, Barbany G, Timmusk T, Zachrisson O, Verge V M, Persson H. Differential expression of mRNase for neurotrophins and their receptors after axotomy of the sciatic nerve. J Cell Biol 1993;123:455-465.
39. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. Cell Biol 1992;119:45-54
40. Funakoshi H, Frisen J, Barbany G, Timmusk T, Zachrisson O, Verge V M, Persson H. Differential expression of mRNase for neurotrophins and their receptors after axotomy of the sciatic nerve. J Cell Biol 1993;123:455-465.
41. Bolin L M, Verity A N, Silver J E, Shooter E M, Abrams J S. Interleukin-6 production by Schwann cells and induction in sciatic nerve injury. J Neurochem 1995;64:850-858.
42. Saade A, Reichert F, Rotschenker S. Granulocyte macrophage colony stimulating factor produced in lesioned peripheral nerves induces the up-regulation of cell surface expression of MAC-2 by macrophages and Schwann cells. J Cell Biol 1996;133:159-167.
43. Friedman B, Scherer S S, Rudge J S, Helgren M, Morrisey D, McClain J, Wang D Y, Wiegand S J, Furth M E, Lindsay R M et al. Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells in vivo. Neuron 1992;9:295-305.
44. Apfel S C, Arezzo J C, Moran M, Kessler J A. Effects of administration of ciliary neurotrophic factor on normal motor and sensory peripheral nerves in vivo. Brain Res 1993;604:106-116.1.
45. Hofer M, Pagliusi S R, Hohn A, Leibrock J, Barde YA. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 1990;9:2459-2464.
46. Honie H, Voshimasa Y, Sohma Y, Nozawa R, Okawa K, Hasegawa M, Muramatsu N, Kawano H, Horie M, Koyama H, Sakai I, Takeshita K, Kowada Y, Takano M, Kadoya T. Galectin-1 regulates initial axonal growth in peripheral nerve after axotomy. J Neurosci 1999;19:9964-9974.
47. Sondell M, Lundborg G, Kanje M. Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. J Neurosci 1999;19:5731-5740.
48. Ip N Y, Nye S H, Boulton T G, Davis S, Taga T, Li Y, Birren S J, Yasukawa K, Kishimoto T, Anderson D J, et al. CNTF and LIF act on possible transcriptional regulatory factors. Science 1992;69:1121-1132.
49. Patterson P H. Leukemia inhibitory factor, a cytokine at the interface between neurobiology and immunobiology. Proc Natl Acad Sci (USA) 1994;91:7833-7835.
50. Rao M S, Sun Y, Escary J L, Perreau J, Tresser S, Patterson P H, Zigmond R E, Brulet P, Landis S C. Leukemia inhibitory factor mediates an injury response but not a target-directed developmental transmitter switch in sympathetic neurons. Neuron 1993;11: 1175-1185.
51. Curtis R, Scherer S S, Somogyi R, Adryan K M, Ip N Y, Zhu Y, Lindsay R M, DiStefano P S. Retrograde axonal transport of LIF is increased by peripheral nerve injury; correlation with increased LIF in distal nerve. Neuron 1994;12:191-204.1.
52. Sendtner M, Stöckli K A, Thoenen H. Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during regeneration. J Cell Biol 1992;118:139-148.
53. Scherer S S, Kamholz J, Jakowlew S B. Axons modulate the expression of transforming growth factors betas in Schwann cells. Glia 1993;8:265-276.
54. Cheng H L, Randolph A, Yee D, Delafontaine P, Tennekoon G, Feldman G. Characterization of insulin-like growth factor-1 and its receptor and binding proteins in transected nerves and cultured Schwann cells. J Neurochem 1996;66:525-536.
55. Pu S F, Zhuang H X, Ishii D N. Differential spatio-temporal expression of insulin like growth factor genes in regenerating sciatic nerves. Mol Brain Res 1995;34:18-28.
56. Zuo J, Hernandez Y J, Muir D. Chondroitin sulfate proteoglycan with neurite-inhibiting activity is up-regulated following peripheral nerve injury. J Neurobiol 1998;34:41-54.
57. Muir D, Engvall E, Varon S, Manthorpe M. Schwannoma cell-derived inhibitor of the neurite-promoting activity of laminin. J Cell Biol 1989;109:2353-2362.
58. Braunewell K H, Pesheva P, McCarthy J B, Furcht L T, Schmitz B, Schachner M. Functional involvement of sciatic nerve-derived versican- and decorin-like molecules and other chondroitin sulphate proteoglycans in ECM-mediated cell adhesion and neurite outgrowth. Eur J Neurosci 1995;7:805-814.
59. Braunewell K H, Martini R, LeBaron R, Kresse H, Faissner A, Schmitz B, Schachner M. Up-regulation of a chondroitin sulphate epitope during regeneration of mouse sciatic nerve: evidence that the immunoreactive molecules are related to the chondroitin sulphate proteoglycans decorin and versican. Eur J Neurosci 1995;7:792-804.1.
60. Zuo J, Neubauer D, Dyess K, Ferguson T A, Muir D. Degradation of chondroitin sulfate proteoglycan enhances the narrator promoting potential of spinal cord tissue. Exp Neurol 1998;154:654-662.
61. Zuo J, Ferguson T A, Hernandez Y J, Stetler-Stevenson W G, Muir D. Neuronal matrix metalloproteinase-2 degrades and inactivates a neurite-inhibiting chondroitin sulfate proteoglycan. J Neurosci 1998;18:5203-5211.
62. David S, Braun P E, Jackson D L, Kottis V, McKerracher L. Laminin overrides the inhibitory effects of peripheral nervous system and central nervous system myelin-derived inhibitors of neurite growth. J Neurosci Res 1995;42:594-602.
63. Agius E, Cochard P. Comparison of neurite outgrowth induced by

