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Sensory axon regeneration: rebuilding functional connections in the spinal cord

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Abstract

Functional regeneration within the adult spinal cord remains a formidable task. A major barrier to regeneration of sensory axons into the spinal cord is the dorsal root entry zone. This region displays many of the inhibitory features characteristic of other central nervous system injuries. Several experimental treatments, including inactivation of inhibitory molecules (such as Nogo and chondroitin sulfate proteoglycans) or administration of neurotrophic factors (such as nerve growth factor, neurotrophin3, glial derived neurotrophic factor and artemin), have been found to promote anatomical and functional regeneration across this barrier. There have been relatively few experiments, however, to determine if regenerating axons project back to their appropriate target areas within the spinal cord. This review focuses on recent advances in sensory axon regeneration, including studies assessing the ability of sensory axons to reconnect with their original synaptic targets.

Introduction

Recovery after injuries to the spinal cord is severely limited. Even 3000 years ago, physicians noted that the prognosis for improvement was poor, as evidenced by this quote from Edwin Smith Surgical Papyrus, circa 2500-1600 BC: “Diagnosis: You should say of him: ‘One having a crushed vertebra in his neck; he is unconscious of his two arms, his two legs, he is speechless. An ailment not to be treated.”. During the last several decades, various therapies have been found to promote some degree of anatomical and functional recovery in animal models of spinal cord injury (SCI) [1-4], yet the prognosis for recovery in humans remains poor [5]. Although patients with severe spinal cord injuries now often have nearly normal life spans [6], there is little or no improvement in function after the first few months following injury.

For many years it was not known if neurons in the central nervous system (CNS) were intrinsically incapable of regeneration or if an unsupportive environment was responsible for their lack of growth within the CNS. Seminal experiments utilizing peripheral nerves as

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bridges within the injured spinal cord provided convincing evidence that at least some CNS neurons were capable of regenerating [7, 8]. Spinal cord neurons extended axons for several centimeters into a peripheral nerve bridge, suggesting that peripheral nerves contain factors that up-regulate growth processes in CNS neurons and/or that the CNS environment contains factors that inhibit axonal growth. Since that time, research has focused on the identification and blockade of inhibitory factors in the CNS as well as the administration of exogenous neurotrophic factors to stimulate axon growth.

The dorsal root crush model of spinal injuries

One experimental model that is particularly well suited to study axon regeneration within the CNS is the regrowth of sensory axons into the spinal cord after crush lesions of the dorsal root. Of the various axon tracts within the cord, sensory axons are among the ones that respond best to experimental therapies to promote regeneration [4]. Dorsal root disruption occurs commonly in brachial plexus injuries and leads to disuse of the affected arm, even when motor roots remain intact. In these injuries, regenerating sensory axons are normally unable to grow back into the spinal cord when they reach the transition between the peripheral nervous system (PNS), and the CNS, termed the dorsal root entry zone (DREZ) [9]. Over the past ten years, various treatments have been found to promote growth past the DREZ and into the spinal cord; this model has therefore provided a useful system to study the relative advantages of different therapies (Table 1).

Sensory neurons are located in dorsal root ganglia (DRG), which lie within the PNS, allowing access to the neurons without disrupting the spinal cord. Their cell bodies are pseudo unipolar, with a peripheral and a central branch. The peripheral location of sensory axons makes them easily available for anatomical labeling and electrophysiological stimulation to test for functional synapses within the spinal cord. The DRG contain many functional classes of sensory neurons that convey specific sensory modalities, innervate a variety of peripheral targets, and project to specific locations within the spinal cord (Box 1). These characteristics make it possible to assess whether different classes of regenerating axons project back to their original target areas. Regeneration following dorsal root crush occurs rapidly until the axons reach the DREZ, where a variety of inhibitory factors block further growth (Figure 1). Most of these factors are the same ones responsible for blocking regeneration in the cord itself, but this model avoids the mechanical trauma associated with spinal lesions, thus allowing experimental manipulation of these components to better understand inhibitory mechanisms within the CNS. Following treatments that allow sensory axons to grow through the DREZ, these axons need to regenerate only a relatively short distance to project to their original targets and form synapses that support functional recovery of well-defined behavioral outcome measures. This makes it possible to assess functional regeneration directly with electrical recording and to address issues of specificity in terms of the anatomical projections of different classes of sensory neurons within the spinal cord. Recent studies have shown that regeneration of individual axons can be followed over time in living mice in which subsets of DRG neurons are genetically labeled with a green fluorescent protein. [10]. That study suggests that some regenerating axons form synaptic-like terminals on glia that stabilize axon terminals at the DREZ, similar to those observed previously [11, 12]. The present review will discuss the advantages of using the dorsal root crush model in detail and explore how this model is being used to provide insights into axon regeneration within the CNS and potential development of molecular therapies.

