Section 1 Neural plasticity: cellular and molecular mechanisms of neural plasticity

Chapter 1

Degenerative changes and reactive growth responses of neurons following denervation and axotomy: historical concepts and their modern embodiments

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Introduction

Today, we tend to think of the nervous system as a highly plastic structure in which connections are continually being modified. With this view, we are not surprised by reports of neuronal growth following injury, and indeed, are perhaps surprised that it does not occur more extensively. This is in stark contrast to the view during the first half of the twentieth century, based on the extensive work of Cajal [1], that the nervous system was "fixed and immutable," and that neurons of the adult mammalian central nervous system (CNS) were incapable of any more than very limited and abortive growth. The origins of the shift in viewpoint can be traced to reports in the late 1960s and early 1970s that documented the formation of novel synaptic connections following CNS injury, especially the landmark study [2] that provided the first electron microscopic evidence that neurons in the septal nucleus were reinnervated after their normal connections had been disrupted by lesions. Similar evidence was obtained in studies of the superior colliculus [3] and olfactory bulb [4]. What made these reports noteworthy was the demonstration of novel synaptic connections that had the potential of modifying circuit function.

Initially, these reports of neuronal growth in the mature nervous system were viewed with skepticism, and many felt that the growth occurred only in special circumstances, or was very limited in extent. Indeed, some opined that there was no growth at all – that the images suggestive of reinnervation reflected nothing more than a passive shift of presynaptic terminals from one postsynaptic site to another that had been left unoccupied by the removal of a degenerating synapse. There were doubts that the apparent synapses were physiologically functional. Nevertheless, these reports motivated subsequent studies that provided more and more examples of synaptic reorganization following injury. Now, as a consequence of hundreds of studies over the past 40 years, we know that reorganization of circuitry after CNS injury is the norm rather than the exception, that new connections are made that are capable of synaptic transmission, and that reorganization of circuitry can contribute to functional recovery.

Another important discovery was that there is some ongoing neurogenesis in the mature nervous system, and that newly formed neurons extend axons for long distances and form synaptic connections (see elsewhere in this volume). These examples of growth potential raise the question of why growth and repair following injury is not more extensive, and why the prognosis following CNS injury still remains rather bleak.

It is clear that part of the reason that recovery is limited is that although new neurons can be generated, the process is very limited, and does not occur to a degree that is sufficient to replace neurons that die as a consequence of injury or disease. In addition, axon growth that does occur is generally short distance, and true regeneration of injured axons is very rare in the mature mammalian nervous system. Nevertheless, the spatially limited growth that occurs naturally without any therapeutic intervention can have functional consequences, and recent studies indicate that the growth can be amplified by various manipulations including simple behavioral experience.

This chapter will describe the reactive changes that CNS neurons exhibit following injury – both degenerative responses that occur following denervation and axotomy, and reactive growth that may contribute to recovery of function. Other chapters consider the many new approaches to enhance regeneration or replace tissue destroyed by trauma through molecular manipulations, transplants, stem cell technologies, and by harnessing the potential for cell replacement that exists in the mature nervous system.

How injuries affect neurons and their connections

Damage to the CNS affects all cell populations in the brain including neurons, glia, ependymal cells, and vascular elements. In addition to directly damaging neurons, traumatic

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injuries disrupt blood flow, disrupt the blood–brain barrier, interfere with the manufacture, distribution, or reabsorption of cerebrospinal fluid (CSF), produce widespread changes in metabolism, and damage myelin-forming oligodendrocytes. In addition, injuries trigger delayed death of neurons and glia through apoptotic and other mechanisms, as well as eliciting invasion of inflammatory cells from outside the CNS. While all of these may directly or indirectly alter neuronal function, we will focus here on the direct injury to neurons and their interconnections.

In this light, CNS trauma affects neuronal circuitry by: (1) interrupting axonal projections, (2) denervating certain populations of neurons, and (3) removing some neurons entirely. Indeed, most traumatic injuries produce all of these effects. Because of the loss of some neurons, even if CNS neurons were capable of axonal regeneration, some regenerating axons would find nothing to innervate upon reaching the area of damage. Thus, when considering repair mechanisms following CNS trauma, the important issues concern the fate of the tissue that survives and the mechanisms that contribute to the salvage of neurons that have lost either their normal targets or their normal inputs.

The fate of neurons that lose their normal inputs

The consequences of synapse loss for CNS neurons range from subtle changes in specific receptive elements (alterations in neurotransmitter receptors, modifications in the postsynaptic specialization, disappearance of dendritic spines, etc.) to changes that culminate in the disappearance of entire dendrites or even in the death of the denervated neurons (transneuronal atrophy or degeneration, respectively).

Alterations in neurotransmitter receptors

There is now considerable evidence that neurotransmitter receptor number at individual synapses can be regulated on an ongoing basis by the overall level of synaptic activation and also by the activity of the individual synapse. Recent studies have led to the concept of "homeostatic plasticity" in which the level of activity of CNS neurons is controlled within a particular range by adjustments in the number of neurotransmitter receptors at synapses (for a recent review, see [5]). Prolonged decreases in overall synaptic activation lead to increases in the number of receptors for excitatory neurotransmitters whereas prolonged periods of increased synaptic input lead to down-regulation of receptors. The cellular and molecular mechanisms underlying these homeostatic adjustments are being worked out currently, and in general, involve receptor trafficking to and from the postsynaptic membrane [6].

This concept of homeostatic plasticity has a historical precedent in the concept of denervation supersensitivity, which was first characterized through studies of denervated muscle and peripheral ganglia [7]. Denervation supersensitivity is a phenomenon in which the postsynaptic cell becomes more sensitive to a neurotransmitter following denervation. In muscle, denervation supersensitivity results from an increase in the number and a change in the distribution of acetylcholine (ACh) receptors [8]. In normally innervated muscle, receptors are selectively localized at the end plate beneath the motor nerve terminal. Following denervation, there is a dramatic induction of ACh receptor expression, and the newly synthesized receptors are inserted all along the muscle fiber, making the muscle fiber sensitive to ACh all along its length. These changes are associated with overall atrophy of the muscle fibers, and other major changes in gene expression and muscle morphology. The alterations in receptor expression and distribution as well as the other changes can be reversed if the muscle fiber is reinnervated either as a result of regeneration of the original axon or collateral sprouting of nearby axons that innervate nearby muscle fibers.

