The Formation and Regeneration of Synapses

Interactions Between Motor Neurons and Skeletal Muscles Organize the Development of the Neuromuscular Junction

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An Overall View

In the three previous chapters (52–54) we described the early events in the development of the mammalian nervous system: the formation of the neural tube, its subdivision along the anterior-posterior and dorsoventral axes, the birth and differentiation of neurons and glial cells, and the extension of axons. After axons reach appropriate targets they begin to form synapses, the structures that permit signaling between nerve cells. Synapse formation completes the hard wiring of the nervous system. Once synapses have formed, an information-processing circuit begins to function. Thereafter, the information-processing capacity of each circuit is refined through use. In this sense, the nervous system continues to develop throughout life.

Synapse formation involves three key events: the formation of selective connections between the developing axon and its target, the differentiation of the axon’s growth cone into a nerve terminal, and the elaboration of a postsynaptic apparatus in the target cell. These steps depend critically on intercellular interactions. Indeed, the current consensus is that a series of signals between cells is responsible for the axon’s recognition of an appropriate postsynaptic cell (and sometimes even a specific domain on the postsynaptic cell’s surface) and the coordinated differentiation of pre- and postsynaptic elements of the synapse. In this chapter we therefore focus on the intercellular signaling that underlies formation of the synapse.

Most of what we know about these developmental interactions comes from studies of the neuromuscular junction, the synapse that motor neurons make on skeletal muscle fibers. The simplicity and accessibility of this synapse have made it a useful model for ultrastructural and electrophysiological studies of chemical synapses.
Figure 55-1 The neuromuscular junction develops in a series of steps.

A. At the mature neuromuscular junction, pre- and postsynaptic membranes are separated by a synaptic cleft containing extracellular material. Vesicles are clustered at a presynaptic release site, transmitter receptors are clustered in the postsynaptic membrane, and nerve terminals are coated by glial processes.

B. Stages in the development of the neuromuscular junction:
1. A growth cone approaches a newly formed myotube and (2) forms a morphologically unspecialized but functional contact.
2. The terminal accumulates synaptic vesicles and a basal lamina forms in the synaptic cleft. (4) As the muscle matures, multiple axons converge on a single site. (5) Finally, all axons but one are eliminated and the survivor matures. (Based on Hall and Sanes 1993.)

(see Chapter 11). These same features, as well as the wealth of information on the adult neuromuscular junction, have made it a powerful system for the analysis of synaptic development. We therefore use the neuromuscular synapse here to exemplify key features of synaptic development. We then apply what has been learned from this model synapse to other, less tractable synapses that form between neurons in the peripheral and central nervous systems. Finally, we discuss the limited ability of the central nervous system to form new synapses after injury limits.
Interactions Between Motor Neurons and Skeletal Muscles Organize the Development of the Neuromuscular Junction

The mature neuromuscular junction comprises parts of three types of cells—a motor nerve terminal, a muscle fiber, and a few Schwann cells. All three cells are highly differentiated in their region of apposition (Figure 55-1A).

The terminals of the motor neuron are rich in synaptic vesicles that contain the neurotransmitter acetylcholine (ACh). Many of these vesicles are clustered at dense patches on the presynaptic membrane, called active zones, where they fuse with the plasma membrane of the nerve terminal and release their contents into the synaptic cleft (see Chapter 4). The nerve terminal is also rich in mitochondria, which provide the energy required to synthesize, package, and release neurotransmitter and to recover and recycle membrane after vesicle fusion. In contrast, the segments of the motor axon that lead to the terminals are rich in neurofilaments but contain few vesicles or mitochondria and no active zones.

Schwann cells are glial cells that insulate the entire motor axon, from the point at which it exits the spinal cord to its nerve terminals. Preterminal Schwann cells form the myelin sheath, whereas terminal Schwann cells extend thin processes that form a continuous nonmyelin layer over the nerve terminals.

The surface of the muscle fiber is indented directly opposite the active zones in the nerve terminal, forming a series of postsynaptic sites on the membrane called the junctional folds. The postsynaptic sites are rich in ACh receptors and include an elaborate cytoskeleton that holds the receptors in place, as well as a number of specialized adhesion and signaling molecules. In contrast, nonsynaptic portions of the muscle membrane are not folded, contain very few ACh receptors, and have a markedly different cytoskeleton.

A layer of extracellular material called basal lamina ensheaths the entire muscle fiber. The synaptic portion of the basal lamina is continuous with, and ultrastructurally similar to, the nonsynaptic portion, but the two regions have distinctive molecular structures. For example, synaptic basal lamina is rich in the enzyme acetylcholinesterase, which hydrolyzes released ACh and thus terminates transmitter action.

The process of synapse formation is initiated when a motor axon, guided by the multiple factors described in Chapter 54, reaches a developing skeletal muscle and approaches an immature muscle fiber or myotube. Contact is then made and the process of differentiation begins. The growth cone begins its transformation into a nerve terminal while the portion of the muscle surface opposite the nerve terminal becomes distinct from the nonsynaptic regions. As development proceeds, synaptic components are added and ultrastructural signs of
Figure 55-3 Although synaptic differentiation requires intercellular interactions, nerve and muscle cells can assemble synaptic components on their own.