Blockade of inhibitory factors in the DREZ

The DREZ provides a unique environment to study mechanisms that induce axon regeneration within the spinal cord in the absence of a zone of mechanical trauma, extensive cellular disruption and the formation of a dense glial scar [13]. Following dorsal root lesions in adult rodents, severed axons terminating within the spinal cord degenerate and induce an injury cascade that includes recruitment of macrophages [14, 15] and proliferation of Schwann cells [16]. Astrocytes at the transition zone become reactive, increasing proliferation rates, expression of glial fibrillary acidic protein (GFAP), and secretion of pro-inflammatory cytokines, causing them to undergo hypertrophy and extend processes deeper into the dorsal roots [17]. Reactive astrocytes also up-regulate extracellular molecules (i.e. chondroitin sulfate proteoglycans, hyaluronic acid and tenascin) that are associated with regeneration failure in damaged axonal areas [18].

Lesioned myelinated sensory afferents degenerate, releasing several myelin-associated proteins that inhibit axon growth. These include Nogo, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) [19]. These proteins bind and signal through a receptor complex on neurons that includes the Nogo receptor (NgR), leucine-rich repeat and Ig domain containing 1 (LINGO-1), and p75/Troy [20]. The myelin inhibitory proteins also contain several growth inhibitory domains that may signal through other receptors such as Paired-immunoglobulin-like-receptor-B (PirB) [21]. Individually or together, these myelin inhibitory proteins dramatically reduce the outgrowth of axons from DRG neurons *in vitro* [22]. Multiple reagents have been deployed to block signaling from myelin inhibitory proteins including the blocking antibody IN-1 [23] and the soluble Nogo-receptor peptide (sNgR) [24]. Treatment of the injured spinal cord with sNgR promotes sprouting of several axonal populations [25-27], and intrathecal application of sNgR after dorsal root lesions leads to regeneration through the DREZ of large myelinated, but not unmyelinated, axons [28]. Regenerating sensory axons arborize both within the white matter of the dorsal columns and in the dorsal horn, but their pattern of arborization is disorganized, unlike the normal patterning of sensory projections (compare Figures 1A and 2C). Nevertheless, stimulation of peripheral nerves evokes synaptic potentials within the spinal cord and there is a progressive functional return of forepaw sensory function over several weeks. These results have recently been repeated using herpes simplex virus to express the soluble Nogo-receptor in DRG neurons [29].

Another important class of inhibitory proteins is the family of chondroitin sulfate proteoglycans (CSPGs), which are up-regulated after dorsal root crush [30-34]. Cytokines produced by activated microglia, particularly Transforming Growth Factor β1 (TGF-β1), contribute to the up-regulation of CSPGs and tenascin by astrocytes [35-37]. CSPGs are major inhibitors of axonal regeneration, as their presence halts sensory axon growth past the DREZ [38]. Although the mechanism by which CSPGs inhibit regeneration is unclear [39], they may act by signaling through the protein tyrosine phosphatase-sigma receptor [40] or inactivating integrin's [41], which are involved in axon guidance and regeneration. To date, the most successful method for blocking CSPG-mediated inhibition is the use of a bacterial enzyme, chondroitinase ABC (ChABC) that cleaves the reactive glycosaminoglycan (GAG) side chains from the core protein. Treatment with ChABC has been particularly successful in promoting sensory axon regeneration, although in most cases it must be combined with another treatment to promote growth [42, 43]. For example, ChABC administration following pretreatment with zymosan promotes anatomical and functional regeneration of sensory axons into the spinal cord [44]. In this case, zymosan acts like a conditioning lesion to increase the intrinsic growth capacity of the sensory axons. Likewise, following dorsal root crush in brevican/Neurocan knockout mice, few axons grow through the DREZ unless a conditioning lesion is also performed [45]. In these examples, conditioning lesions alone

promote only minimal regeneration through the DREZ [46, 44, 45], further reinforcing the concept that axonal regeneration requires interventions that affect both the growth state of the environment and the intrinsic growth state of the neuron.

