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Repairing the Damaged Brain

For much of its history, neurology has been a discipline of outstanding diagnostic rigor but little therapeutic efficacy. Simply put, neurologists have been renowned for their ability to localize lesions with great precision but until recently have had little to offer in terms of treatment. This situation is now changing.

Advances in our understanding of the structure, function, and chemistry of the brain's neurons, glial cells, and synapses have led to new ideas for treatment. Many of these are now in clinical trials, and some are already available to patients. Developmental neuroscience is emerging as a major contributor to this sea change for three main reasons. First, efforts to preserve or replace neurons lost to damage or disease rely on recent advances in our understanding of the mechanisms that control the generation and death of nerve cells in embryos (Chapters 45 and 46). Second, efforts to improve the regeneration of neural pathways following injury draw heavily on what we have learned about the growth of axons and the formation of synapses (Chapters 47 and 48). Third, there is increasing evidence that some devastating brain disorders, such as autism and schizophrenia, are the result of disturbances in the formation of neural circuits in embryonic or early postnatal life. Accordingly, studies of normal development provide an essential foundation for discovering precisely what has gone wrong in disease.

In this chapter, we focus on the first two of these issues: how neuroscientists hope to augment the limited ability of neurons to recover normal function. We shall begin by describing how axons degenerate following the separation of the axon and its terminals from the cell body. The regeneration of severed axons is robust in the peripheral nervous system of mammals and in the central nervous system of lower vertebrates, but very poor in the central nervous system of

mammals. Many investigators have sought the reasons for these differences in the hope that understanding them will lead to methods for augmenting recovery of the human brain and spinal cord following injury. Indeed, we shall see that several differences in regenerative capacity of mammalian neurons have been discovered, each of which has opened promising new approaches to therapy.

We shall then consider an even more dire consequence of neural injury: the death of neurons. The inability of the adult brain to form new neurons has been a central dogma of neuroscience since the pioneering neuroanatomist Santiago Ramón y Cajal asserted that in the injured central nervous system, "Everything may die, nothing may be regenerated." This pessimistic view dominated neurology for most of the last century despite the fact that Ramón y Cajal added, "It is for the science of the future to change, if possible, this harsh decree." Remarkably, in the past few decades, evidence has accumulated that neurogenesis does occur in certain regions of the adult mammalian brain. This discovery has helped accelerate the pace of research on ways to stimulate neurogenesis and to replace neurons following injury. More than a century later, neuroscientists are finally beginning to reverse Cajal's "harsh decree."

Damage to the Axon Affects Both the Neuron and Neighboring Cells

Because many neurons have very long axons and cell bodies of modest size, most injuries to the central or peripheral nervous system involve damage to axons. Transection of the axon, either by cutting or by crushing, is called *axotomy*, and its consequences are numerous.

Axon Degeneration Is an Active Process

Axotomy divides the axon in two: a proximal segment that remains attached to the cell body and a distal segment that has lost this crucial attachment. Axotomy dooms the distal segment of the axon because energy supplies dwindle during a short-lived latent period. Soon the alterations become irreversible. Synaptic transmission fails at severed nerve terminals, and calcium levels increase within the axon. The calcium activates proteases, initiating a program of cytoskeletal disassembly and degradation, and physical degeneration of the axon ensues. Once the denervation begins, its progression is relatively rapid and inexorably proceeds to completion (Figure 50–1). This degenerative response is the first step in an elaborate constellation