Neurons in peripheral ganglia also exhibit denervation supersensitivity, and if denervated cells survive and some projections are spared, the supersensitivity makes existing synapses more powerful [9]. In this way, denervation supersensitivity may be a homeostatic mechanism that contributes to the maintenance of transmission when there is a partial loss of connections. Interestingly, loss of input from a particular neurotransmistter system does not necessarily lead to supersensitivity of all the receptor subtypes activated by that neurotransmitter. For example, removal of cholinergic input to neurons in the superior cervical ganglion (which normally transmit via both nicotinic and muscarinic mechanisms) leads to increased sensitivity to muscarinic but not nicotinic agonists [10]. In fact, the response to nicotinic agents is diminished.

An example of denervation supersensitivity in the CNS is the increase in dopamine sensitivity in the striatum after the destruction of dopaminergic nigrostriatal projections [9,11]. Early studies using ligand binding techniques did not reveal increases in dopamine receptors [12], suggesting that the mechanisms of the functional supersensitivity appear to be different than in muscle. More recent studies, however, have revealed that this example of functional supersensitivity involves increases in both the number of D_2 receptors, and the coupling of D_1 and D_2 receptors to their respective G-protein signaling partners [13].

There has been speculation that the spasticity that develops in the chronic period after spinal cord injury (SCI) reflects, in part, a supersensitivity phenomenon in which neurons that lose descending input become more sensitive to neurotransmitters. For example, in the chronic period following SCI, activation of sensory afferents to segments caudal to the injury elicits long-duration muscle spasms indicating enhanced excitability of segmental reflex circuitry. Recently, an in vitro model has been developed to explore the cellular mechanisms of this spasticity, in which the sacral spinal cord of rats that had received full transactions at S2 one month previously are maintained in recording chambers. In these preparations, stimulation of dorsal roots elicits prolonged discharges of

ventral root axons, mimicking the prolonged muscle spasms elicited by afferent stimulation in vivo. These chronically denervated sacral segments also exhibited prolonged discharges in response to application of the neuromodulators norepinephrine and serotonin, indicating supersensitivity to the neuromodulators.

Most of the well-characterized examples of denervation supersensitivity in the CNS involve neuromodulators like dopamine, norepinephrine, and serotonin, but there are examples of denervation supersensitivity in the glutamatergic systems in the CNS (that is, increases in glutamate receptor expression or sensitivity to glutamatergic agonists following loss of glutamatergic inputs). One interesting example involves the nucleus tractus solitarius (NTS), which becomes supersensitive to glutamate after the extensive denervation produced by removal of the nodose ganglion [14]. It is noteworthy that the NTS receives a substantial proportion of its innervation from the nodose ganglion, and hence the degree of denervation would be extensive.

Most CNS neurons receive tens of thousands, sometimes hundreds of thousands, of synapses, and it is hard to imagine an injury that would cause the degeneration of a majority of the glutamatergic inputs to individual neurons. There is surprisingly little information about whether there are adjustments in receptor number following partial denervation and if so, whether these occur at denervated sites or at other surviving synapses. If at denervated sites, the result could be increased sensitivity to glutamate spillover from nearby synapses. If at other surviving synapses, the result would be increased potency of these other synapses (a form of heterosynaptic receptor plasticity).

Because CNS neurons receive a multitude of individual synapses, it is possible that fundamentally different mechanisms exist to adjust to denervation than in muscle fibers and neurons in peripheral ganglia. At the same time, however, the phenomena encompassed by the concept of "homeostatic plasticity" indicate the existence of mechanisms that could up-regulate sensitivity in the face of prolonged decreases in excitatory synaptic drive as would be expected to occur with denervation.

There has been a surge in understanding of mechanisms regulating receptor trafficking during homeostatic plasticity based on studies of neurons in culture. Studies of responses to denervation in vivo will be more challenging, especially because adjustments in receptors occur over the course of many hours or even days. It is also possible that homeostatic plasticity as such is largely restricted to the developmental period. For example, it has been reported that the capacity for synaptic scaling decreases as circuits mature [15]. At the same time, there are other reports that the capacity for scaling persists through adulthood, at least in some areas [16]. Whatever the case with activity-dependent scaling, it will be important to explore whether similar mechanisms are engaged when neurons are denervated. New live imaging techniques may provide ways to explore the question directly in living animals. It may be possible, for example, to transfect neurons with genetically tagged receptor subunits, and then assess receptor trafficking at both denervated and nondenervated synaptic sites after experimental injuries.

Structural modifications of denervated neurons

Receptor trafficking occurs at existing synapses, but denervation can lead to the loss of postsynaptic membrane specializations and spines, sometimes involves loss or shrinkage of dendrites, and can even result in the death of the postsynaptic neuron. Historically, the terms used to refer to these denervation-induced changes are transneuronal atrophy and transneuronal degeneration.

Transneuronal atrophy

Transneuronal atrophy is a general term that refers to disappearance of spines, decreases in the size of dendrites, or decreases in the size of the postsynaptic cell following denervation. Transneuronal atrophy of neuronal somata was studied extensively in sensory relay nuclei of the visual and auditory systems, where the incoming afferents provide a substantial proportion of the input to the relay neurons. For example, destruction of the projections from the eye results in atrophy of neurons in the lateral geniculate nucleus [17], and interruption of the eighth nerve leads to atrophy of neurons in the cochlear nucleus (see later).

Transneuronal atrophy can involve only part of the receptive surface of the postsynaptic cell. For example, if a given projection system terminates on dendritic spines, then the removal of that input will often lead to the disappearance of the denervated spines [18–21]. This usually involves a collapse of the spine into the parent dendrite [18,22]. Postsynaptic membrane specializations may also disappear, although some cells may retain uninnervated membrane specializations for a time [23,24].