Up-regulation of growth pathways in sensory neurons

Earlier experiments using peripheral nerve grafts to bridge lesions of the transected spinal cords suggested not only the possibility that the CNS might contain factors that actively inhibit axonal growth, but also that PNS grafts might contain factors not present in the CNS that stimulate axonal growth [7, 8]. Many neurotrophic factors (NTFs) are known to be up-regulated in peripheral nerves in response to axonal injury, and treatment with these factors has been particularly successful in promoting regeneration of specific sensory axon populations. For example, a four week intrathecal infusion of nerve growth factor (NGF), neurotrophin-3 (NT-3) or glial-derived neurotrophic factor (GDNF), but not brain-derived neurotrophic factor (BDNF), leads to increased regeneration of sensory afferents through the DREZ and into superficial layers of the spinal cord (Figure 2) [47]. Electrical stimulation of peripheral nerves evokes activity in dorsal horn neurons, whose firing patterns suggest that individual NTFs preferentially induced regeneration of axons expressing their requisite NTF receptors. Further anatomical and behavioral tests also suggested the action of individual NTFs on specific classes of sensory neurons. In a separate study, delayed treatment with NT-3 (one week after root crush) resulted in significantly less regenerative capacity [48], perhaps related to the up-regulation of inhibitory factors in the DREZ. Interestingly, these axons initially grow through the DREZ but stop growing when they encounter a region with numerous macrophages. Such macrophages have been observed in tissue culture to inhibit axon growth and induce dystrophic growth cones [49]. These observations suggest that two zones of inhibition occur at the DREZ: an initial transition zone where axons leave the growth-supportive environment of Schwann cells to enter the growth-poor CNS environment, and a second, slightly deeper zone, composed of macrophages and degenerating myelin [48].

Robust regeneration of several classes of sensory axons is also promoted by treatments with the GDNF-family member artemin (ARTN). Systemic injections of ARTN over a two-week period following crush of all brachial dorsal roots results in extensive regeneration of myelinated and unmyelinated sensory axons (Figure 2E) [50]. Stimulation of peripheral forelimb nerves evokes short-latency synaptic potentials in the spinal cord, indicating the reformation of functional synaptic connections by regenerated axons, and a variety of behaviors involving proprioceptive and nociceptive function are restored. Moreover, the recovery is persistent, lasting at least six months following root lesions. The extent and persistence of functional recovery and the ease of systemic application suggest that ARTN has a potential for long-term therapeutic application, although at the present time, the treatment must be initiated shortly after injury to be effective.

Sensory axon regeneration can also be induced with NTFs expressed locally within the cord via viral expression. An early report demonstrated that viral expression of NT-3 within the ventral spinal cord induced regeneration of lesioned sensory axons into the area expressing NT-3, although behavioral recovery was not assessed [51]. The expression of fibroblast growth factor-2 or NGF within the dorsal horn also induces massive growth of nociceptive axons expressing calcitonin gene-related peptide ($CGRP^+$) into the spinal cord following dorsal root crush (Figure 2A) [52]. Robust growth was observed despite a two-week delay in injection of the virus, suggesting that inhibitory factors in either zone of the DREZ are not sufficient to block regeneration of these fibers into the cord. In this study, the expression of NGF lead to the regeneration of peptidergic-nociceptive axons but no other sensory populations, most likely due to restriction of neurotrophic tyrosine kinase receptor type 1

(TrkA receptor) expression to these neurons. $CGRP^+$ axons regenerated throughout the entire dorsal horn, showing no target specificity, which typically is confined to laminae I and II. Whether this absence of topographic specificity results from high levels of generalized expression of NTFs disrupting growth cone guidance or from neurotrophin-trapping remains to be determined (see Box 2).