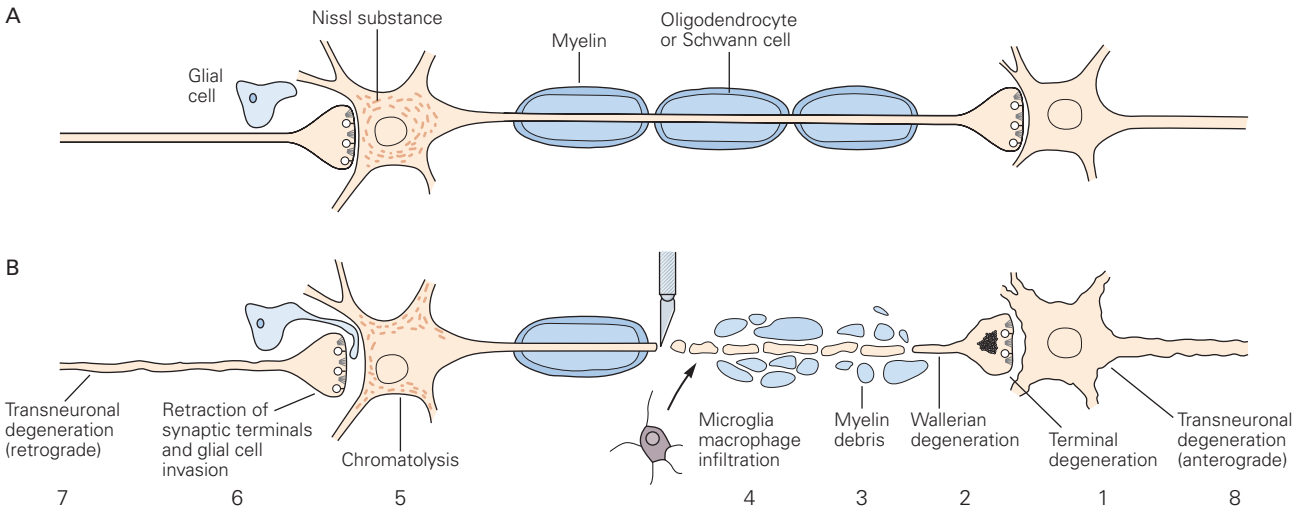

Figure 50–1 Axotomy affects the injured neuron and its synaptic partners.

A. A normal neuron with an intact functional axon wrapped by myelinating cells contacts a postsynaptic neuron. The neuron's cell body is itself a postsynaptic target.

B. After axotomy, the nerve terminals of the injured neuron begin to degenerate (**1**). The distal axonal stump separates from the parental cell body, becomes irregular, and undergoes Wallerian degeneration (**2**). Myelin begins to fragment (**3**) and the lesion site is invaded by phagocytic cells (**4**). The cell body of the damaged neuron undergoes chromatolysis: The cell body swells and the nucleus moves to an eccentric position (**5**). Synaptic terminals that contact the damaged neuron withdraw and the synaptic site is invaded by glial cell processes (**6**). The injured neuron's inputs (**7**) and targets (**8**) can atrophy and degenerate.

of changes, called *Wallerian degeneration*, that were initially described in 1850 by Augustus Waller.

The degeneration of transected axons was long thought to be a passive process, the consequence of separation from the cell body, where most of the cell's proteins are synthesized. Lacking a source of new protein, the distal stump was thought to simply wither away. But the discovery and analysis in mice of a spontaneously occurring mutation called *Wlds* (Wallerian degeneration slow) challenged this view (Figure 50–2). In *Wlds* mutant mice, the distal stumps of peripheral nerves persist for several weeks after transection, about 10-fold longer than in normal mice. This remarkable finding suggested that degeneration is not a passive consequence of separation from the cell body, but is rather an actively regulated response.

Analysis of the *Wlds* mutant mice led to insights into the nature of this regulation. The mutation led to formation of a mutant form of nicotinamide mononucleotide adenyltransferase 1 (NMNAT1), an enzyme involved in biosynthesis of a metabolic cofactor, nicotinamide adenine dinucleotide (NAD). A related enzyme, NMNAT2, which is normally present in the axon, becomes quite unstable and breaks down rapidly following axotomy, leading to loss of NAD, which is critical for maintenance of energy homeostasis in the axon. Although normal NMNAT1 is confined to the nucleus, the mutant *Wlds* form mislocalizes to the axon, where it substitutes for NMNAT2 to prolong axonal survival. Surprisingly, a main way that both the wild type and *Wlds* forms of NMNAT maintain NAD levels is not by synthesizing it but by inhibiting another protein, SARM1, that breaks down NAD. Thus, loss of SARM protects damaged axons, whereas activation of SARM1 leads to degeneration (Figure 50–3A). Several other proteins modulate this core pathway (Figure 50–3B).