intact and injured sciatic nerves: a confocal and functional analysis. J Neurosci 1998;18:328-338.
64. Kuffler D P, Megwinoff O. Neurotrophic influence of denervated sciatic nerve on adult dorsal root ganglion neurons. J Neurobiol 1994;251267-1282.
65. Xie F, Latalladi G, Kuffler D P. Neurotrophic influence of sciatic nerve-released factors on adult motor neurons in vitro. J Periph Nervous Sys 1998;3:1-10.
66. Richardson P M, McGuinness U M, Aguayo A J. Axons from CNS neurons regenerate into PNS grafts. Nature 1980;284: 264-265.
67. Weinberg E L, Raine C S. Reinnervation of peripheral nerve segments implanted into the rat central nervous system. Brain Res 1980;198:1-11.
68. David S, Aguayo A J. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. Science 1981;214:931-933.
69. Aguayo A J, David S, Bray G M. Influences of the glial environment on the elongation of axons after injury: transplantation studies in adult rodents. J Exp Biol 1981;95:231-234.
70. Benfey M, Aguayo A J. Extensive elongation of axons from rat brain into peripheral nerve grafts. Nature. 1982;296:150-152.
71. So K F, Aguayo A J. Lengthy regrowth of cut axons from ganglion cells after peripheral nerve transplantation into the retina of adult rats. Brain Res 1985;328:349-354.
72. Baehr M, Bunge R P. Functional status influences the ability of Schwann cells to support adult rat retinal ganglion cell survival and axonal regrowth. Exp Neurol 1989;106:27-40.
73. Fawcett J W, Keynes R J. Peripheral nerve regeneration. Annu Rev Neurosci 1990;13:43-60.
74. Schnell L, Schwab M E. Axon regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. Nature 1990;343:269-272.
75. Cadelli D S, Bandtlow C E, Schwab M E. Oligodendrocyte and myelin-associated inhibitors of neurite outgrowth: Their involvement in the lack of CNS regeneration. Exp Neurol 1992; 115:189-192.
76. Bregman B S, Kunkel-Bagden E, Schnell L, Dai N H, Gao D, Schwab M E. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 1995;378:498-501.
77. Thallmair M, Metz G A, Z'Graggen W J, Raineteau O, Kartje G L, Schwab M E. Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions. Nat Neurosci 1998;1:124-131.
78. Z'Graggen W, Metz G A, Kartje G L, Thallmair M, Schwab M E. Functional recovery and enhanced corticospinal plasticity after unilateral pyramidal tract lesion and blockade of myelin-associated neurite growth inhibitors in adult rats. J Neurosci 1998;18:4744-4757.
79. McKerracher L, David S, Jackson D L, Kottis V, Dunn R J, Braun P E. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 1994;13: 80-811.
80. Mukhopadhay G, Doherty P, Walsh F S, Crocker P R, Filbin M T. A novel role for myelin associated glycoprotein as an inhibitor of axonal regeneration. Neuron 1994;13:757-767.
81. Li Y, Raisman G. Schwann cells induce sprouting in motor and sensory axons in the adult rat spinal cord. J Neurosci 1994;14:4050-4063.
82. Shen Y J, DeBellard M E, Salzer J L, Roder J, Filbin M T. Myelin-associated glycoprotein in myelin and expressed by Schwann cells inhibits axonal regeneration and branching. Mol Cell Neurosci 1998;12:79-91.
83. Shibata A, Wright M V, David S, McKerracher L, Braun P E, Kater S B. Unique responses of differentiating neuronal growth cones to inhibitory cues presented by oligodendrocytes. J Cell Biol 1998;142:191-202.