Extensive denervation of a dendrite can result in atrophy of the entire dendrite. This phenomenon has been particularly well documented in the auditory pathways of the chick [25,26]. For example, neurons of the avian homolog of the medial superior olive have bipolar dendritic trees that receive most of their innervation from the cochlear nucleus. One side of the bipolar dendritic arbor is innervated by the ipsilateral cochlear nucleus, while the opposite arbor receives contralateral input. When the inputs to one dendritic arbor are damaged, the denervated dendrites on one side of the cell body undergo substantial atrophy whereas the normally innervated dendrites extending from the opposite pole are preserved. When the denervation is partial, there is a partial preservation of the denervated dendrite [27]. Thus, in this system, the degree of dendritic atrophy is related to the extent of denervation.

The atrophy of denervated portions of postsynaptic cells can be a transient phenomenon, in that dendrites and their

spines can be reconstructed if the dendrite is reinnervated. For example, denervation of the granule cells of the hippocampal formation results in a loss of spines and atrophy of affected dendrites at early post-lesion intervals, but these changes are reversed as synapses are replaced [18,20]. In the auditory system, however, rapid dendritic atrophy may prevent reinnervation that could otherwise occur. For example, dendrites are preserved when denervation occurs gradually over a prolonged period of time, and in this case, the dendrites are partially reinnervated [27]. Thus, the final extent of transneuronal atrophy may depend upon the timing of atrophy in relation to the timing of reinnervation.

Live imaging techniques offer the promise of achieving a far greater understanding of these dynamic structural alterations than has been possible to date. For example, one recent study has used time-lapse live imaging of neurons in slice cultures to reassess spine loss and reacquisition in the dentate gyrus following removal of entorhinal cortical input [28]. Interestingly, denervation did not affect the rate of new spine formation, but did alter spine turnover. Early after denervation, spines were less stable leading to spine loss; then spine stability increased leading to restoration of normal spine density. One caveat is that this study involved slice cultures rather than living animals. It will be of considerable interest to use live cell imaging approaches to assess directly the timing of spine loss and reappearance, atrophy of dendrites, time course of reinnervation, and the relationship between the different processes in living animals.

Transneuronal degeneration

In some cases, denervation results in the death of the affected neuron, which is termed transneuronal degeneration [29]. Some of the early examples of transneuronal degeneration came from studies in sensory systems. For example, destruction of the projections from one eye results in the death of some neurons in the lateral geniculate nucleus [17]. In the olfactory system, removal of the olfactory epithelium results in transneuronal degeneration of cells in the olfactory bulb [30], and interruption of the lateral olfactory tract leads to transneuronal degeneration of neurons in the pyriform cortex [31]. In the auditory system, neurons in the cochlear nucleus exhibit rapid transneuronal degeneration when input from the cochlea is disrupted in young animals [32,33]. In mature animals, the same lesion causes transneuronal atrophy, but minimal degeneration. Thus, the extent of transneuronal degeneration depends critically upon developmental age. Indeed, in chicks and mice, neurons become resistant to transneuronal degeneration over the interval of a few days. The mechanisms underlying the development of resistance remain to be defined.

There have been extensive studies of the mechanisms underlying transneuronal degeneration in the auditory system of the chicken [34–38] and more recently in rodents [39,40]. Removal of the cochlea in young chicks leads to the death of about 30% of the neurons in the nucleus magnocellularis (the avian homolog of the cochlear nucleus). In this system, transneuronal degeneration is triggered by the cessation of synaptic activity because transneuronal degeneration can be induced by infusing tetrodotoxin into the cochlea, which silences activity in the eighth nerve [37]. In contrast, there is minimal cell death after cochlear removal in adult animals, indicating that this form of transneuronal degeneration is restricted to a critical period of development. Studies in mice have defined changes in gene expression in neurons at the end of the critical period [39], and that denervation triggers a different transcriptional response after the end of the critical period, which is associated with neuronal survival [40].

An interesting feature about transneuronal degeneration in the chick auditory system is that it occurs very rapidly. Within hours after removal of the cochlea, neurons in the nucleus magnocellularis cease producing protein [38]. The cessation of protein synthesis is one of the earliest signs of the impending degeneration. Interestingly, in parallel with the disruption of protein synthesis, there is a dramatic loss of ribosomes within the affected cells [41]. Mechanisms underlying the loss of ribosomes remain to be established.

Transneuronal degeneration is not invariably observed, even when the loss of input is substantial. For example, in mammals, the dorsal cochlear nucleus receives a substantial projection from the cochlea, but does not degenerate along with the ventral cochlear nucleus following interruption of cochlear input [42]. It may be that other inputs sustain these cells, or that elimination of eighth nerve activity does not affect postsynaptic activity to the same degree as in the ventral cochlear nucleus. Alternatively, it may be that certain neuron types are inherently more able to survive the loss of inputs than others.

Fate of neurons following axotomy

Trauma can cause physical transection of axons (axotomy), causing the portion distal to the injury to degenerate (Wallerian degeneration). The affected neurons are thus both physically damaged and disconnected from the targets that the damaged axon normally contacts. Neurons exhibit a range of responses following axotomy from atrophy and death (retrograde atrophy and degeneration, respectively) to survival with minimal obvious consequences [29,43,44]. Obviously, neurons that die cannot regenerate their axons or receive new connections that might contribute to recovery of function, and hence protection from retrograde degeneration is a potential target for therapeutic interventions to preserve or improve recovery after trauma.

Retrograde atrophy and degeneration

Three factors appear to influence the degree of retrograde atrophy and degeneration following axotomy. First, the degree of atrophy and degeneration is greatest if axons are damaged proximal to the cell body than if the injury occurs more distally. Second, retrograde atrophy and degeneration are more likely if a substantial proportion of connections with

targets are interrupted. This is thought to reflect the fact that retrograde atrophy and degeneration occur because neurons depend on trophic factors supplied by the target (the topics of neuronal death and rescue and the role of target-derived trophic factors are considered elsewhere in this volume). Additionally, neurons with collateral projections to a number of different targets are more likely to survive the loss of one of these targets than neurons that project predominantly or exclusively to one site. This is the "principle of sustaining collaterals" [45]. Third, retrograde degeneration is usually more severe in young animals. Retrograde atrophy and degeneration are most likely in young animals and following proximal axonal injury, but there have been reports of retrograde degeneration in mature animals following distal axonal injury.