A. Demonstration of the release of ACh from growth cones. An electrode tip is coated with an outside-out patch of muscle membrane containing a high density of ACh receptors.

B. A microelectrode is used to stimulate an isolated cultured motor neuron; the evoked action potentials are shown in the top trace. The membrane patch containing ACh receptors is activated by the ACh released from the neuron upon stimulation. Bottom trace shows ACh receptor activity within the membrane patch. The receptor-bearing micropipette serves as a sensitive biodetector of released ACh. Use of this method showed that motor axons synthesize and release ACh even in the absence of myotubes. (Based on Hume et al. 1983.)

Three general features of neuromuscular development give us clues about the mechanisms that underlie synapse formation. First, nerve and muscle organize each other's differentiation. In principle, the precise apposition of pre- and postsynaptic specializations might be explained by independent programming of nerve and muscle properties. However, studies of cultured cells show that this is not how it happens. When motor neurons and myoblasts from embryonic chicks or rodents are cultured in isolation, the myoblasts fuse to form myotubes and the motor neurons extend axons to the myotubes. At sites where the two cells meet, both acquire specializations that are found at synapses in vivo (Figure 55-2). The initial contact of the cells is essentially random; thus the site of synaptic specialization is not predetermined. Such specializations are instead initiated and organized by molecular signals that pass between the nerve and muscle.

The second key feature of neuromuscular development is that new synaptic components are added in a series of defined developmental steps. Thus the newly formed neuromuscular junction is not simply an immature version of the fully developed synapse. For example, although the nerve and muscle membrane form contacts at an early stage in synaptogenesis, only at a later stage does the synaptic cleft widen and the basal lamina appear. Similarly, ACh receptors accumulate in the postsynaptic membrane before acetylcholinesterase accumulates in the synaptic cleft. Active zones form in the nerve terminal only after vesicles have accumulated and junctional folds form in the postsynaptic membrane only after the nerve terminal has matured. Around the time of birth several different axons innervate each myotube, but during early postnatal life all but one withdraw (see Figure 55-1B). This elaborate sequence is unlikely to be organized by simple contact between...
nerve and muscle. More probably, signals pass between the cells: The nerve sends a signal to the muscle that triggers the first steps in postsynaptic differentiation, which generates a retrograde signal that triggers the initial steps of nerve terminal differentiation. The nerve then sends further signals to the muscle and this reverberative interaction continues.

The third critical feature of neuromuscular development is that most synaptic components of the motor neuron and myotube develop on their own. Motor axons, for example, can form synaptic vesicles and synthesize neurotransmitter in the absence of muscle. In fact, clever electrophysiological detection methods have revealed that vesicles in growth cones can release ACh in response to electrical stimulation, even before the growth cones have reached their target cells (Figure 55-3A). Similarly, uninnervated myotubes can synthesize functional ACh receptors. Moreover, some of these receptors cluster into high-density aggregates, much like those found in the postsynaptic membrane, and these aggregates become associated with components of the cytoskeleton and basal lamina that are localized at synapses in vivo (Figure 55-3B). When synapses form, however, these specializations disperse and new ones are assembled at sites of nerve muscle contact. These observations tell us that the developmental signals that pass between nerve and muscle are unlikely to be signals that induce wholesale changes in cell properties, such as those that regulate neurogenesis at earlier stages (Chapter 52). Instead, the role of the developing nerve and muscle is to assure that components of the pre- and postsynaptic apparatus are formed at appropriate levels, at the correct time, and in correct places. For this reason, it is helpful to think of the intercellular signals that control synaptogenesis as organizers rather than inducers.

The Motor Nerve Organizes Differentiation of the Postsynaptic Muscle Membrane

To illustrate the ways in which axons organize postsynaptic differentiation, we will consider the development of the ACh receptors of the neuromuscular junction. As we have seen in Chapter 11, ACh receptors are pentamers, composed of α, β, δ, γ, or ε subunits encoded by related genes. The subunits form a barrel-like structure that spans the membrane; the channel formed by
the plasma membrane. Although some receptors spontaneously cluster into aggregates, the majority are distributed throughout the membrane at a density of about 1000 per \( \mu m^2 \). Once synapse formation is complete, the distribution of the receptors changes drastically: The receptors become highly concentrated in the synaptic sites of the membrane (to a density up to 10,000 per \( \mu m^2 \)) and depleted in the nonsynaptic membrane (being present at 10 per \( \mu m^2 \) or less). This thousand-fold difference in ACh receptor density occurs within a few tens of microns at the edge of the nerve terminal (see Figure 55-4B).

The redistribution of ACh receptors is the result of the combined effects of three distinct processes: translocation of ACh receptors from the membrane to nonsynaptic to synaptic regions; a transcriptional activation of the expression of genes for the ACh receptor subunits in the few nuclei that lie directly beneath the postsynaptic membrane; and repression of the expression of receptor subunit genes in the nuclei of nonsynaptic regions. The motor nerve controls the entire redistribution program but uses distinct signals to regulate each process. We will consider each process in turn.