84. Li Y, Raisman G. Sprouts from cut corticospinal axons persist in the presence of astrocytic scarring in long-term lesions of the adult rat spinal cord. Exp Neurol 1995;134:102-111.
85. Vanek P, Thallmair M, Schwab M E, Kapfhammer J P. Increased lesion-induced sprouting of corticospinal fibers in the myelin-free rat spinal cord. Eur J Neurosci 1998;10:45-56.
86. Bartsch U, Bandtlow C E, Schnell L, Bartsch S, Spillmann A A, Rubin B P, Hillenbrand R, Montag D, Schwab M E, Schachner M. Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. Neuron 1995;15:1375-1381.
87. Davies S J, Goucher D R, Doller C, Silver J. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. J Neurosci 1999;19:5810-5822.
88. Chen M S, Huber A B, van der Haar M E, Frank M, Schnell L, Spillmann A A, Christ F, Schwab M E. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 2000;403:434-439.
89. Thallmair M, Metz G A, Z'Graggen W J, Raineteau O, Kartje G L, Schwab M E. Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions. Nat Neurosci 1998;1:124-131.
90. Buffo A, Zagrebelsky M, Huber A B, Skerra A, Schwab M E, Strata P, Rossi F. Application of neutralizing antibodies against NI-35/ 250 myelin-associated neurite growth inhibitory proteins to the adult rat cerebellum induces sprouting of uninjured Purkinje cell axons. J Neurosci 2000;20:2275-2286.
91. Fok-Seang J, Smith-Thomas L C, Muir E, Du J.-S, Housden F, Johnson A R, Faissner A, Geller H M, Keynes R J, Rogers J H, Fawcett J W. An analysis of astrocytic cell lines with different abilities to promote axon growth. Brain Res 1995;689:207-223.
92. Liuzzi F J, Lasek R J. Astrocytes block axonal regeneration in mammals by activating the physiological stop pathway. Science 1987;237:642-645.
93. Liuzzi F J. Proteolysis is a critical step in the physiological stop pathway: mechanisms involved in the blockade of axonal regeneration by mammalian astrocytes. Brain Res 1990;512: 277-283.
94. McKeon R I, Schreiber R C, Rudge J S, Silver J. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. J Neurosci 1991;11:3398-3411.
95. Pindzola R R, Doller C, Silver J. Putative inhibitory extracellular matrix molecules at the dorsal root entry zone of the spinal cord during development and after root and sciatic nerve lesions. Dev Biol 1993;156:34-48.
96. Ghirnikar R S, Eng L F. Astrocyte-Schwann cell interactions in culture. Glia 1994;11:367-377.
97. Davies S J A, Fitch M T, Memberg S P, Hall A K, Raisman G, Silver J. Regeneration of adult axons in white matter tracts of the central nervous system. Nature 1997;390:680-684.
98. Golding J, Shewan D, Cohen J. Maturation of the mammalian dorsal root entry zone from entry to no entry. Trends Neurosci 1997;20:303-308.
99. Golding J P, Shewan D, Berry M, Cohen J. An in vitro model of the rat dorsal root entry zone reveals developmental changes in the extent of sensory axon growth into the spinal cord. Mol Cell Neurosci 1996;7:191-203.
100. Fitch M T, Silver J. Glial cell extracellular matrix: boundaries for axon growth in development and regeneration. Cell Tissue Res 1997;290:379-384.
101. Carlstedt T, Cullheim S, Risling M, Ulfhake B. Nerve fibre regeneration across the PNS-CNS interface at the root-spinal cord