One CNS system that has served as a model for studies of retrograde atrophy and degeneration is the magnocellular division of the red nucleus following damage to rubrospinal axons in the spinal cord. Following interruption of the rubrospinal tract in the spinal cord, there is a dramatic decrease in the number of red nucleus neurons that can be seen in histological preparations. Although it was initially thought that many neurons in the red nucleus died after axotomy, subsequent studies revealed that the neurons actually undergo extreme retrograde atrophy, and that there is little or no actual cell death. For example, following delivery of brain-derived neurotrophic factor (BDNF) to the area of the brainstem containing the red nucleus one year after injury, red nucleus neurons again became visible in Nissl stains, indicating that the neurons had atrophied to the extent that they were undetectable in routine histological preparations [46]. It remains to be seen how many other examples of apparent retrograde degeneration will turn out to involve extreme atrophy rather than actual neuronal death.

Retrograde changes in red nucleus neurons can be reversed by transplanting fetal spinal cord tissue into the injury site in the spinal cord [47]. The neurons that are rescued are ones that have axon collaterals to rostral CNS areas, exemplifying the principle of sustaining collaterals [48]. In keeping with the idea that retrograde atrophy is due to the loss of target-derived trophic support, retrograde atrophy of red nucleus neurons can be reduced by delivering neurotrophic factors (BDNF or neurotrophin [NT-3]) [49], or by transplanting fibroblasts that have been genetically modified to secrete BDNF [50].

One system that is of considerable interest in terms of retrograde degeneration is the corticospinal tract (CST). There have been conflicting reports for decades regarding the fate of cortical motoneurons following damage to the CST after SCI (for references, see [51]). A recent study seemed to provide compelling evidence for substantial loss of cortical motoneurons that project to thoracic levels and below following an injury to their axons in the dorsal funiculus at thoracic levels [52]. In this study, cortical motoneurons were retrogradely labeled by injecting tracers into the spinal cord at the time of the injury, and then counts were made of the number of retrogradely labeled neurons at different times post-injury. There was an approximately 40% decrease in the number of retrogradely labeled cortical motoneurons in the hindlimb region, which was interpreted as retrograde degeneration. Many cortical motoneurons were also TUNEL positive and exhibited increased staining for caspase-3 and Bax, suggesting apoptosis. These striking results were of major importance in terms of developing therapies to promote axon regeneration after SCI. Therapies to promote axon regeneration require that the cells of origin survive, because when a cell body dies, the axon dies as well, which would make therapeutic intervention to induce axon regeneration futile.

Follow-up studies failed to confirm the reported retrograde degeneration of cortical motoneurons following SCI, however. In one follow-up study, the question was addressed using a different approach than had been used previously [51]. Instead of analyzing the cell bodies of cortical motoneurons, this study assessed axons of the CST in the one site that they are found in a single, definable tract – the medullary pyramid. Essentially all CST axons travel through the medullary pyramid en route to the spinal cord. If CST cell bodies undergo retrograde degeneration following SCI, their axons would undergo Wallerian degeneration. This is called "indirect Wallerian degeneration" [53]. There should also be time-dependent decreases in axon number in the medullary pyramid. Quantitative analyses of the medullary pyramid after the same type of lesions of the dorsal funiculus at the T9 level as in Hains et al. [52], or lateral hemisections at C5 revealed no evidence for Wallerian degeneration at any time post-SCI. Moreover, axon counts revealed no decrease in axon number in the medullary pyramid after SCI, regardless of injury level, severity, or time post-injury.

In a second follow-up paper [51], the question was reassessed using the same techniques as had been used in Hains et al. [52], by counting the number of retrogradely labeled cortical motoneurons at different times following lesions of the dorsal funiculus at T9 and staining for TUNEL. There was no evidence for any loss of retrogradely labeled cortical motoneurons or increased TUNEL staining following either T9 lesions [52]) or C5 lateral hemisections. Similarly, Brock et al. [54], used a stereological technique to count cortical motoneurons after dorsal column lesions at C5, and found no evidence of cortical motoneuron loss. Other studies have also failed to detect any loss of cortical motoneurons following cervical hemisections in primates [55].

Taken together, the evidence now seems compelling that there is minimal if any loss of cortical motoneurons following injury to their axons as a result of lesions in the spinal cord, at least within the time frame of the studies that have been undertaken (approximately one year post-injury, in the case of that by Nielson et al. [51]). Nevertheless, there has been confirmation of previous findings of retrograde atrophy of the cell bodies of cortical motoneurons following SCI [54,56,57]. Interestingly, two of these studies also showed that retrograde atrophy of cortical motoneurons could be reversed by treatments that may enhance regenerative growth of axons. For example, retrograde atrophy of cortical motoneurons after cervical SCI in either rats or primates was reduced in animals that received

BDNF-expressing adeno-associated virus (AAV) vectors into the spinal cord [54]. In addition, retrograde atrophy after thoracic SCI in mice was reversed by delivering chondroitinase ABC either into the cerebral ventricles or the spinal cord [56]. It will be of interest to determine whether the reversal of retrograde atrophy is correlated with enhanced axon growth in these situations.

Synapse stripping

One interesting manifestation of the retrograde response to axotomy is synapse stripping, also called bouton shedding, in which presynaptic inputs to axotomized neurons disconnect and withdraw [58–60]. Studies of axotomized motoneurons reveal that even before physical withdrawal, there are decreases in synaptic potency [61,62]. Often, glial processes are interposed between presynaptic profiles and their former site of termination [60]. Synapse stripping can cause a substantial disruption of synaptic transmission along pathways that are otherwise intact [58]. Nevertheless, disconnected synapses can re-establish contact with the axotomized neuron if the axotomized neuron successfully regenerates its axon, restoring synaptic communication [58,60]. The disconnection persists, however, if the axotomized neuron is prevented from reconnecting [59]. Interestingly, there are reports that the extent of stripping of synapses from axotomized facial motoneurons was reduced in rats that received subcutaneous implants of testosterone at the time of facial nerve transection [63], indicating that the process can be modulated by therapeutic interventions. The extent to which such interventions can enhance circuit function remains to be seen.