**Agrin Triggers the Clustering of Acetylcholine Receptors**

To analyze the distribution of ACh receptors on the muscle surface it is first necessary to label them. The most common means of visualizing ACh receptors on muscle cells is by use of a toxin, \( \alpha \)-bungarotoxin, isolated from the venom of the poisonous snake *Bungarus bungaris*. This small protein binds specifically and almost irreversibly to ACh receptors, inactivating them. In the wild the snake uses \( \alpha \)-bungarotoxin to paralyze its prey. In the laboratory neurobiologists conjugate a fluorophore to the toxin, creating a stain for ACh receptors.

Monroe Cohen, Gerald Fischbach, and their collaborators analyzed the distribution of labeled ACh receptors on muscle cells in vitro before and after innervation by motor neurons. Prior to innervation ACh receptors were distributed uniformly on the muscle surface, or in occasional patches, but after innervation prelabeled receptors were concentrated at synaptic sites. The simple observation that receptors present on the muscle surface before the nerve arrives later appeared at synapses indicates that axons can cause the redistribution of ACh receptors within the plane of the membrane. Based on these studies and denervation experiments indicating that the activity responsible for clustering ACh receptors resides in the basal lamina (Figure 55-5), Uel McManah and colleagues searched for factors that might...
**Figure 55-6** Agrin released by nerve terminals acts through MuSK and rapsyn to aggregate ACh receptors at synaptic sites in the muscle fiber.

A. ACh receptors on cultured myotubes were labeled with rhodamine-conjugated α-bungarotoxin. Few receptor clusters form under control conditions, but addition of basal lamina extract induces clustering. Researchers used this assay to purify agrin from the basal lamina extract.

B. Agrin is a large extracellular matrix proteoglycan. Alternative splicing at a site called "z" regulates ability of agrin to cluster ACh receptors. Only neurons synthesize the active isoform containing the z site. Agrin activates the membrane-associated receptor tyrosine kinase MuSK, triggering a cascade of intracellular reactions that results in clustering. Rapsyn, a cytoplasmic ACh receptor-associated protein is essential for clustering.

C. Muscles from a wild-type neonatal mouse and from mutants lacking agrin, MuSK, or rapsyn. Muscles were double labeled for ACh receptors (green) and nerves (brown). By birth, ACh receptor clusters have formed beneath each nerve terminal. Few clusters are present in the agrin mutant, and none are present in the MuSK or rapsyn mutants. In the rapsyn mutant, however, ACh receptor levels are higher in the synaptic area than at the ends of myotubes, reflecting the preservation of synapse-specific transcription. All three mutants also have nerve abnormalities, reflecting the inability of the muscle to supply proper retrograde factors. (Based on Gautam et al. 1995, 1996; DeChiara et al. 1996.)
influence the clustering of ACh receptors in embryonic myotubes. This search led to the isolation of an ~400-kDa proteoglycan termed agrin (Figure 55-6). Agrin is synthesized by motor neurons, transported down the axon, released from nerve terminals, and incorporated into the synaptic cleft. Agrin is also made by muscle cells, but the neuronal isoforms of agrin are a thousand-fold more active in aggregating ACh receptors.

The generation of mutant mice lacking agrin provides strong evidence that agrin has a role in the organization of ACh receptors. Agrin mutants have grossly perturbed neuromuscular junctions and die at birth. The number, size, and density of ACh receptor aggregates is severely reduced in these mice (see Figure 55-6C). All other components of the postsynaptic apparatus—including cytoskeletal, membrane, and basal lamina proteins—are similarly reduced. Interestingly, differentiation of presynaptic elements is also perturbed in the mutant. However, the defects in the presynaptic element probably do not result directly from lack of agrin in the motor neuron but result indirectly from the failure of the disorganized postsynaptic apparatus to generate signals for presynaptic specialization.

How does agrin work? Several molecules that interact with agrin are present in myotube membranes, including dystroglycan, which is critical to maintaining muscle stability, and integrins, which mediate interactions with many extracellular matrix components. Any or all of these molecules may be important, but the best candidate as an agrin receptor is a muscle-specific tyrosine kinase called MuSK. MuSK is normally concentrated at synaptic sites in the muscle membrane, and muscles of mutant mice lacking MuSK have no ACh receptor clusters (see Figure 55-6C). Myotubes cultured from these mutants express normal levels of ACh receptors, but these receptors cannot be clustered by agrin. MuSK therefore appears to be a critical component of the receptor for agrin. However, agrin does not bind directly to MuSK, so the agrin receptor may contain an additional subunit.

One key component in the cascade of reactions initiated by MuSK is a cytoplasmic protein called rapsyn. Rapsyn is colocalized with ACh receptors in vivo and is present at ACh receptor clusters soon after they form. It can induce the aggregation of ACh receptors in vitro. In mice lacking rapsyn, muscles form normally and ACh receptors accumulate in normal numbers but fail to aggregate at the synaptic sites on the membrane (see Figure 55-6C). Thus rapsyn is an essential component of the cytoskeletal apparatus responsible for ACh receptor clustering.