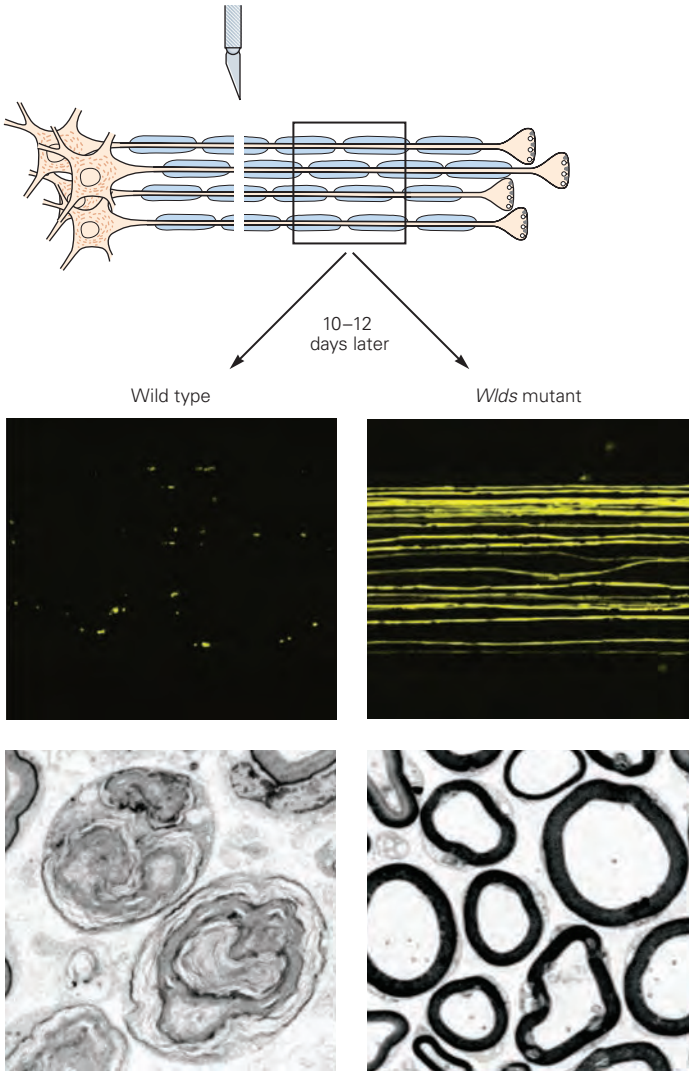

Figure 50–2 Axonal degeneration is delayed in *Wlds* mutant mice. In wild type animals, axons in the distal stump degenerate rapidly after sectioning of a peripheral nerve, as shown by disrupted axonal fragments (yellow) and the lack of myelinated axonal profiles at the electron micrographic level. In *Wlds* mutant mice the distal portion of severed axons persists for a long time. (Confocal micrographs reproduced, with permission, from Beirowski et al. 2004. Copyright © 2004 Elsevier B.V.; electron micrographs reproduced, with permission, from Mack TGA, Reiner M, Beirowski B, et al. 2001. Copyright © 2001 Springer Nature.)

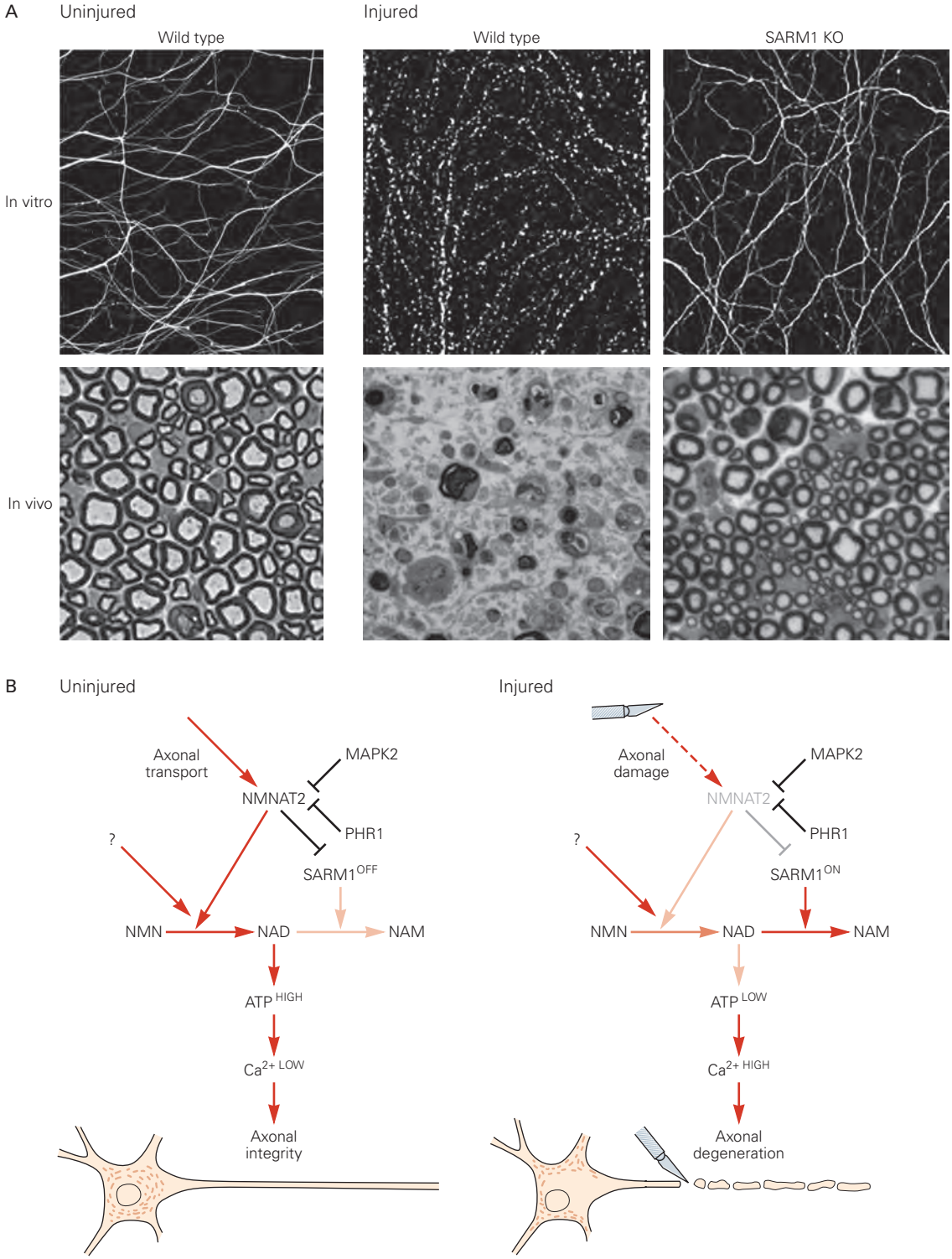

Figure 50–3 A core pathway regulates axon degeneration following axotomy in mice.