Cascading degeneration

Retrograde and transneuronal degeneration are not necessarily limited to one synaptic relay. If denervated neurons die, then their targets are denervated, and depending on the circumstances, the next neuron in the relay may also die. Furthermore, retrograde degeneration will remove the target of axons that normally terminate on the degenerating cells. This may then induce a secondary retrograde degeneration of the cells that normally innervate the neurons actually damaged by the trauma. For example, damage to the limbic cortex results in retrograde degeneration in the anterior thalamic nucleus and secondary retrograde degeneration in the mammillary nucleus [29,64]. This sort of cascading degeneration seems to take place predominantly when lesions occur during development, and in projection systems that are "closed" in that they receive and provide limited connections to other brain regions [29]. This is consistent with the concept of sustaining collaterals, mentioned previously.

Delayed neuronal death following ischemia

An important form of delayed degeneration occurs in some neuronal populations that have suffered transient ischemia [65]. Neurons that are susceptible to this form of degeneration survive the immediate ischemic period, but then die hours or days later. Certain populations of neurons in the cortex and hippocampus are particularly susceptible [66]. This form of degeneration is due in part to excitotoxic injury caused by massive release of glutamate during and after the ischemic insult [67–69], and occurs via apoptosis (see other chapters in this volume for further discussion of ischemic, excitotoxic, and apoptotic processes).

Trauma-induced death of oligodendrocytes and demyelination

Another form of degeneration that is related to axonal damage is a delayed degeneration of oligodendrocytes leading to demyelination of axons (Figure 1.1). This has been especially

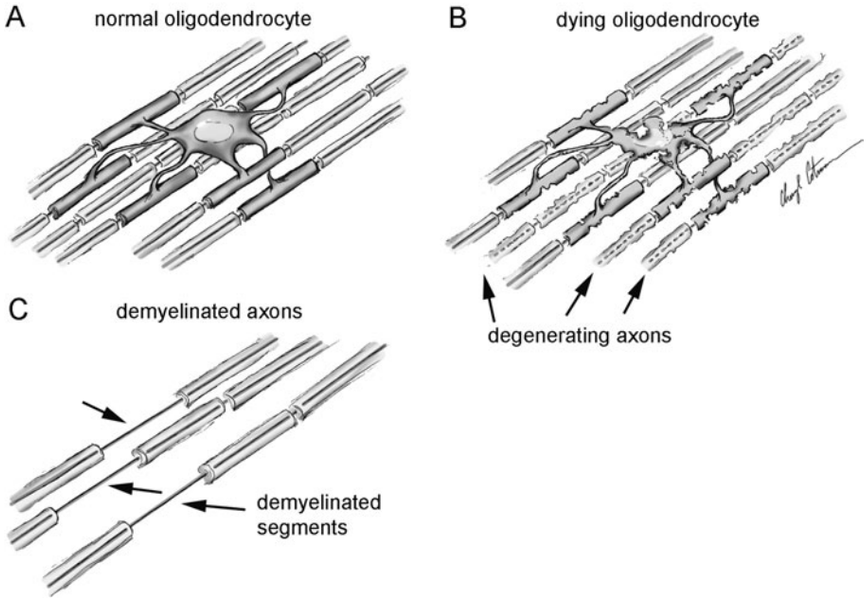

Figure 1.1. Relationship between Wallerian degeneration, death of oligodendrocytes, and demyelination. How much demyelination is there when oligodendrocytes die as a consequence of trauma? A. Normal oligodendrocytes can myelinate multiple axons; the number varies depending on the tract. B. Injury that causes the degeneration of axons in white matter sometimes leads to apoptosis of oligodendrocytes. Oligodendrocytes may die as a consequence of Wallerian degeneration of some of the axons that they ensheath. Alternatively, oligodendrocyte death may be unrelated to Wallerian degeneration, in which case death of the oligodendrocyte would lead to demyelination of all of the axons ensheathed by a particular oligodendrocyte. C. Following death of an oligodendrocyte, axons may survive but have demyelinated segments. (For color image, see color plate section.)

