1

Introduction

This book is about peripheral nerves, their unique biology and how they repair themselves during regeneration. The biology of the peripheral nervous system is not often considered on its own. Much has been learned about the neurosciences of peripheral nerves, specifically during injury and regeneration, but it is my sense that some of this new and exciting information should be consolidated and considered in an overview.

Without nerves, specifically peripheral nerves, there is no movement, no sensation. Peripheral nerves are the essential connections between the body, brain, and spinal cord. The "peripheral nervous system (PNS)" distinguishes itself from the "central nervous system (CNS)" on many levels. Peripheral axons reside in many types of local environments including muscles, connective tissue, skin, and virtually every organ of the body. This reach extends into the meninges that surround the brain, a surprising fact to some. Moreover, peripheral neurons are very different from their CNS counterparts in how they respond to injury or disease, in which cells they partner with and in what axon trees they support. For example, a sensory neuron in the lumbar dorsal root sensory ganglion is required to maintain and support distal axon branches that can extend a meter or more to the skin of the toe. Only a small proportion of CNS neurons have comparable outreach and demands placed upon them.

"Neuropathies," of which there are a large number, are simply disorders of peripheral nerves. A neuropathy might be focal (also known as a mononeuropathy) and involve only a single peripheral nerve, or it might involve peripheral nerves widely (polyneuropathy). Despite being very common problems, comparable in prevalence with stroke and Alzheimer's disease, they are not widely understood by patients, health care providers or neuroscientists! Polyneuropathy can be detected in approximately half of all diabetic subjects, an important issue

to consider in this day of dramatically rising Type 2 diabetes prevalence. Diabetic neuropathy itself, without considering all other forms of neuropathy, has a prevalence of over ten times that of MS.

Consider a few important points. A patient with severe peripheral nerve disease, such as Guillain–Barré syndrome (GBS) can, during the acute phase be completely "locked in," or unable to move a single limb muscle or eye muscle. This patient may require a ventilator to breathe. He may also have lost any sensation to light touch, pain, or temperature. Despite these severe deficits, however, cognition may well be fully retained because the disorder does not involve the brain. It is difficult to conceive of being "locked in" while being fully conscious unless one has suffered from GBS or a comparable disorder of the peripheral nervous system. The reader is referred to books written by patients who have suffered and recovered from GBS [373,374]. GBS is an autoimmune inflammatory polyneuropathy, sometimes triggered by infections or vaccinations that took place 2–3 weeks earlier. Different types of GBS are recognized. Yet it is the unique neurobiology of the nerves damaged during GBS that will most impact how a patient might fare. The most common form of GBS, also known as the classical demyelinating type, involves only the myelin sheath of the peripheral nerve. The underlying axon tree remains intact despite having been rendered nonfunctional by the loss of its myelin sheath. Remyelination of the axons is expected and can be associated with a complete recovery of paralysis and sensation.

Alternatively, a type of GBS recently termed AMSAN (acute motor and sensory axonal neuropathy) primarily attacks the axons and spares the myelin. Recovery is dictated by the rate and likelihood (certainly not guaranteed) that axons will regrow from the injury site to their correct original target, e.g., a small foot muscle, a touch receptor in the finger. The unfortunate result is very limited, delayed, or absent recovery in this severe form of GBS. While axons might be expected to regrow at the rate of about an inch per month in order to reach their targets, this likelihood falls dramatically with time. These limitations will be discussed in subsequent chapters. The tragedy is that, in some instances, severe GBS that primarily attacks axons may not recover at all.

Consider the story of "Nancy B.," a young woman who garnered national attention in Canada because of her peripheral nerve disorder. She developed severe axonal GBS that rendered her "locked in" and ventilator bound without any improvement over 2½ years. While being perfectly lucid about her condition, she made the decision to have her health care workers withdraw her ventilator support. Without this support, she did not survive. The story generated widespread discussion about ethical issues surrounding the maintenance of life support in patients who do not have a chance for recovery. Figure 1.1 is an image of a patient who required intensive care unit hospitalization for 1 year because of GBS.