A. Damage to neurites in vitro leads to degeneration of the portions separated from the cell body. Likewise, axotomy in vivo leads to Wallerian degeneration, as shown by loss of myelin profiles in the cross section. Both in vitro and in vivo axons are spared if the *SARM1* gene is deleted. (From Gerdts et al. 2013.)

B. NMNAT2, closely related to the mutant *Wlds* protein, is normally present in axons. It can generate nicotinamide adenine dinucleotide (**NAD**) and inhibit SARM1, which degrades NAD. High NAD levels are required for energy metabolism, keeping adenosine triphosphate (**ATP**) levels high and calcium levels low in the axon. Following axotomy, NMNAT2 levels decrease rapidly, disinhibiting SARM1. NAD levels fall, ATP is depleted, calcium levels rise, calcium-dependent proteases are activated, and the axon is degraded. Kinases (MAPK) and a ubiquitin ligase (Phr1) regulate the pathway.

Together, these exciting new discoveries provide an answer to the question of why, following axotomy, the distal stump degenerates while the proximal stump is preserved. The conventional explanation that the distal stump is deprived of nutrients normally delivered from the cell body is incomplete. Instead, a signaling pathway in the axon senses damage and rapidly triggers degeneration. In this scenario, the key element supplied by the axon is NMNAT2. Its breakdown following axotomy disinhibits SARM1 and, perhaps in parallel with activation of factors that stimulate SARM1, triggers the loss of NAD, leading to the energy crisis that results in Wallerian degeneration.

These recent discoveries may be useful in devising treatments for neurological disorders in which axonal degeneration is prominent and generally precedes neuronal death. A fatal disease of motor neurons, amyotrophic lateral sclerosis, falls into this category. Other possibilities include some forms of spinal muscular atrophy, Parkinson disease, and even Alzheimer disease. Axon degeneration that occurs in these diseases, as well as after metabolic, toxic, or inflammatory insults, resembles the degeneration that follows acute trauma and may be regulated in similar ways. Thus, while methods for saving transected distal axons are unlikely to be useful clinically for treating patients who have suffered traumatic injury, the same techniques could be useful in treating neurodegenerative diseases.

Even though the proximal portion of the axon remains attached to the cell body, it too suffers. And in some cases, the neuron itself dies by apoptosis, probably because axotomy isolates the cell body from its supply of target-derived trophic factors. Even when this does not occur, the cell body often undergoes a series of cellular and biochemical changes called the *chromatolytic reaction:* The cell body swells, the nucleus moves to an eccentric position, and the rough endoplasmic reticulum becomes fragmented (Figure 50–1B). Chromatolysis is accompanied by other metabolic changes, including an increase in protein and RNA synthesis as well as a change in the pattern of genes that the neuron expresses. These changes are reversed if regeneration is successful.

Axotomy Leads to Reactive Responses in Nearby Cells

Axotomy sets in motion a cascade of responses in numerous types of neighboring cells. Among the most important responses are those of the glial cells that ensheath the distal nerve segment. One is fragmentation of the myelin sheath, which is then removed by phagocytes. This process is rapid in the peripheral nervous system, where the myelin-producing Schwann cells break the myelin into small fragments and engulf it. Schwann cells, which then divide, secrete factors that recruit macrophages from the blood stream. The macrophages in turn assist the Schwann cells in disposing of debris. Schwann cells also produce growth factors that promote axon regeneration, a point to which we will return later.

In contrast, in the central nervous system, the myelin-forming oligodendrocytes have little or no ability to dispose of myelin, and removal of debris depends on resident phagocytic cells called *microglia*. This difference in cellular properties may help explain the observation that Wallerian degeneration proceeds to completion much more slowly in the central nervous system.

Axotomy also affects both the synaptic inputs to and the synaptic targets of the injured neuron. When axotomy disrupts the major inputs to a cell—as happens in denervated muscle, or to neurons in the lateral geniculate nucleus when the optic nerve is cut—the consequences are severe. Usually the target atrophies and sometimes dies. When targets are only partially denervated, their responses are more limited. In addition, axotomy affects presynaptic neurons. In many instances, synaptic terminals withdraw from the cell body or dendrites of chromatolytic neurons and are replaced by the processes of glial cells—Schwann cells in the periphery and microglia or astrocytes in the central nervous system. This process, called *synaptic stripping*, depresses synaptic activity and can impair functional recovery.