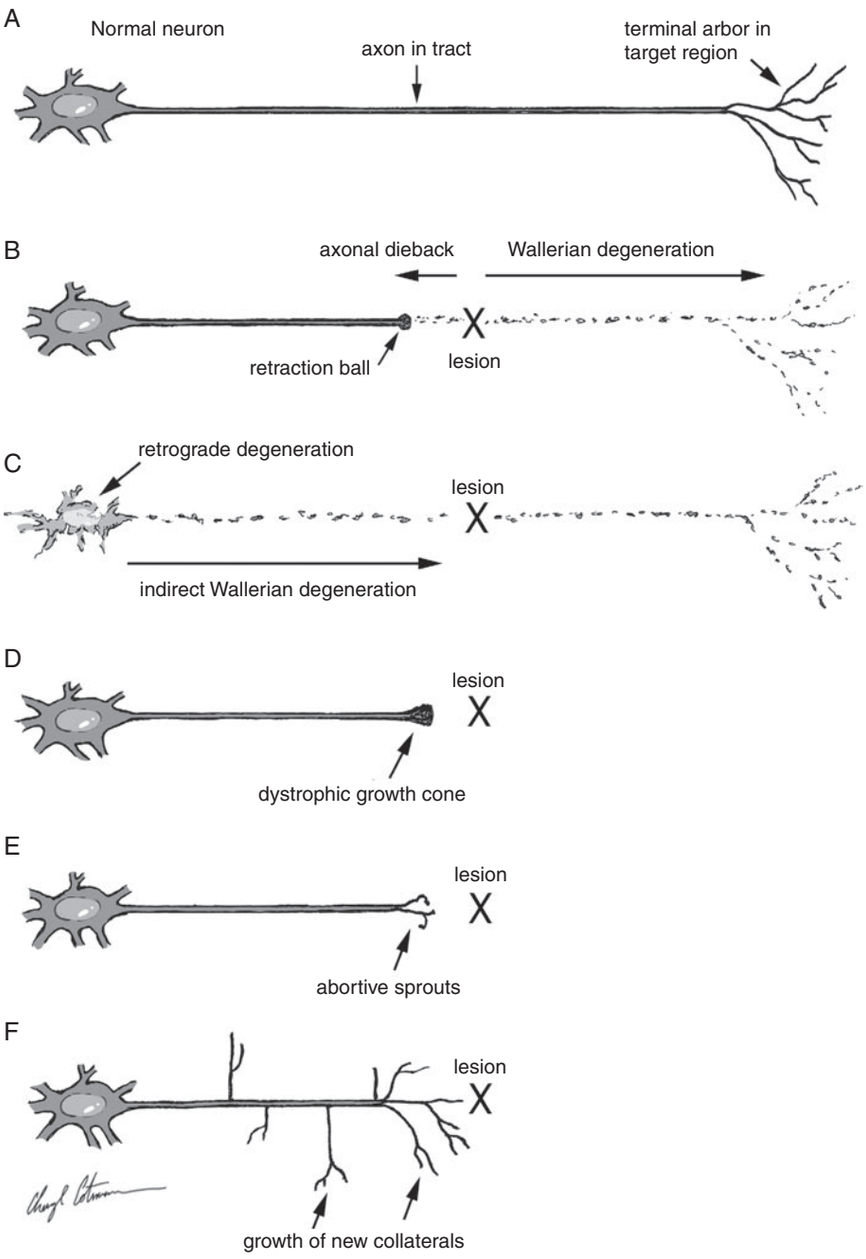

Figure 1.2. Responses of neurons to axotomy. When axons are cut at a particular location, the distal segment degenerates (Wallerian degeneration), and the proximal segment retracts, forming retraction balls (axonal dieback). If neurons die as a result of axotomy (retrograde degeneration), the cell body dies, and the resulting degeneration of the part of the axon proximal to the axotomy is termed "indirect Wallerian degeneration." Abortive growth is reflected by the presence of dystrophic growth cones and tangled arbors. Bona fide regeneration would involve the regrowth of the cut axon without branching. Cut axons may also give rise to new branches (collaterals) at or near the point of injury, or at any point along the axon between the amputation and the cell body. (For color image, see color plate section.)

well documented following SCI, where it has been shown that injuries at a particular segmental level cause the death of oligodendrocytes over many segments [70] (see [71] for a recent review). The oligodendrocytes die days and even weeks after the injury through apoptotic mechanisms [72,73]. The loss of myelin segments from surviving axons (demyelination) is thought to disrupt action potential propagation by the demyelinated axons (see Volume I, Chapter 32 for a further discussion of the consequences of demyelination on axonal function).

If injury leads to extensive death of oligodendrocytes causing demyelination of large numbers of axons, this points to several possible strategies for repair, including transplanting myelin-forming cells. Nevertheless, predicting the relationship between the death of oligodendrocytes and demyelination of surviving axons is not straightforward. One issue is the number of axons that are myelinated by a single oligodendrocyte. In some tracts, oligodendrocytes form myelin on multiple axons (Figure 1.2A); in other tracts, oligodendrocytes myelinate fewer axons, perhaps only one. Understanding the consequences of oligodendrocyte degeneration depends on knowing which of these situations exists in the particular tract under study.

Consider, for example, the death of oligodendrocytes that occurs following SCI. This death is seen in white matter tracts

that contain degenerating axons, and one interpretation is that oligodendrocytes die as a consequence of Wallerian degeneration of the axons that they ensheath [74]. At one extreme, it could be that oligodendrocytes die only if all of the axons that they ensheath degenerate. In this case, there would be *no* demyelination because both axons and their oligodendrocytes die. If oligodendrocytes die when only some of the axons degenerate, other axons would be demyelinated (Figure 1.2B). At the other extreme, it may be that oligodendrocytes die in response to some signal that is not directly related to Wallerian degeneration of the axons they ensheath, in which case *all* of the axons ensheathed by a particular oligodendrocyte would be demyelinated if that oligodendrocyte died. These considerations point to the need for detailed knowledge of the numerical relationship between oligodendrocytes and axons (how many axons a given oligodendrocyte myelinates), in particular white matter tracts.

There is a lack of consensus about the persistence of demyelination after SCI. One study in rats reported unmyelinated axons one year after a contusion injury of the spinal cord, and that the number of demyelinated axons increased during the chronic injury period [75]. This is consistent with the idea that the death of oligodendrocytes is an ongoing process after SCI, and leads to persistent demyelination. A more recent study assessed myelination of axons of the rubrospinal tract after contusion injuries in mice, and found no evidence for chronic demyelination [76]; instead, surviving rubrospinal axons had somewhat thinner myelin sheaths with shorter internodes, suggesting efficient remyelination. As we consider the development of new strategies to restore myelin, it is critical to assess the extent to which demyelination is an important component of the pathology following SCI in humans (for recent reviews, see [71,77,78]). The answer to this question will provide an indication of the degree of functional improvement that might be possible as a result of improving conduction in demyelinated axons or restoring myelin through cell transplantation therapies.

Reorganization of neuronal connections following trauma

Axon regeneration and the restoration of connections

When the axon of a neuron is interrupted, the most functionally beneficial response would be the regeneration of the damaged axon back to its normal target (assuming that the target is still present). This represents canonical axon regeneration. If a projection system is normally highly specified (for example, if the pattern of connectivity is specific between two single cells) then regrowth of an interrupted axon to its normal target represents specific regeneration. A priori, specific regeneration has a high likelihood of restoring function.