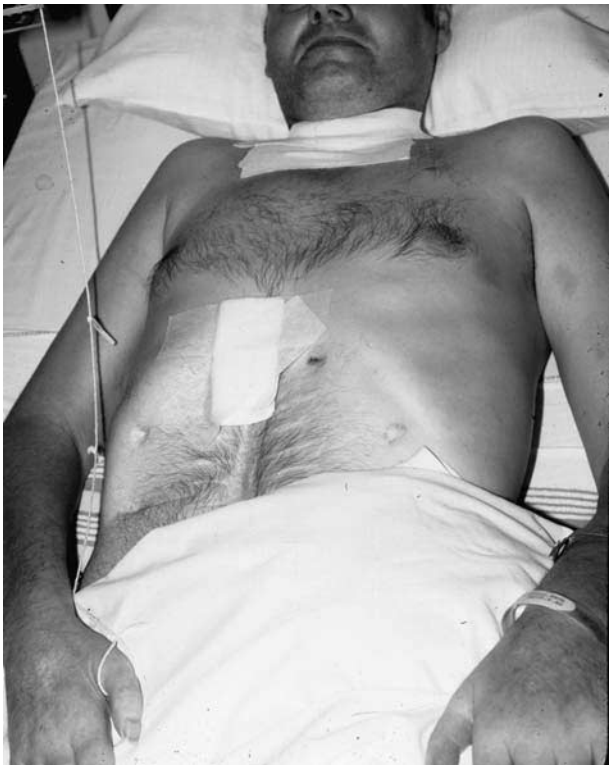

Figure 1.1 A patient with severe Guillain–Barré syndrome with axonal damage and paralysis of all of his limbs. He required intensive care unit support to breathe for a period of 1 year before recovering.

Fortunately, many neuropathies do not render disability as severe as that experienced by "Nancy B." They do, however, impose their own range of disabilities and interference with quality of life. Some are associated with significant loss of function. Consider the diabetic patient seen by the author who developed a focal mononeuropathy of his ulnar nerve at the elbow. This lesion rendered significant, though not complete, hand wasting and weakness. The patient, however, had been a professional tennis player and could not accept his inability to play. The neuropathy compounded underlying depression in this patient and led to suicide. Figure 1.2 is an image of a patient with a focal sciatic nerve injury lesion from a buttock firearm wound, rendering paralysis of muscles in the leg and loss of sensation in the foot.

Loss of sensation is associated with loss of the ability to detect skin and soft tissue injuries. Patients may develop skin ulcers from unrecognized injury to their feet (e.g., stepping on a nail or damaging their skin from overly tight footwear). In some cases these injuries are associated with additional damage, infection,

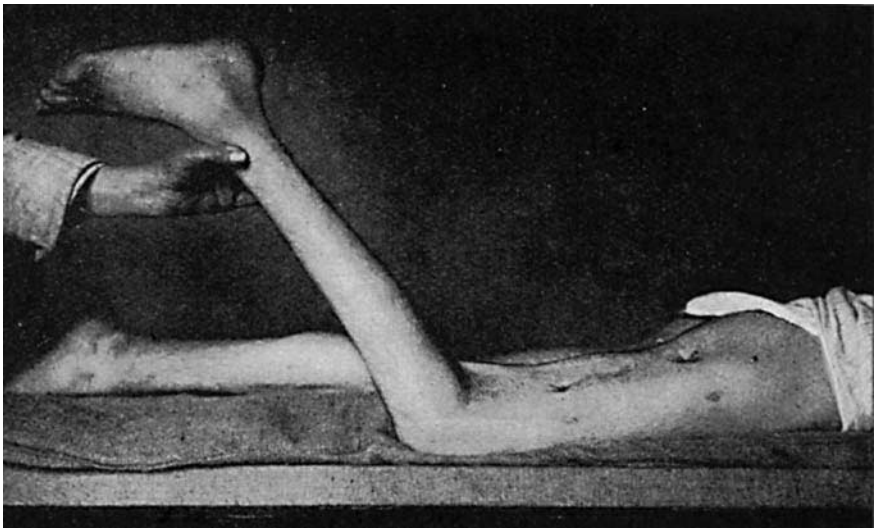

Figure 1.2 A patient with a right buttock bullet wound that damaged his underlying sciatic nerve trunk. The severe axonal disruption caused by the injury resulted in permanent paralysis and atrophy of muscles in the thigh and below the knee with sensory loss in the foot and leg. (From Tinel [681].)

and the need for amputation. Polyneuropathy is a leading cause of lower limb amputation in diabetes. Loss of sensation to position (proprioception) also contributes to falls and injury because it is impossible for a patient to tell where the limbs are in space. Finally, peripheral nerve damage of all types is frequently associated with a severe and debilitating type of pain known as "neuropathic pain." Neuropathic pain can render patients unable to walk, work, sleep, or enjoy life. While this text does not directly address neuropathic pain, full and effective regeneration of the peripheral nervous system usually extinguishes it.