junction. Brain Res Bull 1998;22:93-102.
102.Haas C A, Rauch U, Thon N, Merten T, Deller T. Entorhinal cortex lesion in adult rats induces the expression of the neuronal chondroitin sulfate proteoglycan neurocan in reactive astrocytes. J Neurosci 1999;19:9953-9963.
103.Fawcett J W, Housden E, Smith-Thomas L, Meyer R L. The growth of axons in three dimensional astrocyte cultures. Dev Biol 1989;135:449-458.
104.Fawcett J W. Astrocytic and neuronal factors affecting axon regeneration in the damaged central nervous system. Cell Tissue Res 1997;290:371-377.
105.Geller H M, Dubois-Dalcq M. Antigenic and functional characterization of a rat central nervous system-derived cell line immortalized by a retroviral vector. J Cell Biol 1986;107:1977-1986.
106.Grierson J P, Petroski R E, Ling D S F, Geller H M. Astrocyte topography and tenascin/cytotactin expression: correlation with the ability to support neuritic outgrowth. Dev Brain Res 1990;55:11-19.
107.Powell E M, Fawcett J W, Geller H M. Proteoglycans Provide Neurite Guidance at an Astrocyte Boundary. Mol Cell Neurosci 1997;10:27-42.
108.Fidler P S, Schuette K, Asher R A, Dobbertin A, Thornton S R, Calle-Patino Y, Muir E, Levine J M, Geller H M, Rogers J H, Faissner A, Fawcett J W. Comparing astrocyte cell lines that are inhibitory and permissive for axon growth: the major axon-inhibitory proteoglycan is NG2. J Neurosci 1999;19: 8778-8788.
109.Smith-Thomas L C, Stevens J, Fok-Seang J, Faissner A, Rogers J H, Fawcett J W. Increased axon regeneration in astrocytes grown in the presence of proteoglycan synthesis inhibitors. J Cell Sci 1995;108:1307-1315.
110.Dou C L, Levine J M. Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. J Neurosci 1994;14:7616-7628.
111.Dou C L, Levine J M. Identification of a neuronal cell surface receptor for a growth inhibitory chondroitin sulfate proteoglycan NG2). J Neurochem 1997;68: 1021-1030.
112.Levine J M. Increased expression of the NG2 chondroitin-sulfate proteoglycan after brain injury. J Neurosci 1994;14:4716-4730.
113.Snow D M, Steindler D A, Silver J. Molecular and cellular characterization of the glial roof plate of the spinal cord and optic tectum: a possible role for a proteoglycan in the development of an axon barrier. Dev Biol 1990a;138:359-376.
114.Carbonetto S, Gruver M M, Turner D C. Nerve fiber growth in culture on fibronectin, collagen, and glycosaminoglycan substrates. J Neurosci 1983;3:2324-2335.
115.Snow D M, Lemmon V, Carrino D A, Caplan A L, Silver J. Sulfated proteoglycans in astroglial barriers inhibit neurite outgrowth in vitro. Exp Neurol 1990;109:111-130.
116.Fichard A, Verna J M, Olivares J, Saxod R. Involvement of a chondroitin sulfate proteoglycan in the avoidance of chick epidermis by dorsal root ganglia fibers: a study using beta-D-xyloside. Dev Biol 1991;148:1-9.
117.Friedlander D R, Milev P, Karthikeyan L, Margolis R K, Margolis R U, Grumet M. The neuronal chondroitin sulfate proteoglycan neurocan binds to the neural cell adhesion molecules Ng-CAM/ L1JMLE and N-CAM, and inhibits neuronal adhesion and neurite outgrowth. J Cell Biol 1994;125:669-680.
118.Yamada H, Fredette B, Shitara K, Hagihara K, Miura R, Ranscht B, Stalleup W B, Yamaguchi Y. The brain chondroitin sulfate proteoglycan brevican associates with astrocytes ensheathing cerebellar glomeruli and inhibits neurite outgrowth from granule neurons. J Neurosci 1997;17:7784-7795.
119.Niederöst B P, Zimmermann D R, Schwab M E, Bandtlow C E. Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. J Neurosci 1999;19:8979-8989.
