Model Organisms in Spinal Cord Regeneration

Edited by
Catherina G. Becker and Thomas Becker

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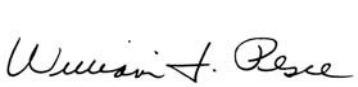

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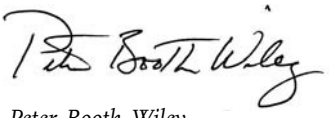

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Contents

Preface

After spinal cord injury in humans severed axons do not regrow. This causes permanent functional deficits, such as loss of sensation and paralysis. These devastating consequences of spinal cord injury have long been thought to be incurable, but this pessimistic view is changing. Over the last decade we have gained a much better insight into the environmental and neuron-intrinsic factors that prevent axon regrowth in the central nervous system (CNS) of mammals. Progress in experimentally eliciting some axon regrowth in mammals is currently leading to therapeutic strategies. In the light of these encouraging findings, it is important to address further challenges for *functional* regeneration. Namely, we may ask how axon regrowth can be made even more robust, what the targets are that regrowing axons have to contact, how axons manage to grow there and how reconnections can lead to functional recovery. These aspects of spinal cord regeneration are difficult to study in mammals that do not normally regenerate their spinal cord and in vitro analyses cannot mimic the complex spinal network. There are, however, other vertebrate model systems that share many basic features of connectivity in the spinal cord with mammals and show robust axon regrowth and functional recovery, such as fish and amphibians. By comparing the mammalian situation, in which enhancement of axon growth seems to be feasible now, with that in functionally regenerating vertebrates, we may learn which mechanisms are important for functional recovery. For these reasons, this volume aims in its first part to give a comprehensive view over the state of the art in research into spinal cord injury in mammals in 2006. The second part is to increase our understanding of the spinal-intrinsic circuitry, the target of regenerating axons. The third part demonstrates how diverse non-mammalian regenerating model systems contribute to our understanding of spinal cord regeneration. Finally, in the fourth part non-mammalian models of optic nerve regeneration are discussed, because this popular and accessible system is likely to yield insights into CNS regeneration that is also relevant for the spinal cord.

In the first part of the book Pat Anderson, *Jez Fabes* and *David Hunt* (Chapter 1) are illuminating recent findings on the array of molecules in the environment of the lesioned CNS of mammals that are inhibitory to axon regrowth. In a complementary review *Ferdinando Rossi* (Chapter 2) gives a current account of the factors intrinsic to neurons that prevent vigorous axon regrowth. *Bhavna Ylera* and *Frank*

Bradke (Chapter 3) show us how the neuron-intrinsic response of axotomized neurons can be enhanced in mammals. Richard Benton and *Scott Whittemore* (Chapter 4) report how the inhibitory environment in the CNS can be replaced by more conducive and growth promoting cellular substrates including promising stem cell approaches. They finish the part on mammalian regeneration research by critically discussing the latest clinical trials.

Stan Grillner and *Peter Wallén* (Chapter 5) begin the second part by describing the spinal-intrinsic neuronal network that produces locomotion-related patterns of activity, the so-called central pattern generator of locomotion, in the lamprey. This jawless vertebrate possesses a simple, yet typical vertebrate spinal network and the authors show us how mathematical modeling increases our understanding of the activity in this network. *Anna Vallstedt* and *Klas Kullander* (Chapter 6) then describe genetic techniques in mice that are currently being used to improve our surprisingly small knowledge of the central pattern generator in mammals. The spinal central pattern generator is a target for regenerating descending axons. *Agustin Gonzáles* and *Hans ten Donkelaar* (Chapter 7) point out how major descending tracts are evolutionarily conserved between non-mammalian vertebrates and mammals. This adds to the significance of findings from non-mammalian vertebrates for regeneration research.

In the third part *Michael Shifman, Li-Quing* and *Michael Selzer* (Chapter 8) demonstrate the power of the lamprey system to understand spinal cord regeneration at the level of individually identifiable neurons. The zebrafish is an important model organisms for developmental biology. *Joe Fetcho, Dimple Bhatt* and *Steven Zottoli* (Chapter 9) continue to show that regeneration can be experimentally augmented in larval zebrafish and the process of axon regrowth can be visualized in the living larva. We show in our own contribution (Chapter 10) that specific genes expressed during spinal cord regeneration in adult zebrafish can be directly manipulated, which leads to alterations in behavioural recovery. Thus the importance of individual molecules for the regenerative outcome can be tested in fish model systems.