Although many components of the synaptic membrane and cytoskeleton in muscle remain diffusely distributed in rapsyn-deficient mice, MuSK nevertheless becomes concentrated at synaptic sites. It is likely therefore, that MuSK is a critical component of a primary synaptic scaffold to which rapsyn recruits ACh receptors and other postsynaptic proteins. Agrin released from the nerve localizes MuSK to synaptic sites in addition to activating MuSK. MuSK in turn stimulates ACh receptor clustering via its kinase activity and plays a structural role in nucleating synapse assembly. In this way the nerve not only stimulates specialization at synaptic sites in the muscle, but also ensures that ACh receptor aggregates are precisely apposed to sites of ACh (and agrin) release from the nerve.

**Neuregulin Stimulates Synthesis of Acetylcholine Receptors**

Motor neurons not only trigger the clustering of ACh receptors in the postsynaptic cell membrane, but also stimulate ACh receptor synthesis. A search for the molecules that mediate this distinct activity of the nerve led to isolation of an ACh receptor-inducing activity (ARIA) later renamed neuregulin. Neuregulin is capable of stimulating expression of the genes for the ACh receptor subunit. Molecular cloning revealed that neuregulin is the product of the gene that encodes a protein previously purified as the ligand of a set of receptor tyrosine kinases called erbB2, erbB3, and erbB4. Like agrin, neuregulin is made by both motor neurons and muscles; like MuSK, the erbB kinases are concentrated in the postsynaptic muscle membrane. Together, these results suggest that neuregulin activates erbB kinases to stimulate ACh receptor synthesis in the muscle (Figure 55-7A). Mutant mice lacking neuregulin, erbB2, or erbB4 die of cardiovascular malformations early in embryogenesis and so are not useful for studies of synaptic differentiation. In neuregulin heterozygotes, however, the density of ACh receptors in the postsynaptic membrane and the abundance of ACh receptor subunit RNA in muscle are both decreased by 50% (Figure 55-7B). These observations support the idea that neuregulin is an activator of postsynaptic specialization. They do not tell us, however, whether nerve- or muscle-derived neuregulin is more important.

Why would the nerve need to stimulate expression of ACh receptor genes when myotubes can express these genes on their own? The answer may lie in the peculiar geometry of the muscle fiber. Individual muscle fibers can be several centimeters long and contain hundreds of nuclei along their length. ACh receptors synthesized near the ends of fibers would never reach the synapse, despite the actions of agrin. However, several nuclei are clustered just beneath the synaptic membrane, and their products do not have far to go.
A Neuregulin controls ACh receptor gene transcription

B A decrease in ACh receptors in neuregulin heterozygote mice

Figure 55-7 Neuregulin stimulates expression of the genes encoding the ACh receptors.

A. Neuregulins are synthesized and secreted by motor axons. Binding of neuregulin to erbB kinases in the postsynaptic membrane activates transcription of ACh receptor genes via a cascade of protein kinases. Neuregulins are also synthesized by muscle, and the neuregulin gene is subject to alternative splicing to generate more than 20 membrane- and matrix-bound forms. Thus neuregulins may influence ACh receptor synthesis both as a nerve-derived messenger and as a muscle-derived second messenger. In addition, three different erbB kinases (erbB2, erbB3, and erbB4) are expressed by muscle, and erbB2 and erbB3 are also expressed by Schwann cells. Neuregulin may stimulate ACh receptor expression through transduction pathways that involve ras, and the raf, and the ETS class transcription factors.

B. A decrease in ACh receptors in heterozygous neuregulin mice. Both the density of the ACh receptors and the amplitude of the miniature end-plate potential (MEPP) is decreased around 50% in the heterozygote compared to wild type mice, suggesting that neuregulin is a rate-limiting factor for ACh receptor expression. (From Sandrock et al. 1997.)
the synapse. These subsynaptic nuclei express the genes encoding ACh receptor subunits (and those encoding some other components of the postsynaptic membrane as well) at far higher levels than nonsynaptic nuclei within the same cytoplasm (see Figure 55-4). The concentration of ACh receptor subunit mRNAs in synaptic areas leads to preferential synthesis and insertion of ACh receptors near synaptic sites. Thus, by acting locally, neuregulin and the erbB kinases activate transcription in synaptic nuclei and thereby stimulate synthesis of ACh receptors specifically in synaptic areas.

Neural Activity Represses Synthesis of Acetylcholine Receptors in Nonsynaptic Areas

Around the time of birth, the density of ACh receptors in nonsynaptic portions of the muscle membrane declines. Yet many more receptors in nonsynaptic areas of the membrane are lost than can be accounted for by clustering at the synapse. The loss of nonsynaptic receptors results primarily from a decrease in the expression of the ACh receptor subunit genes by nonsynaptic nuclei, which decreases the level of ACh receptor mRNA and thus receptor synthesis.