120.Katoh-Semba R, Oohira A. Core proteins of soluble chondroitin sulfate proteoglycans purified from the rat brain block the cell cycle of PC12D cells. J Cell Physiol 1993;156:17-23.
121.Schnell L, Fearn S, Klassen H, Schwab M E, Perry V H. Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. Eur J Neurosci 1999;11:3648-3658.
122.Schnell L, Fearn S, Schwab M E, Perry V H, Anthony D C. Cytokine-induced acute inflammation in the brain and spinal cord. J Neuropathol Exp Neurol 1999;58:245-254.
123.Bartholdi D, Schwab M E. Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: an in situ hybridization study. Eur J Neurosci 1997;9:1422-1438.
124.Bartholdi D, Rubin B P, Schwab M E. VEGF mRNA induction correlates with changes in the vascular architecture upon spinal cord damage in the rat. Eur J Neurosci 1997;9:2549-2560.
125.Oudega M, Vargas C G, Weber A B, Kleitman N, Bunge M B. Long-term effects of methylprednisolone following transection of adult rat spinal cord. Eur J Neurosci 1999;11:2453-2464.
126.Imperato-Kalmar E L, McKinney R A, Schnell L, Rubin B P, Schwab M E. Local changes in vascular architecture following partial spinal cord lesion in the rat. Exp Neurol 1997;45:322-328.
127.Raineteau 0, Z'Graggen W J, Thallmair M, Schwab M E. Sprouting and regeneration after pyramidotomy and blockade of the myelin-associated neurite growth inhibitors NI 35/250 in adult rats. Eur J Neurosci 1999;11:1486-1490.
128.Belien A T, Paganetti P A, Schwab M E. Membrane-type I matrix metalloprotease (MT I-MMP) enables invasive migration of glioma cells in central nervous system white matter. J Cell Biol 1999;144:373-384.
129.Amberger V R, Hensel T, Ogata N, Schwab M E. Spreading and migration of human glioma and rat C6 cells on central nervous system myelin in vitro is correlated with tumor malignancy and involves a metalloproteolytic activity. Cancer Res 1998;58:149-158.
130.Hensel T, Amberger V R, Schwab M E. A metalloprotease activity from C6 glioma cells inactivates the myelin-associated neurite growth inhibitors and can be neutralized by antibodies. Br J Cancer 1998;78:1564-1572.
131.Muir D. Metalloproteinase-dependent neurite outgrowth within a synthetic extracellular matrix is induced by nerve growth-factor. Exp Cell Res 1994;210:243-252.
132.Betancourt A, Reyes O, Kuffler D P. Enhancing the rate of neurite outgrowth in the peripheral nervous system. Proceedings Puerto Rico Neuroscience Conference, San Juan, PR: 1999. Abstract # 7.
133.Bixby J L, Lilien J, Reichardt LF. Identification of the major proteins that promote neuronal process outgrowth on Schwann cells in vitro. J Cell Biol 1988;107:353-362.
134.Kromer L F, Cornbrooks C J. Transplants of Schwann cell cultures promote axonal regeneration in the adult mammalian brain. Proc Natl Acad Sci (USA) 1985;82:6330-6334.
135.Fernandez E, Palliti R, & Mercanto D. Effects of topically administered nerve growth factor on axonal regeneration in peripheral nerve autographs implanted into spinal cord of rats. Neurosurg 1990;26:37-42.
136.Kliot M, Smith GM, Siegal JD, Silver J. Astrocyte-polymer implants promote regeneration of dorsal root fibers into the adult mammalian spinal cord. Expl Neurol 1990;109:57-69.
137.Paino CL, Bunge MB. Induction of axon growth into Schwann cell implants grafted into lesioned adult rat spinal cord. Exp Neurol 1991;114:254-257.
138.Montero-Menei C N, Pouplard-Barthelaix A, Gumpel M,