In the third part we compiled contributions on regeneration in the optic system of non-mammalian vertebrates, which provides the researcher with a relatively homogeneous population of neurons, i.e. retinal ganglion cells, that is readily accessible and easily lesioned by an optic nerve crush. *Sarah Dunlop* (Chapter 11) shows how regenerative capacity for this cell type varies across vertebrate classes from full functional regeneration in fish via axon regrowth without recovery of function in reptiles to no axon regrowth in mammals. *Saturo Kato, Yoshiki Koriyama, Tori Matsukawa* and *Kayo Sugitani* (Chapter 12) demonstrate how the optic system in goldfish can be used to find new regeneration-associated genes and how the function of these genes can be tested in vivo and in vitro. Finally, *Marie-Claude Senut, Blake Fausett, Matthew Veldman* and *Daniel Goldman* (Chapter 13) show how promoter analysis of regeneration-associated genes can be performed in transgenic zebrafish and new regeneration-associated genes can be discovered by gene array analysis. Many of the genes activated in regenerating retinal ganglion cells in fish are also upregulated in regenerating mammalian neurons. Therefore, there is a justified

hope that some of the regeneration-associated genes discovered in gold- and zebrafish are part of a general regeneration program in vertebrates.

Secondary neuron loss around a spinal lesion site in mammals is significant and thought to exacerbate the condition. These neurons are usually not replaced. Transgenic fish used by *Senut* et al. also shed light on gene activation during lesion-induced stem cell proliferation in the CNS of zebrafish, indicating a mechanism by which damaged neurons may be replaced. Salamanders are even able to regenerate the entire spinal cord during tail regeneration. Thus, the analysis of stem cell proliferation and functional integration of newly generated neurons in fish and amphibians may lead to ways to activate similar mechanisms in mammals.

Overall, there is an enormous increase in the number of findings on spinal cord regeneration both from mammalian and non-mammalian systems. Bringing together insights from different vertebrate classes from the molecular to systems level offers an opportunity to identify the critical steps necessary for successful regeneration of highly complex spinal functions. We hope that this book gives an up to date introduction into the many facets of CNS regeneration research for students and provides specialists in the field with a useful entry point to comparative analysis.

We thank Dr. Andreas Sendtko from Wiley-VCH for his initiative and highly professional management of the volume and all the authors for their captivating contributions and excellent cooperation.

Edinburgh, October 2006

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Part I
Mammalian Models of CNS Regeneration

1
The Role of Inhibitory Molecules in Limiting Axonal Regeneration in the Mammalian Spinal Cord

Patrick N. Anderson, Jez Fabes, and David Hunt

1.1
Introduction

In adult mammals axonal regeneration is vigorous following peripheral nerve injury, but meager after injury to the central nervous system (CNS). Several theories seek to explain this situation. First, the cell body response to axotomy may be inadequate in intrinsic CNS neurons. Second, there may be inadequate levels of support in terms of neurotrophic factors and cell adhesion molecules in the CNS. Third, the regeneration of axons in the CNS may be prevented by molecules which inhibit neurite outgrowth *in vitro*. In addition, the absence of a normal wound-healing response in mammalian CNS tissue may limit regeneration; whereas a lesion site in a peripheral nerve is rapidly repopulated by Schwann cells migrating from the two stumps, lesion sites in the CNS expand by secondary degeneration during the first week after injury. None of these hypotheses explains all of the data, but the idea that inhibitory molecules play a major role in preventing axonal regeneration in the CNS has dominated thought in this area for almost two decades. However, there remains much contradictory evidence concerning the roles inhibitory molecules and conflicting views as to their importance in limiting axonal regeneration *in vivo* (e.g., Raisman, 2004; Schwab, 2004).