In most neural circuits, connectivity is probably not specified on a cell-by-cell basis, however. For example, in the case of neuromuscular connections, specificity may be in terms of a muscle, not individual muscle fibers. The same is true of peripheral sensory axons, where sensory representation depends on an appropriate topographic pattern of innervation of the skin. In systems like this, reconnection of an axonal projection system with its normal target region in an appropriate topographic fashion may restore the degree of specificity that is normally present. We call this "region-specific regeneration." If region-specific regeneration occurs in a normal topographic order, we term this "orderly regeneration." If regrowing axons regrow into the appropriate target area, but with a disrupted topographic order, we term this "disorderly regeneration."

In mammals, true regeneration of axonal projections rarely occurs, except in the peripheral nervous system [79–81]. Even in the peripheral nervous system, the regeneration that does occur is usually limited in scope, disorganized, and often of minimal functionality. This is especially true following peripheral nerve injury in humans.

When CNS axons are transected during early development, some systems can grow to their normal targets [82,83], sometimes via abnormal routes [84–86]. In addition, when the targets of growing axons are destroyed in young animals, growing axons are sometimes redirected to structures that they would not normally innervate where they form ectopic connections. For example, following destruction of one side of the superior colliculus of the developing hamster, some retinal axons that would normally terminate on that side grow into the opposite colliculus via a recrossing projection [87]. It is not clear whether these examples of redirected axon growth should be considered "regeneration" (a specialized response to injury) or simply a reflection of a continuation of development, which would not require any particular reactive change in the growing neuron.

It is thought that the lack of regeneration in the mature CNS is primarily due to: (1) the presence of inhibitory molecules in myelin-like Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp); (2) molecules expressed by reactive astrocytes at the site of an injury, such as chondroitin sulfate proteoglycans (CSPG); and (3) a lack of intrinsic growth capacity of mature CNS neurons. It is likely that all three factors are important. The question is whether one of these factors presents a more optimal target for therapeutic intervention than the others, or whether all three will have to be addressed in a combinatorial strategy to achieve optimal regeneration. Other chapters in this text consider these issues in more detail.

Pitfalls for studies of axon regeneration

A steady stream of new studies report that some level of axon regeneration can be induced in the mature mammalian nervous system by novel treatments or genetic manipulations. In assessing these reports, it is important to be mindful of the history of regeneration research, which is littered with the

corpses of studies that reported regeneration that later proved incorrect. The main reason is the "spared axon conundrum," in which axons that survive a lesion are mistakenly identified as having regenerated. Accordingly, it is important to establish rigorous criteria that may be used to identify regenerated versus spared axons in the injured CNS.

On the face of it, a study of axon regeneration in the central nervous system would seem simple to perform. One simply cuts or otherwise damages a population of axons, and then evaluates whether those axons regrow. In a typical experiment involving SCI, for example, one would produce a lesion in the spinal cord, wait for some period of time to allow for possible axon regeneration, and then trace particular spinal tracts using tract tracing techniques. Numerous studies indicate that there is minimal axon regeneration in normal animals. The axons that had been cut retract for some distance from the injury, and persist as retraction balls, perhaps exhibiting regenerative sprouting into nearby territory. In contrast, several recent studies report that in animals that receive some treatment or that have been genetically modified, axons that had been cut regenerate around, beyond, or sometimes even through the lesion site.

What could possibly go wrong in such a simple experiment? The answer is that axons are remarkably resilient, and can survive displacement and stretch (for further discussion and documentation, see [88]). Because of this resiliency, axons that are revealed by tract tracing at some time point after a lesion may not have been cut in the first place, and treatments or genetic manipulations may result in an increased number of spared axons in the experimental group (for an expanded discussion and examples, see [89]). This potential problem is exacerbated by the fact that many recent studies have adopted surgical approaches that are designed to minimize physical damage, in order to lower the bar for successful axon regeneration through the lesion site.

Based on these considerations, we have put forward a set of criteria that can be used to distinguish regenerated from spared axons [88]. These criteria were developed based on studies of regeneration of CST axons following SCI, but the criteria also apply to other sites in the CNS. The proposed criteria to identify a regenerating or regenerated axon are:

1. The axon extends from the CNS into a non-CNS environment; specifically, the tissue environment of the scar that develops at the injury site.
2. The axon extends from the host CNS into a non-host graft or transplant.
3. The axon originates at or near a site of amputation.
4. The axon takes an unusual course through the tissue environment of the CNS.
5. The axon extends no further than could be accounted for by plausible regeneration rates.
6. The axon has a morphology that is not characteristic of normal axons of its type (for example, exhibiting unusual branching patterns).
7. The axon is tipped with a growth cone.

Some of these criteria represent definitive evidence of regeneration (#1 and #2). The other criteria are weaker, but support the decision that given axons are regenerated rather than spared. The more criteria that can be met, the more secure the interpretation can be. Importantly, some of the criteria require detailed reconstructions of axon trajectory, especially as the axon passes the lesion site (#4 and #5), which require a detailed anatomical analysis, including analyses of axons in serial sections. Other criteria require an analysis of the time course of regenerative growth (#5 and #7), which requires that animals be evaluated at different times after the injury. Hopefully, adoption of these and other rigorous criteria will help to avoid the problem of "false resurrections" that has plagued the study of axon regeneration, especially following SCI.

Abortive regeneration: dystrophic growth cones and tortuous axon arbors

Even Cajal in his pessimistic view of neuronal growth capabilities concluded that some axon growth does occur following injury, calling the growth "abortive" because the axons did not regrow to a target. Nevertheless, even "abortive" growth involving the formation of axonal extensions does indicate that neurons possess some growth capacity. Thus, abortive growth provides indirect support for the concept that regeneration is blocked by inhibitors that are present in the tissue environment. Two morphological forms are recognized that suggest an abortive growth response: (1) dystrophic growth cones, and (2) tortuous (tangled) axonal arbors.

When an axon in a long tract (like the CST) is cut, the distal portion undergoes Wallerian degeneration, and the proximal portion dies back over a period of days or weeks. At the distal tip of the amputated axon, there is often an enlarged ball-shaped collection of cytoplasm termed a "retraction ball." Disconnected ball-shaped structures are often seen distal to the tip of the axon, suggesting that retraction balls become physically separated as axons die back.