Baron-Van Evercooren A. Pure Schwann cell suspension grafts promote regeneration of the lesioned septo-hippocampal cholinergic pathway. Brain Res 1992;570:198-208.

139.Guénard V, Xu X M, Bunge M B. The use of Schwann cell transplantation to foster central nervous system repair. Neurosci 1993;5:401-411.

140.Li Y, Field P M, Raisman G. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 1997;277:2000-2002.

141.Xu X M, Guénard V, Kleitman N, Aebischer P, Bunge M B. A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp Neurol 1995;134:261-272.

142.Xu X M, Guénard V, Kleitman N, Bunge M B. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. J Comp Neurol 1995;351:145-160.

143.Cheng H, Cao Y, Olson L. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. Science 1996;273:510-513.

144.Guest J D, Hesse D, Schnell L, Schwab M E, Bunge M B, Bunge R P. Influence of IN-1 antibody and acidic FGF-fibrin glue on the response of injured corticospinal tract axons to human Schwann cell grafts. J Neurosci Res 1987;50:8888-8905.

145.Blesch A, Grill R J, Tuszynski M H. Neurotrophin gene therapy in CNS models of trauma and degeneration. Prog Brain Res 1998;117:473-84

146.Tuszynski M H, Weidner N, McCormack M, Miller I, Powell H, Conner J. Grafts of genetically modified Schwann cells to the spinal cord: survival, axon growth, and myelination. Cell Transplant 1998;7:187-96.

147.Neveu I, Arenas E. Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. J Cell Biol 1996 133;631-646.

148.Lucidi-Phillipi C A, Gage F H, Shults CW, Jones KR, Reichardt L F, Kang U J. Brain-derived neurotrophic factor-transduced fibroblasts: production of BDNF and effects of grafting to the adult rat brain. J Comp Neurol 1995;354:361-376.

149.Kim D H, Gutin P H, Nobel L J, Nathan D, Yu S J. Treatment with genetically engineered fibroblasts producing NGF or BDNF can accelerate recovery from traumatic spinal cord injury in the adult rat. Neuroreport 1996;7:2221-2225.

150.Blesch A, Tuszynski M H. Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells. Exp Neurol 1997;148:444-452.

151.Guest J D, Rao A, Olson L, Bunge M B, Bunge R P. The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord. Exp Neurol 1997;148:502-522.

152.Guénard V, Gwynn L A, Wood P. Astrocytes inhibit Schwann cell proliferation and myelination of dorsal root ganglion neurons in vitro. J Neurosci 1994;14:2980-2992.

153.Brunello N M, Reynolds M, Wrathall J R, Mocchetti I. Increased nerve growth factor receptor mRNA in contused rat spinal cord. Neurosci Lett 1990;118:238-240.

154.Emfors P, Henschen A, Olsen L, Persson H. Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. Neuron 1989;2:1605-1613.

155.Houle J D, Ye J H. Survival of chronically-injured neurons can be prolonged by treatment with neurotrophic factors. Neuroscience 1999;94:929-936.

156.Oppenheim R W, Yin Q W, Prevette D, Yan Q. Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. Nature 1992;360:755-757.

157.Yan Q, Rosenfeld R U, Matheson C R, Hawkins N, Lopez O T, Bennett L, Welcher A A. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience 1997;78:431-448.

158.Goto A, Furukawa S. Experimental changes in BDNF- and NT-3-like immunoreactivities in the spinal cord following its transection. Nippon Seikeigeka Gakkai Zasshi 1995;69:506-516.

159.Houle J D, Ziegler M K. Bridging a complete transection lesion of adult rat spinal cord with growth factor-treated nitrocellulose implants. J Neural Transplant Plast 1994;5:115-124.

160.Oudega M, Vargas C G, Weber A B, Kleitman N, Bunge M B. Long-term effects of methylprednisolone following transection of adult rat spinal cord. Eur J Neurosci 1999;11:2453-2464.