Although the mechanism of synaptic stripping remains unclear, two possibilities have been suggested. One is that postsynaptic injury causes axon terminals to lose their adhesiveness to synaptic sites so that they are subsequently wrapped by glia. The other is that glia initiate the process of synaptic stripping in response to factors released from the injured neuron or to changes in its cell surface. Whatever the trigger, the activation of microglia and astrocytes by axotomy clearly contributes to the stripping process. In addition, biochemically altered astrocytes, called reactive astrocytes, contribute to formation of a *glial scar* near sites of injury.

As a result of these transsynaptic effects, neuronal degeneration can propagate through a circuit in both anterograde and retrograde directions. For example, a denervated neuron that becomes severely atrophic can fail to activate its target, which in turn becomes atrophic. Likewise, when synaptic stripping

prevents an afferent neuron from obtaining sufficient sustenance from its target cell, the afferent neuron's inputs are placed at risk. Such chain reactions help to explain how injury in one area in the central nervous system eventually affects regions far from the site of the injury.

Central Axons Regenerate Poorly After Injury

Central and peripheral nerves differ substantially in their ability to regenerate after injury. Peripheral nerves can often be repaired following injury. Although the distal segments of peripheral axons degenerate, connective tissue elements surrounding the distal stump generally survive.

Axonal sprouts grow from the proximal stump, enter the distal stump, and grow along the nerve toward its targets (Figure 50–4). The mechanisms that drive this process are related to those that guide embryonic axons. Chemotropic factors secreted by Schwann cells attract axons to the distal stump, adhesive molecules within the distal stump promote axon growth along cell membranes and extracellular matrices, and inhibitory molecules in the perineural sheath prevent regenerating axons from going astray.

Once regenerated peripheral axons reach their targets, they are able to form new functional nerve endings. Motor axons form new neuromuscular junctions; autonomic axons successfully reinnervate glands, blood vessels, and viscera; and sensory axons reinnervate muscle spindles. Finally, those axons that lost their myelin sheaths are remyelinated, and chromatolytic cell bodies regain their original appearance. Thus, in all three divisions of the peripheral nervous system—motor, sensory, and autonomic—the effects of axotomy are reversible. Peripheral regeneration is not perfect, however. In the motor system, recovery of strength may be substantial, but recovery of fine movements is usually impaired. Some motor axons never find their targets, some form synapses on inappropriate muscles, and some motor neurons die. Nevertheless, the regenerative capacities in the peripheral nervous system are impressive.