The decreased ACh receptor expression reflects a repressive effect of the nerve, as originally shown by studies of denervated muscle. When muscle fibers are denervated, as happens when the motor nerve is damaged, the density of ACh receptors in the postsynaptic membrane increases markedly, a phenomenon termed denervation supersensitivity. This suppressive effect of the nerve is mediated by electrical activation of the muscle rather than by a protein factor such as agrin or neuregulin. Under normal conditions the nerve keeps the muscle electrically active, and active muscle synthesizes fewer ACh receptors than inactive muscle. Indeed, direct stimulation of denervated muscle through implanted electrodes decreases ACh receptor expression, preventing or reversing the effect of denervation (Figure 55-8). Conversely, when nerve activity is blocked by application of a local anesthetic, the number of ACh receptors throughout the muscle fiber increases, even though the synapse is intact. Blockade of synaptic transmission by application of α-bungarotoxin to the muscle has a similar effect. Thus the number of ACh receptors in extrasynaptic regions of the muscle is controlled by the level of activity of the muscle cell.

In essence then, the nerve uses ACh to repress expression of the ACh receptor genes. Current that passes through the receptor leads to an action potential that propagates along the entire muscle fiber. This depolarization opens voltage-dependent Ca$^{2+}$ channels, leading to an influx of Ca$^{2+}$. The Ca$^{2+}$ influx activates a cascade of protein kinase reactions that transduces signals that reach the nonsynaptic nuclei and regulate the
Figure 55-9 Components of the basal lamina at the synaptic site help organize the nerve terminal.

A. Following nerve damage motor axons regenerate and form new neuromuscular junctions. Nearly all of the new synapses form at the original synaptic sites (left). A strong preference for original sites persists even after the muscle fibers have been removed, leaving behind basal lamina "ghosts" (right). Moreover, the regenerating axons differentiate into nerve terminals when they contact original synaptic basal lamina. Thus components of synaptic basal lamina account for the selective reinnervation of synaptic sites and trigger the differentiation of growth cones into nerve terminals.

B. Micrograph showing a normal neuromuscular junction (left). Micrograph showing nerve terminals that differentiated following reinnervation of a basal lamina ghost (right). (From Glickman and Sanes 1983.)
transcription of ACh receptor genes. Thus the same voltage changes that produce muscle contraction over a period of milliseconds also regulate transcription of ACh receptor genes over a period of days.

Several Aspects of Postsynaptic Differentiation Are Controlled by the Motor Axon

Many components of the postsynaptic apparatus are subject to the organizing influences of the three neural signals (agrin, neuregulin, and ACh) that lead to clustering of ACh receptors. Mice lacking agrin, MuSK, or rap-syn are defective in nearly all aspects of postsynaptic specialization. Nevertheless, not all components are regulated in the same way, because postsynaptic specialization occurs in sequential steps. For example, agrin stimulates aggregation of acetylcholinesterase as well as ACh receptors, but synthesis of acetylcholinesterase also requires muscle activity. The requirement of nerve-evoked muscle activity for the synthesis of acetylcholinesterase but not ACh receptors may explain the sequential expression of these two proteins at synapses.

The Muscle Fiber Organizes the Differentiation of Motor Nerve Terminals

Soon after the growth cone of a motor axon contacts a developing myotube a rudimentary form of neurotransmission begins. The axon releases ACh in vesicular packets, the transmitter binds to receptors, and the muscle responds with depolarization and weak contractions. The onset of transmission at the new synapse reflects the intrinsic capabilities of each synaptic partner.
Figure 55-11 Some synapses are eliminated after birth.

A. As the neuromuscular junction develops, each myotube becomes innervated by several motor axons at a common synaptic site. After birth all terminals but one withdraw from each site, and the survivor grows. This elimination occurs without any overall loss of axons: The axons that “lose” at some muscle fibers “win” at others.

B. Diagrams (B1) and fluorescent images (B2) show that local postsynaptic inactivity can cause elimination of overlying nerve terminals. Some ACh receptors at an adult neuromuscular junction are blocked by focal application of α-bungarotoxin whereas others are left unperturbed. Those nerve terminals opposite the blocked ACh receptors retracted, and those opposite the unblocked receptors maintained synaptic contact. In contrast, blockade of all ACh receptors had little effect (not shown). This result suggests that differential activity by competing inputs (but not activity per se) influences the survival of those inputs. This mechanism may be responsible for synapse elimination during development. In B2, a nerve terminal dye was used to image presynaptic terminals and rhodamine-α-bungarotoxin labeling to map the distribution of ACh receptors. (Adapted from Balice-Gordon and Lichtman 1994.)

Nevertheless, cell-intrinsic behaviors cannot readily explain the marked increase in the rate of transmitter release that occurs after nerve-muscle contact is made, nor can they explain the accumulation of synaptic vesicles and assembly of active zones in the small portions of the motor axon that directly abut the muscle surface. These developmental steps require retrograde signals from muscle to nerve.