161.Oudega M, Hagg T. Nerve growth factor promotes regeneration of sensory axons into adult rat spinal cord. Exp Neurol 1996;140:218-229.

162.Schnell L, Schneider R, Bard Y A, Schwab M E. Neurotrophin-3 enhanced sprouting of corticospinal tract during development and after adult spinal cord lesion. Nature 1994;367:170-173.

163.Zhang Y, Dijkhuizen P A, Anderson P N, Lieberman A R, Verhaagen J. NT-3 delivered by an adenoviral vector induces injured dorsal root axons to regenerate into the spinal cord of adult rats. J Neurosci Res 1998;54:554-562.

164.Tuszynski M H, Peterson D A, Ray J, Baird A, Nakahara Y, Gage F H. Fibroblasts genetically modified to produce nerve growth factor induce robust neuritic ingrowth after grafting to the spinal cord. Exp Neurol 1994;126:1-14.

165.Oudega M, Haag T. Neurotrophins promote regeneration of sensory axons in the adult rat spinal cord. Brain Res 1999;818:431-438.

166.Bennett D L, French J, Priestley J V, McMahon S B. NGF but not NT-3 or BDNF prevents the A fiber sprouting into lamina II of the spinal cord that occurs following axotomy. Mol Cell Neurosci 1996;8:211-220.

167.Bregman B S, McAtee M, Dai H N, Kuhn P L. Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. Exp Neurol 1997;148:475-494.

168.Bregman B S, Broude E, McAtee M, Kelley M S. Transplants and neurotrophic factors prevent atrophy of mature CNS neurons after spinal cord injury. Exp Neurol 1998;149:13-27.

169.Bradbury E J, Khemani S, Von R, King, Priestley J V, McMahon S B. NT-3 promotes growth of lesioned adult rat sensory axons ascending in the dorsal columns of the spinal cord. Eur J Neurosci 1999;11:3873-83.

170.Iwaya K, Mizoi K, Tessler A, Itoh Y. Neurotrophic agents in fibrin glue mediate adult dorsal root regeneration into spinal cord. Neurosurgery 1999;44:589-595.

171.Tuszynski M, Gabriel H K, Gage F H, Suhr S, Meyer S, Rosetti A. Nerve growth factor delivery by gene transfer induces differential outgrowth of sensory, motor, and noradrenergic neurites after adult spinal cord injury. Exp Neurol 1996;137:157-173.

172.Ye J H, Houle J D. Treatment of the chronically injured spinal cord with neurotrophic factors can promote axonal regeneration from supraspinal neurons. Exp Neurol 1997;143:70-81.

173.von Meyenburg J, Broesamle C, Metz G A, Schwab M E. Regeneration and sprouting of chronically injured corticospinal tract fibers in adult rats promoted by NT-3 and the man IN-1, which neutralizes myelin-associated neurite growth inhibitors. Expl Neurol 1998;154:583-594.

174.Grill R, Murai K, Blesch A, Gage F H, Tuszynski M H. Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. J Neurosci 1997;17:5560-5572.

175.Weidner N, Blesch A, Grill R J, Tuszynski M H. Nerve growth factor-hypersecreting Schwann cell grafts augment and guide spinal cord axonal growth and remyelinate central nervous system axons

in a phenotypically appropriate manner that correlates with expression of L1. J Comp Neurol 1999;413:495-506.

176. Kobayashi N R, Fan D P, Giehl K M, Bedard A M, Wiegand S J, Tetzlaff W. BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and alpha 1-tubulin mRNA expression, and promote axonal regeneration. J Neurosci 1997;17:9583-95.

177. Nakahara Y, Gage F H, Tuszynski M H. Grafts of fibroblasts genetically modified to secrete NGF, BDNF, NT-3, or basic FGF elicit differential responses in the adult spinal cord. Cell Transplant 1996;5:191-204.

178. Morales A, Garcia-Castiñeiras S, Kuffler D P. Multiple peripheral nerve-released factors act simultaneously to promote process outgrowth with unique morphologies. Proceedings Puerto Rico Neurosciences Conference, San Juan, PR: 1999. Abstract # 36.