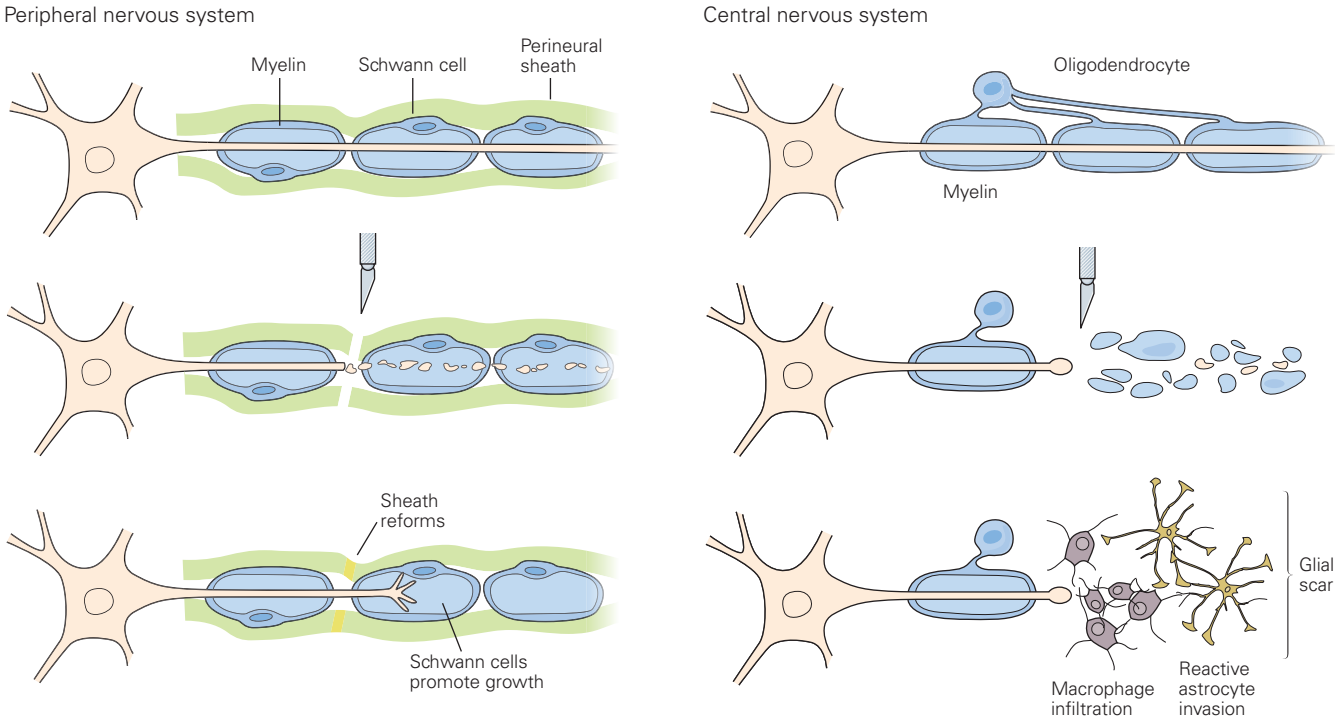

Figure 50–4 Axons in the periphery regenerate better than those in the central nervous system. After sectioning of a peripheral nerve, the perineural sheath reforms rapidly and Schwann cells in the distal stump promote axonal growth by producing trophic and attractant factors and expressing high levels of adhesive proteins. After sectioning of an axona in the central nervous system, the distal segment disintegrates and myelin fragments. In addition, reactive astrocytes and macrophages are attracted to the lesion site. This complex cellular milieu, termed a *glial scar*, inhibits axonal regeneration.

In contrast, regeneration after injury is poor in the central nervous system (Figure 50–4). The proximal stumps of damaged axons can form short sprouts, but these soon stall and form swollen endings called "retraction bulbs", which fail to progress. Long-distance regeneration is rare. The failure of central regeneration is what led to the long-standing belief that injuries to the brain and spinal cord are largely irreversible and that therapy must be restricted to rehabilitative measures.

For some time, neurobiologists have been seeking the reasons why regenerative capacity in the central and peripheral nervous systems differs so dramatically. The goal of this work has been to identify the crucial barriers to regeneration so that they can be overcome. These studies have begun to bear fruit, and there is now cautious optimism that the injured human brain and spinal cord have a regenerative capacity that can eventually be exploited.

Before discussing these new developments, it is helpful to consider the problem of neural regeneration in a broader biological context. Is it the ability of peripheral axons to regenerate that is unusual, or the inability of central axons to do so? It is in fact the latter. Obviously, central axons grow well during development. More surprisingly, axons in immature mammals can also regenerate following transection in the brain or spinal cord. Moreover, regeneration is robust in the adult central nervous systems of lower vertebrates such as fish and frogs, as exemplified by the studies of Roger Sperry on restoration of vision following damage to the optic nerve (Chapter 47).

So why have mature mammals lost this seemingly important capacity for repair? The answer may lie in what the mammalian brain *can* do peerlessly, which is to remodel its basic wiring diagram in accordance with experience during critical periods in early postnatal life, so that each individual's brain is optimized to deal with the changes and challenges of internal and external worlds (Chapter 49). Once remodeling has occurred, it must be stabilized. Although it is obviously useful to reassign cortical space to one eye if the other is blinded in childhood, we would not want our cortical connections similarly rearranged in response to a brief period of unusual illumination or darkness. Maintaining constancy in the face of small perturbations in connectivity may therefore have the unavoidable consequence of limiting the ability of central connections to regenerate in response to injury. In this view, our limited regenerative capacity is a Faustian bargain in which we have sacrificed recuperative power to ensure the maintenance of precisely wired circuits that underlie our superior intellectual capacity.

Therapeutic Interventions May Promote Regeneration of Injured Central Neurons

In seeking reasons for the poor regeneration of central axons, one critical question is whether it reflects an inability of neurons themselves to grow or an inability of the environment to support axonal growth. This issue was addressed by Albert Aguayo and his colleagues in the early 1980s. They inserted segments of a central nerve trunk into a peripheral nerve, and segments of a peripheral nerve into the brain or spinal cord, to find out how axons would respond when confronted with a novel environment.

As expected, axons in the grafts, which were separated from their somata, promptly degenerated, leaving "distal stumps" containing glia, support cells, and extracellular matrix. What was striking was the behavior of axons near the translocated segments. Spinal axons that regenerated poorly following spinal cord injury grew several centimeters into the peripheral graft (Figure 50–5). Similarly, retinal axons, which regenerated poorly following damage to the optic nerve, grew long distances into a peripheral graft placed in their path.