Evidence for the existence of such retrograde signals has come from studies on the reinnervation of adult muscle. Although axotomy leaves muscle fibers denervated, and leads to a proliferation of ACh receptors in nonsynaptic regions, the postsynaptic apparatus remains largely intact. It is still recognizable by its subsynaptic nuclei, junctional folds, and the ACh receptors, which remain far more densely packed in synaptic areas than in extrasynaptic areas of the cell. Damaged peripheral axons regenerate readily (unlike those in the central nervous system) and form new neuromuscular junctions that look and perform much like the original ones. A century ago Fernando Tello, a student of Santiago Ramón y Cajal, noted that most such junctions form at preexisting synaptic sites on the denervated muscle fibers (Figure 55-8A) even though the postsynaptic membrane occupies only 0.1% of the muscle fiber surface (Figure 55-9). Thus motor axons must recognize signals associated with the postsynaptic apparatus. Moreover, presynaptic specialization in the axon occurs only at sites where it contacts the muscle. In fact, active zones form directly opposite the mouths of junctional folds, thus reconstituting normal synaptic geometry at a submicron level of precision.
What factors organize presynaptic differentiation? We know much less about them than we do about the nerve-derived organizers. Candidates include cell adhesion molecules of the type that promote neurite outgrowth. For example, two homophilic cell adhesion molecules, N-CAM and N-cadherin (see Chapter 54), are present on both the motor axon growth cone and the surface of developing myotubes. Both molecules promote outgrowth of motor axons on myotubes, and the adhesive interactions they mediate might stabilize initial nerve-muscle contacts. Other candidates are soluble trophic factors of the type that promote neuronal survival and differentiation (see Chapter 53). Brain-derived neurotrophic factor and ciliary neurotrophic factor lead to a rapid increase in the rate of transmitter release, much like that seen at the early stages of synaptogenesis.

Additional candidates have been suggested by experiments exploring the ability of adult axons to reinnervate muscle fibers and form synapses at original postsynaptic sites. Muscles were damaged in vivo in ways that killed the muscle fibers but left their basal lamina intact. The necrotic fibers were phagocytized, leaving behind basal lamina sheaths on which synaptic sites were readily recognizable. The nerve was cut at the same time that the muscle was damaged, and time was allowed for regeneration. Under these conditions motor axons reinnervated the empty basal lamina sheaths and contacted synaptic sites as precisely as they had done when muscle fibers were present. Moreover, nerve terminals developed at regions of contact with synaptic basal lamina, and active zones even formed opposite struts of basal lamina that once lined junctional folds (see Figure 55-9).

Components of the basal lamina therefore appear to be organizers of presynaptic specialization.

One such organizer is an isoform of laminin. Laminins are major components of all basal laminae and are potent promoters of axon outgrowth from many neuronal types (see Chapter 54). They are heterotrimers of α, β, and γ chains, comprising a family of at least five α, four β, and three γ chains. Laminin 11 (α5/β2/γ1) is a major laminin of synaptic basal lamina; Laminin 2 (α2/β1/γ1) is the major laminin of nonsynaptic basal lamina (Figure 55-10).

Motor axons that encounter a deposit of synaptic laminin in vitro stop growing, accumulate synaptic vesicles, and acquire the ability to release neurotransmitter. Furthermore, the development of nerve terminals and Schwann cells is markedly aberrant in mutant mice that lack the laminin β2 chain (Figure 55-10B). Thus laminin-11 is a retrograde synaptic organizer. However, because presynaptic specialization does proceed to a considerable extent in the absence of laminin-11, additional retrograde organizers of axonal specialization must exist.

Many Neuromuscular Junctions That Form in the Embryo Are Eliminated After Birth

In adult mammals a one-to-one relationship exists between motor nerve terminals and muscle fibers. Each motor axon branches to contact tens of hundreds of muscle fibers, but each muscle fiber bears only a single synapse. However, this is not the condition in the embryonic nervous system.
bryo. At intermediate stages of development several axons converge on each myotube and form synapses at a common site. Soon after birth all inputs but one are eliminated.

This process of synapse elimination is not a consequence of neuronal death. In fact, synapse elimination takes place long after the period of naturally occurring cell death (see Chapter 53). Instead, each motor axon withdraws branches from some myotubes and strengthens its connections with others, thus focusing its increasing capacity for transmitter release on a decreasing number of targets. Moreover, the elimination process is not targeted at defective synapses; all the inputs to a neonatal myotube are morphologically and electrically differentiated and each can activate the postsynaptic cell (Figure 55-11).

The purpose of this transient polyneuronal innervation remains a mystery. One possibility is that it provides a way to ensure that each muscle fiber is innervated. A second is that it allows all axons to capture appropriate numbers of target cells. A third and more intriguing idea is that synapse elimination provides a means by which activity can change the strength of specific synaptic connections. We will revisit this idea in Chapter 56.

Like synapse formation, synapse elimination results from intercellular interactions. Two observations support this point. First, every muscle fiber ends up with exactly one input: none have zero and very few have more than one. It is difficult to imagine how this could occur without some feedback from the muscle cell. Second, if some axons are removed by partial denervation at birth, each remaining axon maintains a larger number of synapses. Thus synapse elimination must be in part a competitive process.