Unlike many other disorders, neuropathies impose a burden of neurological deficit that requires nerve regeneration, irrespective of what caused the damage. Therapy for an active peripheral nerve disease, or microsurgical repair of a transected peripheral nerve trunk (we use the term "trunk" to refer to a peripheral nerve branch containing hundreds to thousands of individual axons and their supporting cells) may address the inciting lesion that caused damage, yet it is regeneration that must ensue to restore proper function. For example, vasculitic neuropathy is a disorder that damages peripheral nerve axons through inflammation of nutrient feeding vessels of the peripheral nerve trunk. It may be "cured" with a course of immunosuppressive therapy, a treatment that arrests the inflammation but does not restore function. This is unsatisfactory to many patients who suffer from neuropathy; previously damaged axons must now regrow to

reverse the deficits that have developed. At the time of this writing, specific therapy designed to coax more complete and effective recovery of nerves is unavailable.

In the neurosciences literature, peripheral neurons have been highlighted as examples of neurons whose axons can regenerate, unlike those of the CNS. Indeed, significant excitement has resulted from findings that injured CNS neurons in the spinal cord can regenerate into and through peripheral nerve grafts. Peripheral neurons have been seeded on substrates of CNS myelin to demonstrate its property to inhibit regrowth. Without diminishing the importance of these findings, however, they do not address the realities of peripheral nerve disease. In neuropathies, the fate of axons regenerating in their own peripheral microenvironment is the important consideration. Recovery is slow, and if the distances to the target tissues are long, regeneration may never occur. Such catastrophic failure occurs despite the fact that axons have a "denervated" distal stump into which to grow. Distal to an axonal injury, axons undergo the process of "Wallerian degeneration" in which disconnected branches are phagocytosed and eventually disappear. They leave behind a denervated distal stump that includes a connective scaffold and supporting Schwann cells. In this text, my intent is to dispel the idea that peripheral neurons serve simply as a model for understanding issues in CNS regeneration. Rather, I seek to convince the reader that PNS disease poses its own unique burdens on a substrate of neurons and supporting cells and has its own, separate but compelling regeneration issues. It deserves an equal and focused place at the neurosciences research table.

Exciting new aspects of peripheral nerve behavior challenge traditional concepts. One such example involves how axons interact with their basement membranes. Extracellular basement membrane constituents of nerve trunks expose specific ligand (e.g., the RGD, or Arg–Gly–Asp tripeptide sequences) moieties that interact with integrin receptors of adjacent axons. Local signaling cascades within axons are triggered by this interaction. These cascades have the capability of altering growth cone behavior and influencing regeneration yet may be completely independent of changes within their cell body. The idea that growth cones and axons *locally* might signal and react is novel. Not only do such signals influence axon behavior but in this scenario they also alter local *protein synthesis*, previously considered the sole purview of the cell body. To coordinate regeneration, the axon and perikarya also sense that there is injury, alter the pattern of nuclear gene expression in the cell body and change the repertoire of proteins they transport down the axon to the injury site. They do this while managing to signal local axons to synthesize regeneration-related molecules. How the whole family of regeneration molecules is coordinated between local synthesis or transport from the cell body is not known at this time. For example, local axon synthesis might act as a "rapid response" program for injured axons,

later supplemented by reinforcements shipped from the cell body. One might imagine that nerve surgeons could one day be capable of implanting regeneration conduits with graded release of signals that would "shore up" such local axonal events.