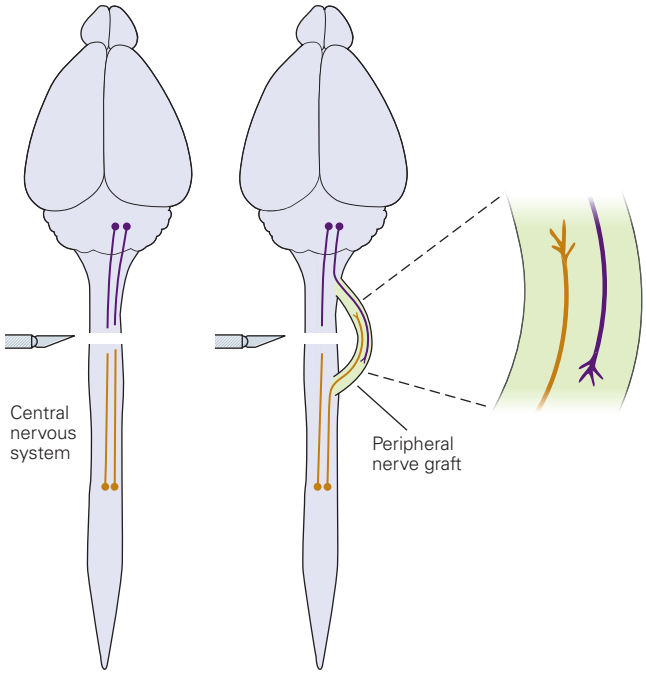

Figure 50–5 A transplanted peripheral nerve provides a favorable environment for the regeneration of central axons. *Left*: After sectioning of the spinal cord, ascending and descending axons fail to cross the lesion site. *Right*: Insertion of a peripheral nerve graft that bypasses the lesion site promotes regeneration of both ascending and descending axons. (Adapted from David and Aguayo 1981.)

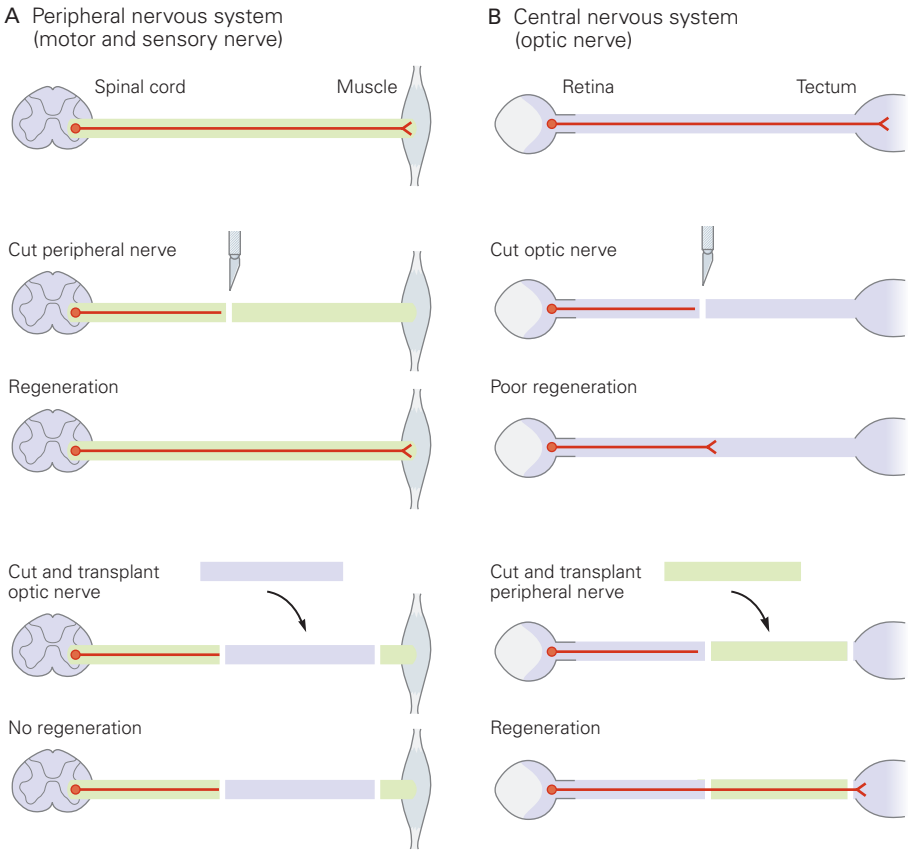

Figure 50–6 Peripheral and central nerves differ in their ability to support axonal regeneration.

A. In the peripheral nervous system, severed axons regrow past the site of injury. Insertion of a segment of optic nerve into a peripheral nerve suppresses the ability of the peripheral nerve to regenerate.

B. In the central nervous system, severed axons typically fail to regrow past the site of injury. Insertion of a section of peripheral nerve into a central nerve tract promotes regeneration.

Conversely, peripheral axons regenerated well through their own distal nerve trunk, but fared poorly when paired with a severed optic nerve (Figure 50–6).

Aguayo extended these studies to show that axons from multiple regions, including the olfactory bulb, brain stem, and mesencephalon, could all regenerate long distances if provided with a suitable environment. Even an optimal environment cannot fully restore the growth potential of central axons for reasons we will discuss in a later section. Nevertheless, these pioneering experiments focused attention on components of the central environment that inhibit regenerative ability and motivated an intensive search for the molecular culprits.

Environmental Factors Support the Regeneration of Injured Axons

In probing the differences between peripheral and central growth environments, initial searches were influenced by the results of experiments performed by Ramón y Cajal's student Francisco Tello nearly a century before Aguayo's studies. Tello transplanted segments of peripheral nerves into the brains of experimental animals and found that injured central axons grew toward the implants, whereas they barely grew when implants were not available.