What does competition in synapse elimination mean? Who is competing for what? Jeff Lichtman has drawn a provocative analogy to athletic competitions, which can be of two types. In one, for example, a wrestling match, the participants compete by interacting with each other. In others, such as diving contests, participants compete by performing on their own.

Most students of synapse elimination have proposed models akin to the first type of competition. In these models the mechanisms that determine the maintenance and elimination of synapses are similar to those that determine whether neurons live or die. For example, the muscle might provide limited amounts of a trophic substance for which the axons compete. As the winner grows it either deprives the loser of its sustenance or gains enough strength to mount an attack on its competitor. Alternatively, the muscle might release a toxic or punitive factor. In these scenarios the muscle only contributes a factor in the competition; the outcome is entirely dependent on differences between axons. These differences could be related to activity. The more active axon might, for example, be better able to take up trophic factor or resist a toxin. Such positive and negative competitive interactions have been demonstrated in Xenopus nerve-muscle cocultures but not in vivo.

In vivo synapse elimination appears to be more akin to the second type of competition. In this model the axons need not interact directly with each other—that is, they are divers, not wrestlers—and muscle plays a selective role in synapse elimination rather than just providing a broadly distributed signal. This model is based on studies of living mice in which individual neuromuscular junctions were observed at close intervals during the process of synapse elimination. ACh receptors and components of the postsynaptic cytoskeleton begin to disappear from parts of the maturing neuromuscular junction before the nerve terminals actually withdraw. This observation suggests that differences in activity among competing axons may elicit different responses in the postsynaptic ACh receptors. For example, the more active axon might trigger the generation of a local retrograde signal that strengthens its adhesive interactions with the synaptic cleft, whereas the less active axon might elicit a retrograde signal that weakens its adhesive interactions. In support of this model, blockade of one synaptic region of ACh receptors by focal application of α-bungarotoxin leads to elimination of the portion of the nerve terminal Arbor overlying the blocked receptors without obvious effects on other portions of the Arbor (see Figure 55-11). One implication of this model is that components of synaptic basal lamina may regulate not only the assembly of the presynaptic nerve terminal but also its demise.

Central Synapses and Neuromuscular Junctions Develop in Similar Ways

Synapses in the central nervous system are structurally similar to neuromuscular junctions in many respects (Figure 55-12). Moreover, like neuromuscular junctions, central synapses develop in a series of steps that imply the exchange of signals between the synaptic partners. To what extent can insights into the development of the neuromuscular junction guide our understanding of synaptic development in the brain?

Central Nerve Terminals Develop Gradually and Are Subject to Elimination

Nerve terminals of neuromuscular junctions and central synapses are quite similar, perhaps reflecting the fact
Figure 1.

A. Synaptophysin and GABA receptors, Merged images.

B. GABA receptors and GABA receptors.

C. GAD and GABA receptors.

D. GAD and Glutamate receptors.

Neural crest cells at Cc

The neural crest cells develop into a variety of cell types, including neurons and glial cells. The diagram illustrates the distribution of synaptophysin and GABA receptors in presynaptic terminals. Synaptophysin labeling is present in presynaptic terminals, while GABAergic terminal labeling is indicated by GAD labeling. Glutamate receptors are also shown in the merged images for comparison.

Note: The image contains detailed micrographs and diagrams that are not transcribed here.
that the motor axon is part of a central neuron. Most of the major protein components of synaptic vesicles have now been isolated and appear to be identical at both types of synapses. Likewise, the mechanisms of transmitter release differ only quantitatively, not qualitatively. Because only a few active zone components and retrograde synaptogenetic signals have been identified, it is not yet clear whether the mechanisms that underlie presynaptic organization have been conserved.

The complexity of the brain makes direct demonstration of synapse elimination problematic, but electrophysiological evidence for elimination has been obtained in the cerebellum, and counts of synapse density as a function of developmental age suggest that a similar process occurs in the cerebral cortex. Synapse elimination in autonomic ganglia has been documented directly and is similar to that seen at neuromuscular junctions. Individual axons withdraw from some postsynaptic cells while simultaneously increasing the size of the synapses they form with other neurons.

**Neurotransmitter Receptors Cluster at Central Synapses**

The concentration of neurotransmitter receptors in the postsynaptic membrane is a feature shared by many synapses. In the brain, receptors for glutamate, glycine, γ-aminobutyric acid (GABA), and other neurotransmitters are concentrated in patches of membrane directly underlying nerve terminals that contain the corresponding transmitter. The processes by which these receptors become localized may be similar to those at the neuromuscular junction. In cultures of dissociated hippocampal neurons, for example, both glutamatergic and GABA-ergic nerve terminals appear to stimulate clustering of appropriate receptors in the postsynaptic membrane (Figure 55-13). The mediators of these effects are unknown, but it is noteworthy that agrin, which triggers the clustering of ACh receptors at the neuromuscular junction, is abundant in the brain. Moreover, nerves can induce expression of genes encoding central glutamate receptors, much as occurs for ACh receptors in muscle, and neuregulin has been implicated as a mediator of this effect in cerebellum. Finally, electrical activity regulates neurotransmitter receptor expression in neurons as it does in muscle.