There are intriguing discoveries in how surrounding and supporting cells of the peripheral nervous system interact with damaged neurons. For example, a remote injury of a sensory neuron axon branch (an "axotomy" is a transection of an axon branch of a neuron) is sufficient to send information to its parent cell body in the dorsal root ganglion (DRG) up to a meter away. In response, perineuronal satellite cells in the ganglion that have not been directly involved in the injury dramatically change their phenotype. Satellite cells are cousins of Schwann cells and both cell types appear in sensory ganglia. Satellite cells, however, are interesting cytoplasmic poor cells that closely surround individual sensory neurons. They exhibit a dynamic form of life and death plasticity with ongoing apoptosis and division within "stable" ganglia. Their plasticity contrasts sharply with the apparent immutability of their neighbor neurons. Within a defined time course, satellite cells enlarge and proliferate around closely associated but axotomized neurons. How neurons communicate with these important and pervasive neighbors is unknown. Communication between neurons and satellite cells is likely to be reciprocal. For example, satellite cells are known to provide trophic molecules such as CNTF (ciliary neurotrophic factor) to support neurons and protect them from injury.

In contrast to sensory neurons, injured motor neurons have cell bodies that are official residents of the CNS, in the anterior horn of the spinal cord. When their remote axons are injured, their dendrites in the gray matter of the spinal cord retract. This accompanies "synaptic stripping" of their connections with other spinal cord neurons. How the loss of these dendritic connections occurs and how they might be restored is uncertain.

The Schwann cell (SC) is a type of glial cell, unique to the peripheral nervous system, that supports all types of axons: sensory, motor, and autonomic. Its roles can be surprisingly multifaceted and differ from those of their CNS myelinating counterpart, the oligodendrocyte. Early after nerve injury, SCs can serve as local inflammatory cells by generating cytokines, inducible nitric oxide synthase (iNOS; an inflammatory enzyme that generates nitric oxide (NO)) and other inflammatory molecules well before macrophages from the bloodstream enter the nerve and assume this function. SCs can interconvert from "stable" myelinating phenotypes to highly plastic proliferating and migrating cells that may direct appropriate and directional axon regrowth after injury. They offer the peripheral nerve a range of trophic molecules but not necessarily simultaneously. In other words, SCs appear to have a sense of timing and coordination

in what they synthesize. While the exact mechanism is unclear, there is evidence that they accurately guage their local microenvironment and respond accordingly. Moreover, like the neuron and its perineuronal satellite cell in the ganglion, there is intimate and bi-directional talk between the axon and the SC. Cross talk is likely critical during regeneration but may also be a feature of normal uninjured nerve trunks.

Axons elaborate neuregulins, potent molecules capable of altering SC protein synthesis, myelin synthesis, and their likelihood to proliferate. Neuregulins, in turn, instruct SCs to synthesize a series of molecules, such as neurotrophins that encourage axon regrowth in a highly directional manner. When peripheral nerve trunks are transected, the tension normally present within them causes the distal and proximal stumps to retract from one another. While such lesions are incompatible with full axon regrowth, the stumps can reconnect by a connective tissue bridge if they are apposed to one another in a graft or conduit. From the proximal stump of the transected nerve, axons then enter the bridge and begin to grow across it. These early events offer fascinating opportunities as to how axons navigate new territories. One interesting finding is that SCs appear to lead axons through complex, three-dimensional trajectories. Their relationship, closely linked with local trails of laminin, is so encompassing and intimate that it might be called the "axon-SC" dance! SC partnership is critical to the success of axon regrowth. Not surprisingly, a group of colleagues interested in SCs call themselves the "Friends of Schwann"!

The purpose of this text is to emphasize the unique structure, plasticity, and challenges of regrowing peripheral nerves. Excepting focal neuropathy associated with direct injury to the nerve, neuropathies are not addressed directly. We refer readers to other comprehensive texts addressing peripheral nerve disorders and peripheral nerve surgery [159,421].

We begin by examining properties of the peripheral nervous system in nerve trunks that house axons, Schwann cells and other tissue components, and ganglia that house cell bodies, or perikarya. Next we address how peripheral nerves are injured by trauma. What are the resulting injuries, their implications, and the barriers to regrowth? We then address experimental approaches to peripheral nerve regeneration. We ask how does nerve regeneration evolve through its early events and later consolidation? Special consideration will be given to the microvascular supply and its impact on regenerative events. Finally, we address important aspects of regrowth: the impact of long-term denervation, the actions of growth factors and molecular barriers of regrowth.