Many NTFs can guide growing axons as well as stimulating the growth process directly; that is, they can act as chemoattractive or neurotropic agents. For example, sensory axons grow up gradients towards sources of NGF and NT-3. In contrast, other guidance molecules, such as semaphorin 3A, can act as chemorepulsive factors, causing axons to turn and grow away from the source of expression. The poor targeting of regenerating $CGRP^+$ axons to dorsal spinal laminae (see above) can be improved, for example, by establishing slightly overlapping gradients of NGF (dorsally) and semaphorin 3A (ventrally) [53]. Therefore, local expression of these agents by viral delivery can be used to guide regenerating axons to more appropriate target areas (Figure 2B). Interestingly, although the addition of semaphorin significantly reduced the overall number of axons that regenerated, the targeting of synapses to more appropriate laminae resulted in a similar extent of functional recovery. These results emphasize the utility of targeting regenerating axons to more appropriate synaptic targets, particularly for primary sensory afferents that can induce detrimental behavioral effects if misdirected [54, 55].

Neurotrophic factors also display neurotropic effects on other regenerating axon populations. Viral delivery of NT-3 in either the nucleus gracilis or the reticular formation combined with injection of bone marrow stromal cells expressing NT-3 into the lesion site and a conditioning lesion of the sciatic nerve stimulate and direct the regeneration of proprioceptive sensory axons across a lesion of the dorsal columns and back to these targets in the brainstem [56]. For either site of viral injection, axons grew into the source of NT-3 regardless of whether it was an appropriate target (nucleus gracilis) or an inappropriate target (reticular formation). Furthermore, the density of regenerating axons within the nucleus gracilis was robust, about 27% of the original axon density. Although synapses were identified morphologically, sciatic nerve stimulation did not evoke synaptic potentials, most likely due to conduction blockade from lack of myelination of these regenerated axons. Interestingly, this triple combination of treatments induced sensory axon regeneration when administration started either 6 weeks or 15 months after mid cervical lesions [57], suggesting the potential therapeutic usefulness of this technique.

Specificity of regeneration

An issue that is seldom addressed in studies of CNS regeneration is whether the formation of new synaptic connections, either by regenerating or sprouting axons, results in functionally appropriate neural circuits. Studies of regeneration of sensory fibers lend themselves particularly well to this issue. There are many well-characterized functional classes of sensory neurons, which innervate a variety of peripheral targets and make central projections to specific populations of target neurons in the spinal cord. Systemic ARTN treatments result in regeneration that is topologically specific: three different classes of sensory axons each regenerate to their appropriate area in the spinal cord (compare Figures 1A and 2E). Unmyelinated $CGRP^+$ afferents are restricted to the most dorsal laminae of the dorsal horn, myelinated cutaneous afferents project to the topographically correct location in deeper dorsal horn laminae and myelinated muscle afferents project through the dorsal horn towards the ventral horn [58]. In contrast, although sensory regeneration promoted by the Nogo receptor peptide sNgR is also functional, different classes of axons do not project to their former locations in the cord [28, 58]. NGF expression within the spinal cord also leads to widespread distribution of regenerating $CGRP^+$ axons rather than restricted targeting to

dorsal laminae [52]. The patterns of regeneration promoted by these different treatments are illustrated schematically in Figure 2.

These findings suggest that molecular guidance cues persist in the adult mammalian spinal cord, but these cues can guide regenerating axons correctly only with certain therapeutic treatments or by targeting the treatment to the appropriate location (for example to the cell bodies vs. the site of regeneration; see Box 1). A more general implication of these results is that other guidance cues within the gray matter also may be available to guide the regeneration of other classes of spinal axons, such as those damaged in contusion injuries. Therapeutic strategies that act directly on the cell body of regenerating axons, such as the up-regulation of the mammalian target of rapamycin (mTOR) protein pathway in corticospinal neurons [59], rather than modifying the environment of the growing axons, may be useful in promoting regeneration without disrupting the action of intrinsic guidance cues.