1.1.1
CNS Neurons Have Widely Differing Phenotypes

The heterogeneity of CNS neurons and their responses to injury greatly complicates the evaluation of hypotheses on CNS regeneration. This is best illustrated by the results of grafting peripheral nerves into the CNS. Richardson et al. (1980) showed that many adult mammalian CNS neurons could regenerate axons through a suitable environment in the form of a peripheral nerve graft. However, subsequent studies showed that many, perhaps most, neurons in the brain are very poor at regenerating axons, even into nerve grafts (Anderson et al., 1998; Anderson and Lieberman, 1999). This may be because CNS neurons differ dramatically in

their sensitivity to neurotrophic factors, the strength of their cell body responses to axotomy, and in their expression of receptors for inhibitory molecules (Hunt et al., 2002a; Josephson et al., 2002; Lauren et al., 2003; Pignot et al., 2003).

1.2 Difficulties in Assessing Axonal Regeneration in the Mammalian Spinal Cord

A characteristic of scientific progress is that novel techniques that initially appear difficult become commonplace within a few years. This has not been the case with experimental studies of axonal regeneration in the mammalian spinal cord. There have been many claims of treatments resulting in successful axonal regeneration in the mammalian CNS, but there is a paucity of cases where those claims have been replicated in other laboratories, or have even developed into a series of confirmatory observations from the same laboratory. This may be because of the ease with which some axons can be left intact when lesioning tracts in the CNS; spared axons can be misinterpreted as regenerated axons.

1.2.1 Experimental Lesions and Problems of Interpretation

Probably the best model for producing reliable complete lesions of a CNS tract is provided by the optic nerve, which can be completely sectioned or crushed by an experienced operator, with little chance of axonal sparing. This has allowed major discoveries to be made on the influence of neurotrophic stimuli (Berry et al., 1996; Logan et al., 2006) and inflammation (Leon et al., 2000; Lorber et al., 2005) on the vigor of axonal regeneration within CNS tissue. Yet even in the optic nerve, reports of remarkable axonal regeneration (Eitan et al., 1994) have sometimes gone without apparent replication or further development.

The mammalian spinal cord can be transected, contused or compressed to produce a lesion. Transection or partial transection lesions (Fig. 1.1) have the advantage that the site of initial injury can be accurately estimated. The lesion sites are filled with blood and macrophages and then invaded by meningeal cells, endothelial cells and Schwann cells, together with axons, some of which are of peripheral origin (Zhang et al., 1997). Meningeal cells are the source of several molecules that can inhibit or repel regenerating axons (Zhang et al., 1997; Pasterkamp et al., 2001; Niclou et al., 2003). Astrocyte processes extend into the lesion sites, but few astrocyte or oligodendrocyte cell bodies are present. A region of reactive gliosis characterized by hypertrophic astrocytes develops rostral and caudal to the lesion site where CSPGs are up-regulated (Davies et al., 1999; Tang et al., 2003), and there may be cavitation, particularly if the lesion involves the central canal. Complete transection of the mammalian spinal cord should allow axonal regeneration to be studied without the complication of spared fibers. However, the animals require considerable care after surgery, including regular manual emptying of the bladder, and permission to perform such experiments can be difficult to obtain in Europe.

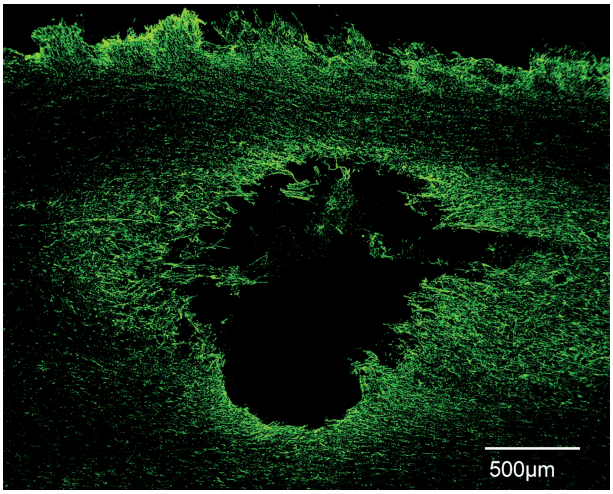

Fig. 1.1. GFAP immunohistochemistry identifying astrocytes in a horizontal section of the cervical spinal cord of an adult rat one week following dorsal column transection. The wound has enlarged since the initial injury and is characterized by the absence of CNS glia. The GFAP-negative "space" at the center is occupied by macrophages, other invading non-glial cells, some axons, and fluid-filled cysts. Reactive astrocytes are present bordering the lesion.