It is my intent that this text might be a project in evolution, consolidating what has been discovered to date, as well as being a catalyst for new ideas and approaches toward resolving the burden of peripheral nerve damage.

2

The intact peripheral nerve tree

A thorough appreciation of the unique anatomy of the peripheral nervous system is essential in understanding how it regenerates. This information, already described in several texts, is nonetheless summarized here to prepare the reader for later chapters. There are many facets to peripheral nervous system anatomy that have a bearing on its response to injury including the multiplicity of neuron subtypes and the qualities of their housing.

Overall structure

The peripheral nervous system (PNS) is complex. The peripheral nerve "trunk" refers to a cable of tissue in which hundreds to thousands of axons may travel. Peripheral nerve trunks form connections from the brain and spinal cord to all skeletal muscles in the body through motor axons. They also connect all sensory organs to the brain and spinal cord through sensory axons (Figure 2.1). Finally, they connect the CNS to smooth muscles, sweat glands, blood vessels, and other structures through axons of the autonomic nervous system. Axons traveling through nerve trunks originate from cell bodies of neurons (perikarya) in the brainstem, spinal cord, and ganglia. Motor neuron cell bodies found in cranial motor nuclei supply the head and neck, and those in the anterior gray matter horn of the spinal cord supply the limbs and trunks. Motor neuron cell bodies have their greatest numbers in the cervical and lumbar enlargements of the spinal cord so that they can supply the large number of muscles in the upper limbs and lower limbs, respectively. Sensory neuron perikarya are found in cranial sensory ganglia and paraspinal ganglia from approximately T1 through to L1 levels. Autonomic neurons are housed in a variety of sites: cranial and cervical ganglia, paraspinal sympathetic ganglia, and a variety of ganglia in the