Restoration of connectivity sufficient for functionally appropriate behavior can also occur when regenerated projections are not topographically correct. For example, the widespread distribution of $CGRP^{+}$ axons following viral expression of NGF in the dorsal horn restores normal nociceptive responses to thermal stimuli without causing hyperalgesia [52]. Similarly, behavioral recovery is as complete following treatment with sNgR as it is with ARTN treatment, despite the absence of topographic specificity [28, 58]. However, it has not been determined if these axon terminals or synaptic connections undergo refinement over time. There is also increasing evidence for significant plasticity of synaptic connections following CNS injuries and for the importance of neural activity in functional recovery [2-4]. Rehabilitative training or exercise increases sprouting and return of function by refining synaptic connections [60]. Such responses are further enhanced following the application of ChABC, which supports sprouting and potential reorganization of synaptic connections associated with improved functional recovery [61, 62]. The effects of enforced neural activity via training with or without the application of ChABC have not yet been explored for recovery of function following damage to dorsal root sensory axons, but this issue will be important to pursue in future experiments.

Conclusions

The recovery of significant function following injuries to the spinal cord remains a daunting challenge. One aspect of this recovery is the regeneration of lesioned CNS axons back to their appropriate synaptic targets. For regeneration to occur, growth programs intrinsic to the neurons must be sufficient to overcome the growth-inhibiting environment of the mature mammalian CNS and/or the inhibitory nature of the environment needs to be attenuated. Among different classes of CNS axons, sensory axons within the spinal cord are particularly responsive to treatments that block inhibitory factors in the CNS environment and up-regulate growth pathways within the sensory neurons. Blockade of the Nogo signaling pathway is sufficient to promote some functional recovery of sensory function, and digestion of the sugar moieties on chondroitin sulfate proteoglycans also facilitates growth of lesioned sensory axons back into the spinal cord. A variety of neurotrophic factors can also stimulate sensory axon growth into and within the spinal cord. These factors can be delivered by intrathecal injection, viral expression directly within the spinal cord, or even by systemic injection. Some of these factors also act neurotropically, so that axons can be guided back to appropriate target regions by localized viral expression. The topographically specific regeneration of sensory axons promoted by systemic ARTN treatment is particularly interesting in this regard, as it suggests that molecular guidance cues persist in the adult mammalian spinal cord. If other therapies are found that act similarly on other classes of

CNS axons, it may be possible to develop generalized strategies for promoting topographically specific regeneration following other types of spinal injury.

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Box 1

A Diversity of Neuronal Cell Types in the Dorsal Root Ganglia

The diversity of sensory information entering the spinal cord is dependent on the wide variety of sensory neurons within dorsal root ganglia. Multiple subpopulations of neurons have been identified based on their peripheral and central projections, function, sensitivity to specific NTFs, and expression of neuropeptides. The identification of these subpopulations provides unique advantages to target the growth and regeneration of specific neuronal types.

Nociceptive neurons are small, unmyelinated cells mediating pain sensation. Peptidergic nociceptors all express CGRP and many also express substance-P. They also express the TrkA receptor and respond to the neurotrophin NGF [66]. Many $CGRP^{+}$ neurons also express receptors for ARTN and GFRα3 [50]. Their central projections terminate largely in laminae I and II [67]. Non-peptidergic nociceptive neurons respond to several GDNF family members, including GDNF and ARTN, via GFRα receptors signaling in conjunction with RET (a receptor tyrosine kinase) [68, 69, 50]. Their axons terminate only in the inner portion of lamina II.

Tactile mechanosensitive sensory axons are larger than nociceptive axons and are well myelinated. Peripherally they project via cutaneous nerves to a variety of specialized receptors in skin. Their central terminations are restricted to the dorsal horn but in deeper laminae than nociceptive axons, and these projections are arranged topographically, reflecting the specific area of skin they innervate peripherally [70]. Many of these neurons express TrkB and respond to the neurotrophin BDNF.