It is worth noting that even with attempts at complete transection, spared fibers can be left at the ventral surface of the cord (You et al., 2003).

Contusion and compression lesions are good models of common types of spinal injury in the western world, but the lesion size and position are more difficult to control. In such lesions it is not possible to be precise about the position of the axotomy, and the possibility of spared fibers is more difficult to eliminate than with transection injuries. Contusion/compression lesions develop from the center of the spinal cord where there is extensive cell death and an invasion of hematogeneous cells (Popovich et al., 1997). Subsequently, large injury sites with cavitation around the central canal, spreading several millimeters rostral and caudal to the site of impact, develop in rats (Bresnahan et al., 1991). Fibrotic tissue instead of cavities is found in most strains of mice (Ma et al., 2001; Stokes and Jakeman, 2002). Typically, the dorsal corticospinal tracts are destroyed in all but the mildest contusion injuries with loss of much gray matter and sparing of a variable amount of peripheral white matter. The axons in the lesioned tracts often terminate well short of the region of primary impact. Although contusion and compression injuries are often less than ideal for studying axonal regeneration, they are excellent models in which to study effects of treatments on behavioral recovery. In all but the most severe lesions, functional recovery occurs to some extent. A number of behavioral tests have been developed for such purposes, including the BBB score (Basso et al., 1995) of open field motor function, grid and rope walking (for a re-

cent use of such tests, see Hendriks et al., 2006). Many more treatments including steroid treatment (Young, 1991) and environmental enrichment (Lankhorst et al., 2001) have been found to enhance behavioral recovery than to promote axonal regeneration. Such treatments presumably act through neuroprotection and/or enhancing plasticity in surviving connections between the rostral and caudal parts of the spinal cord. As the histopathological features differ from those of transection injuies, it would be reasonable to suggest that all potential therapies should be tested on contusion lesions prior to clinical use. However, whether the lesions are produced by transection, contusion or compression, and despite the structural differences between these lesions, regeneration of intrinsic CNS axons across the lesion site is always very poor.

In summary, complete transection lesions are the best for proving axonal regeneration has taken place, but compression or contusion injuries are excellent for studying functional recovery.

1.2.2 Tracing Regenerating Axons

Anterograde tracing of axons provides the ''gold standard'' for assessing the extent of regeneration following injury because it allows the course of regenerating axons to be followed around or through a lesion site. Retrograde tracing has the disadvantage that cell bodies may become labeled by spread of tracer through the tissues, necessitating careful analysis of the injection site. Anterograde tracing of descending axons is usually performed using biotinylated dextran amine (BDA; Fig. 1.2) (Li et al., 1997) or sometimes cholera toxin subunit B (CTB) (Hagg et al., 2005), injected near the cell bodies of the injured neurons. Enhanced green fluorescent protein (EGFP) delivered by lentiviral vectors is also useful for tracing axons from brainstem nuclei, and labels only those axons that arise from neurons in the region where the vector is applied (Fabes et al., 2006; Fig. 1.1). Ascending dorsal column axons may be labeled with CTB or CTB-HRP (Chong et al., 1996, 1999), injected into peripheral nerves.

1.2.2.1 Regeneration of Corticospinal Axons is Difficult to Assess

Corticospinal tract axons are widely distributed through a transverse section of the spinal cord of rodents (Fig. 1.2). Although most corticospinal tract axons passing through a segment of cord are found in the dorsal funiculus, others are present in the lateral and ventral funiculi of the white matter, and these fibers – if spared – will also send branches into the gray matter below a lesion. Hence, it is difficult to eliminate all corticospinal tract projections without a complete lesion, and sprouting of surviving axons caudal to a lesion – an interesting neurobiological phenomenon in its own right – may be confused with axonal regeneration. As corticospinal tract axons are present in much of the gray matter, it is particularly difficult to distinguish any regenerating axons that might grow through the gray matter around a partial lesion, from axons that were undamaged. Rubrospinal tracts (Fig. 1.2) are located entirely within the dorsal part of the lateral funiculus in ro-