This result implied that peripheral cells provide growth-promoting factors to the injured areas, factors normally absent from the brain. Ramón y Cajal reasoned that central nerve pathways lacked "substances able to sustain and invigorate the indolent and scanty growth" similar to those provided by peripheral pathways. Numerous studies over the succeeding century identified constituents of peripheral nerves that are potent promoters of neurite outgrowth. These include components of Schwann cell basal laminae, such as laminin, and cell adhesion molecules of the immunoglobulin superfamily. In addition, cells in denervated

distal nerve stumps begin to produce neurotrophins and other trophic molecules of the sort described in Chapter 46. Together, these molecules nourish neurons and guide growing axons in the embryonic nervous system, so it makes sense that they also promote the regrowth of axons. By contrast, central neuronal tissue is a poor source of these molecules, containing little laminin and low levels of trophic molecules. Thus, in the embryo, both central and peripheral nervous systems provide environments that promote axon outgrowth. But only the peripheral environment retains this capacity in adulthood or is able to regain it effectively following injury.

The practical implications of this view are that supplementing the central environment with growth-promoting molecules might improve regeneration. To this end, investigators have infused neurotrophins into areas of injury or inserted fibers rich in extracellular matrix molecules such as laminin to serve as scaffold for axonal growth. In some attempts, Schwann cells themselves, or cells engineered to secrete trophic factors, have been grafted into sites of injury. In many of these cases, injured axons grow more extensively than they do under control conditions. Yet regeneration remains limited, with axons generally failing to extend long distances. More important, functional recovery is minimal.

Components of Myelin Inhibit Neurite Outgrowth

What accounts for such disappointingly limited regeneration? One part of the explanation is that the environment encountered by severed central axons is not only poor in growth-promoting factors but also rich in growth-inhibiting factors, some of which are derived from myelin. In culture, fragments of central but not peripheral myelin potently inhibit neurite outgrowth from co-cultured central or peripheral neurons. Conversely, sprouting of spinal axon collaterals following injury is enhanced in rats treated to prevent myelin formation in the spinal cord (Figure 50–7).

These findings implied that although both central and peripheral environments might contain a supply of growth-promoting elements, central nerves also contain inhibitory components. The fact that myelin inhibits neurite growth may seem peculiar, but not if we consider that myelination normally occurs postnatally, after axon extension is largely complete.

Searches for the inhibitory components of central myelin turned up an embarrassment of riches. Several classes of molecules that occur at higher levels in central myelin compared to peripheral myelin are able to inhibit neurite outgrowth when presented to cultured neurons. The first to be discovered was identified when an antibody generated against myelin proteins proved

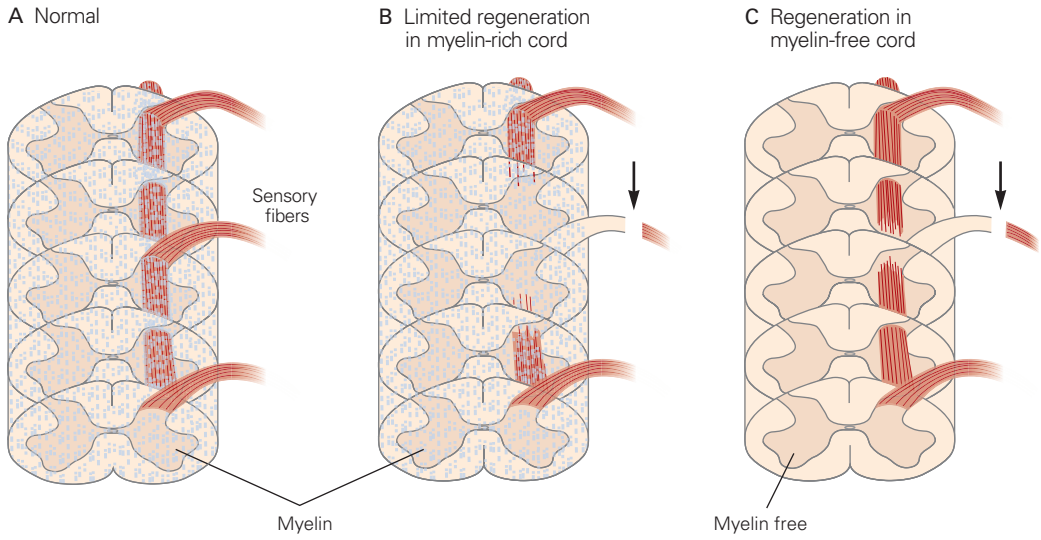

Figure 50–7 Myelin inhibits regeneration of central axons. (Adapted, with permission, from Schwegler, Schwab, and Kapfhammer 1995.)

A. Sensory fibers normally extend rostrally in a myelin-rich spinal cord.

B. Right dorsal root fibers were sectioned in 2-week-old normal rats. Regeneration of the fibers was assessed histochemically 20 days later. The central branches of the sectioned axons degenerated, leaving a portion of the spinal cord denervated. Little regeneration occurred in the myelin-rich cord.

C. Some littermates received local x-irradiation to block myelination. In these animals, sensory fibers that entered the cord through neighboring uninjured roots sprouted new collaterals following denervation.