The largest sensory neurons are proprioceptive, projecting peripherally via muscle nerves and supplying muscle spindles and Golgi tendon organs. They provide the CNS with information regarding body position and muscle length and tension. These neurons respond to the neurotrophin NT3 via TrkC receptors [71, 72], and many of them also express GFRα1, the receptor for GDNF. Their central axons project to more ventral layers of the spinal cord than mechanoreceptors do, where they provide direct excitatory input to motoneurons (mediating the simple stretch reflex) as well as a variety of interneurons involved in locomotion [73].

Box 2

Location, Location, Location: Getting regenerating axons back to their appropriate locations

The re-establishment of the correct patterns of synaptic connections is an important consideration for reconstruction of neural circuits during regeneration. During development, spatially precise expression of targeted-derived growth factors and chemotropic molecules helps establish specific projection patterns of growing axons[74]. Following CNS injury in adult mammals, exogenous growth factors induce regeneration and sprouting, but only rarely are the new projections topographically appropriate. Many neurotrophic factors (NTFs) have well-established chemoattractive properties; growth cones will turn and follow a concentration gradient of NGF, NT-3, or BDNF [75, 76]. Sensory axons grow into peripheral nerve grafts or transplants expressing high levels of NTFs, but then remain in the graft rather than projecting back out and into the host tissue [77, 76]. The application of additional NTFs is required to entice growth back into the host [78, 76, 79]. Moreover, axons grow into the region of the exogenous NTFs whether or not these factors are placed at the appropriate target location [56].

Does the localized expression of NTFs affect topographic organization? Following dorsal root rhizotomy, localized infusion of NGF, NT-3, or GDNF all induce sensory axon regeneration through the DREZ, but there has been little assessment if the regenerating axons project correctly within the spinal cord [47]. Indeed, virally mediated expression of NGF within the dorsal horn following lumbar root rhizotomies promotes robust regeneration of peptidergic-nociceptive axons, but these axons grow throughout the entire region of NGF expression (Figure 2A) [52]. The application of NTFs intrathecally or by viral-mediated expression creates a regional rather than cell-specific distribution, and the supra-physiological concentration of NTF may overwhelm cues from other guidance molecules. For example, NGF shows a dose-dependent neutralization of semaphorin 3A chemorepulsion both *in vitro* [80] and *in vivo* [55]. Slightly overlapping gradients of chemoattractive (eg. NGF) and chemorepulsive (eg. Semaphorin 3A) factors, however, can promote more appropriately targeted projections (Figure 2B) [53]. Although an improvement over expression of NGF alone, this is still a crude method to recapitulate a precise developmental guidance pattern. Intrathecal application of NT-3 promotes regeneration of myelinated sensory axons but not to specific locations in the cord (Figure 2C) [63].

Interestingly, the systemic application of ARTN after dorsal root injury is the only known example in which treatment with a NTF results in appropriate and precise topographic organization of sensory input (Figure 2E) [50, 58]. Systemic ARTN does not enter the spinal cord and presumably acts directly on the sensory cell bodies within dorsal root ganglia [58], suggesting that its ability to promote topographically specific regeneration may be a consequence of its site of action. A puzzling aspect of ARTN's ability to promote regeneration of myelinated sensory axons is that its best-known receptor, GFRα3, is expressed primarily on unmyelinated sensory neurons; relatively few large sensory neurons are immunopositive for GFRα3 [81, 82]. It is possible that GFRα3 may be up-regulated in large neurons following dorsal root crush and/or ARTN treatment; alternatively other receptors may mediate the action of ARTN on these neurons. Further studies are required to explore these possibilities.