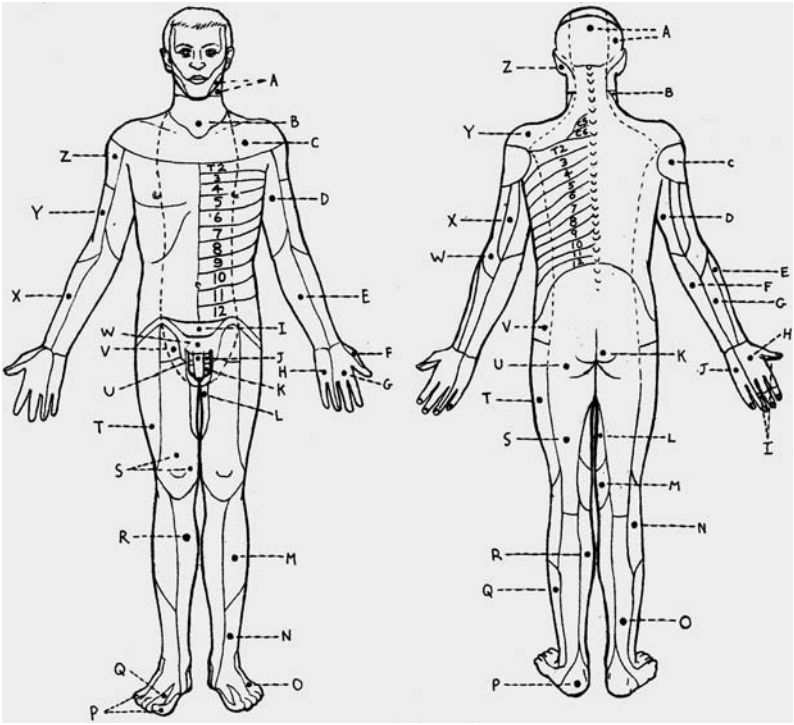

Figure 2.1 An illustration of the peripheral sensory nerve territories of the human body. *Left panel, anterior body:* A – greater auricular nerve; B – anterior cutaneous nerve of neck; C – supraclavicular nerves; D – medial cutaneous nerve or arm and intercostobrachial nerve; E – medial cutaneous nerve of the forearm; F – radial nerve; G – median nerve; H – ulnar nerve; I – iliohypogastric nerve; J – genital branch of genitofemoral nerve; K – scrotal branch of perineal nerve; L – oburator nerve; M – lateral cutaneous nerve of calf; N – superficial peroneal nerve; O – sural nerve; P – medial and lateral plantar nerves; Q – deep peroneal nerve; R – sapenous nerve; S – intermediate and medial cutaneous nerves of the thigh; T – lateral cutaneous nerve of the thigh; U – dorsal nerve of penis; V – femoral branch of genitofemoral nerve; W – ilioinguinal nerve; X – lateral cutaneous nerve of forearm; Y – lateral cutaneous nerve of arm; Z – axilllary nerve. *Right panel, posterior body:* A – greater and lesser occipital nerves; B – anterior cutaneous nerve of neck; C – axillary nerve; D – medial cutaneous nerve of arm and intercostobrachial nerve; E – lateral cutaneous nerve of forearm; F – medial cutaneous nerve of forearm; G – posterior cutaneous nerve of forearm; H – radial nerve; I – median nerves; J – ulnar nerve; K – inferior medial clunical nerve; L – obturator nerve; M – medial cutaneous nerve of thigh; N – lateral cutaneous nerve of calf; O – sural nerve; P – calcaneal branches of sural and tibial nerves; Q – superficial peroneal nerve; R – saphenous nerve; S – posterior cutaneous nerve of thigh; T – lateral cutaneous nerve of thigh; U – inferior lateral clunical nerve; V – iliohypogastric nerve; W – lower lateral cutaneous nerve of arm; X – posterior cutaneous nerve of arm; Y – supraclavicular nerves; Z – greater auricular nerve. (Illustration by Scott Rogers, based on previous illustrations by Haymaker and Woodall [254].)

abdomen that include the celiac, mesenteric, para-aortic, hypogastric, and others. The enteric nervous system includes a large number of neurons within the walls of the gastrointestinal system.

Nerve "trunks" originate through a confluence of branches that supply them. Motor neurons send axons to nerve trunks through the ventral roots of the spinal cord and motor branches of cranial nerves. Sensory neurons have an initial single branch that emerges from the cell body and then divides into two branches, an arrangement called "pseudounipolar" (see below). From the initial single branch, a central branch is directed to the spinal cord entering the dorsal horn and posterior columns and a peripheral branch is sent to the nerve trunks. The peripheral sensory branch in the dorsal root joins motor axons from the ventral roots as they exit the neural foramina of the bony spinal column and together form the mixed spinal nerve. It is at this site that the meningeal sheath (dura and arachnoid) surrounding the spinal cord and its roots forms pockets or sleeves that blend into the epineurial sheath of the peripheral nerve. Mixed spinal nerves then send branches posteriorly to innervate the paraspinal muscles and anteriorly where they form the major nerve trunks of the body. From both the cervical enlargement and the lumbar enlargement, anterior spinal nerve branches merge and intermingle to form the brachial and lumbosacral plexus, respectively. From each plexus, peripheral nerves are then formed from a mixture of motor, sensory, and autonomic axons arising at different root levels. Despite the mixture, most major nerve trunks include axons from only a few spinal root levels. Thus, for example, the human median nerve is composed almost exclusively of axons from the C8 and T1 spinal root level, while the musculocutaneous nerve arises from C5 and C6.

Given this overall arrangement then, most major nerve trunks house a variety of axon types. For example, there are likely no "pure" motor nerve trunks since nerves associated with muscles include both motor axons and a large complement of sensory axons sensitive to muscle pain, or to stretch in muscle spindles. Cutaneous nerves do not include motor axons but do contain both autonomic and sensory axons. In any major peripheral nerve trunk, therefore, there are larger myelinated axons (α motor axons and large Aα sensory axons), small myelinated axons (smaller Aβ and Aδ sensory axons, γ motor axons – see below), and unmyelinated axons (C sensory and autonomic axons). Classical histological approaches do not distinguish whether myelinated axons are motor or sensory or whether unmyelinated axons are sensory or autonomic. Thus, when a transverse section of a peripheral nerve trunk is examined, it is not possible to identify what class a given axon may belong to. In humans the sural nerve is most often harvested for diagnostic purposes and its axons are sensory or autonomic only.