Amputated axons may also be tipped by structures that resemble growth cones, however, and it is thought that these are "dystrophic" in the sense that their extension is impeded (see Volume I, Chapter 27 for further discussion of dystrophic growth cones). Structures resembling growth cones are evident even months after an injury, suggesting that there may be a continuous low-level attempt of the axon to regrow (or at least a capacity for regrowth if growth inhibition could be removed). Indeed, these ideas form the basis for the optimistic view that it may be possible to stimulate axon regeneration even in the chronic post-injury period.

It should be noted that there are no definitive criteria for distinguishing between retraction balls and growth cones, especially at the light microscopic level. Using electron microscopy, certain characteristic features can be identified to bolster the interpretation that a given structure is a growth cone, but even then, differential identification of retraction balls and growth cones is a matter of some interpretation. In addition, electron

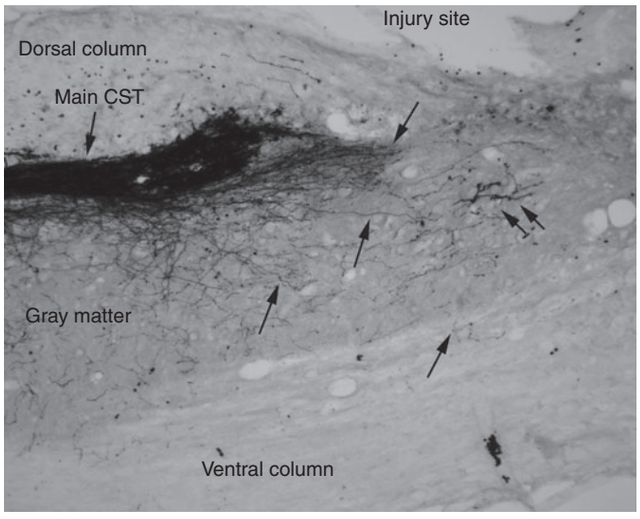

Figure 1.3. Formation of new collaterals by CST axons after SCI. The figure illustrates an experiment in which CST axons are traced by injecting BDA into the sensorimotor cortex after a dorsal hemisection at the thoracic level in a mouse. The SCI and BDA injections were made during the same operation, and mice were allowed to survive for 18 days. In mice and rats, the CST is localized in the ventral portion of the dorsal column (main CST). This is a sagittal section that contains the labeled CST; dorsal is above, rostral is to the left. Note the sprays of axons extending ventrally and caudally from the labeled CST (unlabeled arrows). The double arrow indicates one of the tangled arbors that are also found in areas of collateral sprouting. (For color image, see color plate section.)

microscopy is a very inefficient analytical tool. Some have used immunocytochemical markers (for example, the presence of the growth-associated protein GAP-43) as a marker for growth cones, but the general validity of GAP-43 as a marker for growth cones has not been established. GAP-43 is normally present in certain axons including the CST, so is not a definitive marker of a growth cone. This is another situation in which live imaging combined with genetic labeling will likely be highly revealing.

Another structure suggestive of abortive growth is a branched arbor at the end of an axon. An example of one of these at the end of a CST axon can be seen in Figure 1.3 (double arrow). Because axons in long tracts are unbranched, highly branched arbors at the end of an axon that has been cut clearly indicate some sort of growth response at the damaged tip of the axon. It should be noted that it cannot be determined with certainty whether the example shown in Figure 1.3 originates from the cut parent axon or from a collateral of the main axon. This can be determined with certainty only by reconstructing the course of the axon. It is noteworthy that similar structures are seen in areas in which there is collateral sprouting following denervation (see the following section).

Formation of ectopic connections by regenerating axons

In some cases, cut axons begin to regenerate, but extend for only short distances before forming ectopic synaptic connections. The formation of ectopic connections by regenerating axons may remove a stimulus for growth because the axon reconnects with some target, which may provide trophic support to the neuron. An early concept that has not been explored further is that premature formation of ectopic connections may be part of the reason that regenerating axons fail to grow for long distances [90].

Recent studies have revealed that after a lesion of the dorsal column of the spinal cord, growing tips of cut axons (dystrophic) also make long-lasting contacts with NG2-positive glial progenitors [91]. Over the course of a few days, these contacts mature into synapse-like structures. Similar synapse-like structures are seen normally between axons and glial cells in vivo [92,93]. Formation of contacts with glia may explain the fact that dystrophic axons can survive in a lesion environment (for further discussion, see Volume I, Chapter 27). Again, however, this formation of premature connections may remove a stimulus for growth because the NG2-positive cell provides trophic support.

Forms of axonal growth that would be most appropriate for long-tract regeneration

Are there different modes of axon growth? When growing for long distances in tracts, axons extend with little branching (what might be termed a "tract mode"). In contrast, upon reaching the target region, axons often exhibit a branching form of growth involving the formation of complex terminal arbors. This arborizing form of growth may reflect the axons' response to cues presented in the target region (including cues that trigger synapse formation on target neurons). Efficient long-distance regeneration may require reinitiation of the tract mode of growth. An interesting question is whether the two forms of axon growth require a different program of gene expression by the neuron.

Recent advances in promoting axon regeneration after CNS injury

Because this chapter considers historical concepts and their modern embodiments, it is worth noting that there have been major advances since the publication of the original version of this chapter in 2006. In particular, robust regeneration has been achieved finally, including the regeneration of the CST after complete spinal cord injury. This was achieved through genetic modifications that led to an enhanced intrinsic growth capacity by adult neurons.

As noted previously, one factor that is thought to limit regenerative ability is the limited growth capacity of adult neurons. Developmental decline of axon growth ability mirrors the decline in growth capacity of other cell types. Active growth during development is shut down at maturity so that cell size and number are maintained at a set point characteristic for the organism. Extensive studies in the fields of developmental biology and cancer biology have identified a number of genes that play a role in shutting down cellular