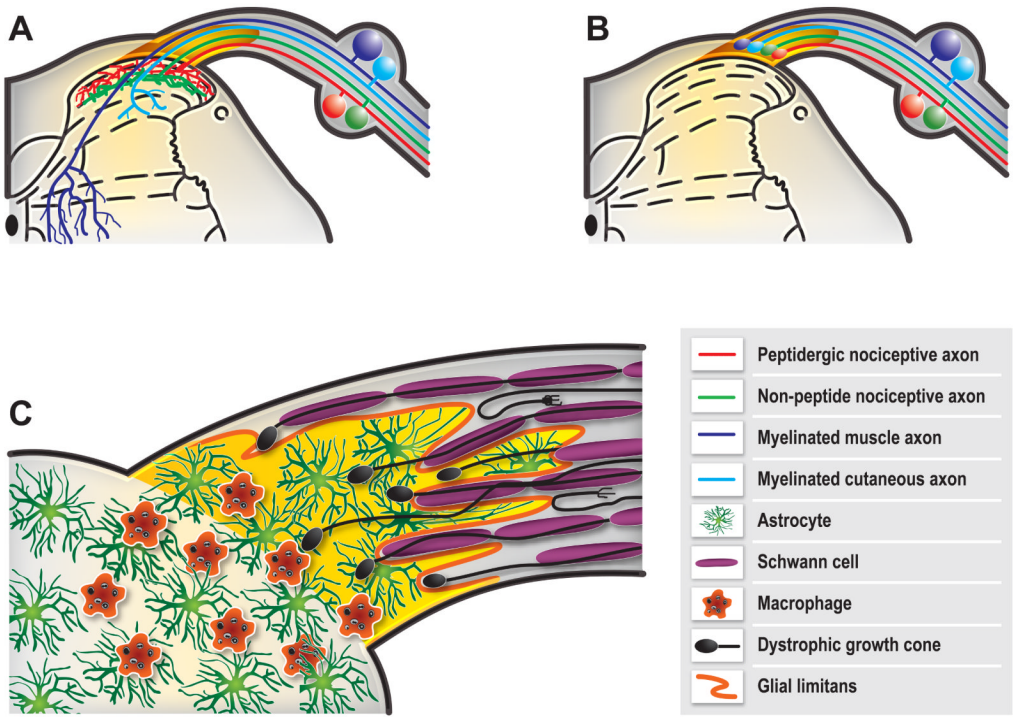

Figure 1.

Spinal projection patterns of various classes of sensory axons. Unmyelinated peptidergic and non-peptidergic nociceptive axons are shown in red and green, and myelinated cutaneous (blue) and proprioceptive (purple) axons are shown in blue and purple. A. Sensory projections in a normal, un-injured spinal cord. Peptidergic nociceptive axons primarily terminate in laminae I and II, whereas non-peptidergic nociceptive axons terminate more ventrally in lamina II. Projections from large myelinated proprioceptive axons terminate within the deeper dorsal laminae and into ventral motor neuron pools, whereas cutaneous tactile axons terminate in laminae III - V. Broken lines in the spinal cord represent different laminae of Rexed, starting from the upper dorsal lamina I. B. Several weeks after sensory axons are lesioned in the dorsal roots, axons regenerate up to the dorsal root entry zone (DREZ, highlighted in yellow), but then stop growing and form dystrophic growth cones or turn around and grow distally within the nerve [9, 10]. C. Higher magnification of the DREZ showing two regions of the dorsal root: a PNS region containing Schwann cells, and a CNS region containing oligodendroglia (not shown), astrocytes and macrophages. The border between Schwann cells and astrocytes is delineated by a basal limitans (orange). The DREZ and dorsal spinal cord contain degenerative myelin, tenascin, and CSPGs which act as presumptive inhibitory components that block further progression of growth cones. Without treatment, growth cones remain in this dystrophic state.

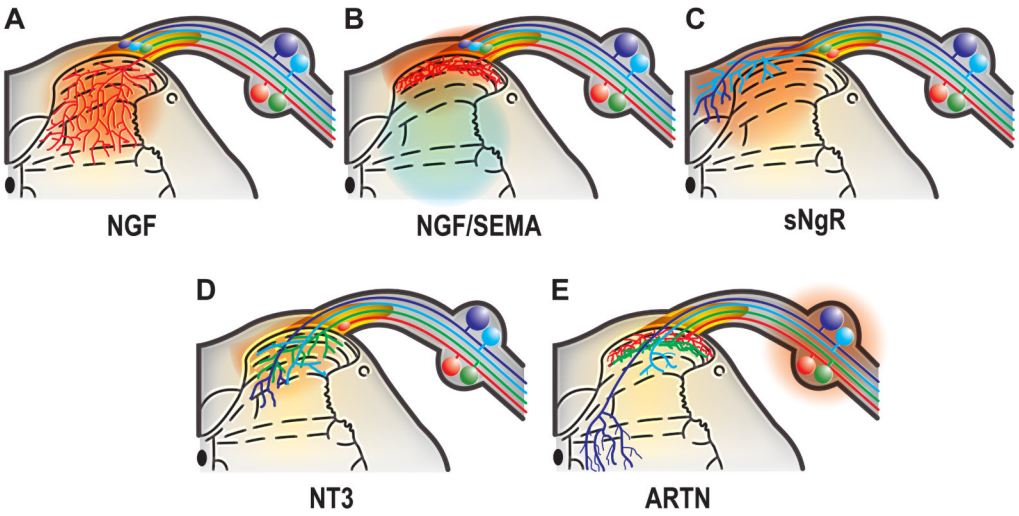

Figure 2.

Pattern of regeneration of different sensory afferent axon populations promoted by different therapeutic treatments after dorsal root lesions in rodents. Presumptive gradients of the highest concentrations of neurotrophic factors or soluble Nogo receptor (sNgR) are shown in orange. A. Viral-mediated expression of NGF centered within the dorsal horn induces regeneration of peptidergic nociceptive axons throughout the entire region of NGF expression. Non-peptidergic and myelinated sensory axons do not regenerate [52]. B. Slightly overlapping expression of NGF (orange) and Semaphorin 3A (SEMA, blue) restrict the growth of these axons to the upper dorsal horn [53]. C. Intrathecal administration of sNgR induces regeneration of myelinated but not unmyelinated sensory axons. Myelinated axons project to ectopic locations within the dorsal horn [28]. D. Intrathecal administration of NT-3 induces regeneration primarily of myelinated sensory axons, with some regeneration of non-peptidergic unmyelinated axons. Myelinated axons projected to both normal and ectopic regions of the cord [47, 63]. E. Systemic application of artemin (ARTN) induces robust regeneration of all sensory axon populations, and the axons project to their topographically appropriate locations [58].

Table 1
Treatments Promoting Regeneration Through the Dorsal Root Entry Zone[1]

Agent / Treatment	Axonal regeneration[2]	Functional recovery[3]	Refs
NGF / Adenovirus	Peptidergic-Nociceptive	Thermal Nociceptive	[52, 55, 53]
/ Intrathecal infusion	Unmyelinated axons	Thermal Nociceptive	[47]
		Electrophysiology	
NT-3 / Adenovirus	Proprioceptive axons	ND	[51]
/ Intrathecal infusion	Large myelinated,	Electrophysiology	[47]
	Proprioceptive axons	Beam walking, Paw placement	[63]
BDNF / Intrathecal infusion	No regeneration observed		[47]
GDNF / Intrathecal infusion	Multiple sensory populations	Thermal and mechanical nociception, grid walking	[47]
		Electrophysiology	
ARTN / Systemic	All sensory axon populations.	Thermal/mechanical nociception and proprioception	[50, 58]
		Electrophysiology	
sNgR / Intrathecal infusion	Cutaneous and proprioceptive myelinated axons	Paw preference and grip strength, electrophysiology	[64, 28, 29]
/ Herpes Simplex Virus	CTB-labeled myelinated axons	Proprioceptive function	[29]
Peripheral nerve lesion			
/ alone – rat	Little regeneration	none (electrophysiology)	[44]
/ alone – mouse	Some regeneration into DREZ	ND	[45]
with CSPG deletion			
/ rat	Dextran labeled myelinated axons	Electrophysiology	[44]
/ mouse	Small increase in regeneration into DREZ	ND	[45]
RAR / lentivirus	CTB-labeled myelinated axons	Paw placement, proprioceptive function;	[65]
		Electrophysiology	

[1] Effects of various treatment paradigms on anatomical and functional regeneration of sensory axons into the spinal cord. All experiments were performed on rats except those with peripheral nerve lesions, which were performed on rats and mice, as indicated. Abbreviations: CTB, cholera toxin subunit B; RAR, retinoic acid receptor β2.

[2] Axonal regeneration indicates the types of sensory axons that regenerated as determined anatomically.

[3] Functional recovery was assessed behaviorally or by electrophysiological recordings from the spinal cord, as indicated.