In forming receptor clusters central neurons face an obvious challenge that myotubes do not: They are contacted by a variety of inputs mediated by different neurotransmitter types. Thus it would seem essential that the nerve terminal have an instructive role in managing the clustering of appropriate receptors. Indeed, in cultures of the hippocampal neurons, where glutamatergic and GABA-ergic axons innervate adjacent regions of the same dendrite, initially dispersed glutamate and GABA receptors each cluster selectively beneath terminals that release the appropriate neurotransmitter. This implies the existence of multiple clustering signals with parallel pathways of signal transduction.

Consistent with this idea, several distinct proteins in central neurons have been found to play a role similar to that of rapsyn at the neuromuscular junction. One, gephyrin, is highly concentrated in the synaptic densities at glycineric and some GABA-ergic synapses (Figure 55-14). Gephyrin is not structurally homologous to rapsyn but appears to be a functional analog: It links the receptors to the underlying cytoskeleton, its overexpression in nonneural cells leads to clustering of glycine receptors, and gephyrin-deficient mutant mice fail to form glycine receptor clusters at inhibitory synapses (Figure 55-14). Similarly, a class of proteins that share conserved segments called PDZ domains, the prototype being PSD-95 or SAP-90, facilitates clustering of NMDA-type glutamate receptors (Figure 55-14). Still other PDZ proteins interact with AMPA-class and metabotropic glutamate receptors. An attractive hypothesis is that distinct presynaptic signals activate the pathways that lead to the expression and localization of gephyrin, PSD-95, and other such proteins.

**Figure 55-13** (Opposite) Neurotransmitter receptors cluster at synaptic sites on central neurons. (Images provided by A. M. Craig 1994.)

A. Localization of glutamate receptors and synaptophysin at synapses formed between hippocampal neurons in culture. Glutamate receptors are clustered underneath synaptophysin-labeled nerve terminals, but not all nerve terminals are associated with clusters of glutamate receptors.

B. Localization of glutamate and GABA receptor cluster. These two classes of receptors cluster at different sites on the neuronal membrane.

C. GABA receptors are clustered beneath GABA-releasing nerve terminals that express the (transmitter synthetic enzyme) glutamatergic acid decarboxylase (GAD).

D. Glutamate receptors are located at postsynaptic sites spatially distinct from GABA-releasing nerve terminals.

**The Synaptic Cleft Differs at Central and Neuromuscular Synapses**

Although the pre- and postsynaptic membranes of neuromuscular and central synapses are generally similar, the synaptic cleft differs dramatically. Whereas muscle fibers are ensheathed by a basal lamina that has a distinctive molecular structure at the neuromuscular junction, central neurons do not have a basal lamina. Central
synaptic clefts contain no detectable laminin, or collagen. Instead, intercellular adhesion at central synapses may involve the interaction of matched adhesion molecules on pre- and postsynaptic membranes, with no intermediate matrix (Figure 55-15A). For example, the cell adhesion molecule N-cadherin is present in some central synaptic clefts, and the related E- and R-cadherins are present at others; both are homophilic adhesion molecules that adhere far better to their own kind than to each other. Proteins called neurexins and neuroligins that bind to each other are also present at synapses. The cytoplasmic segments of these proteins bind to PDZ-class proteins, such as PSD-95, which bind to neurotransmitter receptors (Figure 55-15B). Neurexin-neuroligin interaction could therefore provide a means of coupling the intercellular interactions required for synaptic recognition to the intracellular interactions required to cluster synaptic components within the cell membrane.
The Recognition of Synaptic Targets Is Highly Specific

Perhaps the most amazing physical feature of the nervous system is the specificity of its connections. This specificity arises from several developmental processes, including the generation of appropriate numbers and types of neurons, their migration to appropriate nuclei or laminae, and the guidance of their axons to appropriate target areas. In addition, synapse formation itself is a selective process. Unfortunately, although we know an impressive amount about the molecular cues that guide axons (see Chapter 54) and the intercellular signals that regulate synapse formation, we know distressingly little about the molecules that drive target recognition. Nevertheless, we can at least enumerate some of the synaptic choices for which molecular explanations will need to be found.

The specificity with which connections form is particularly evident when intertwined axons select subsets of interspersed postsynaptic cells. For example, autonomic preganglionic axons that arise from distinct rostrocaudal levels of the spinal cord enter sympathetic ganglia together but then synapse on distinct ganglion cells. The axons of rostral cells synapse on ganglion cells that project their axons to relatively rostral targets, whereas those of caudal cells synapse on caudally projecting neurons. This preference is apparent from the initial stages of innervation, even though the postsynaptic cells are more or less randomly distributed within the ganglion. Moreover, this preference is reestablished during reinervation in adults following nerve damage. Likewise, sensory axons contact specific motor neurons in the spinal cord, even though the dendrites of motor neurons that innervate neighboring muscles have over-
Figure 55-16 Sensory neurons that innervate muscle spindles selectively synapse on appropriate motor neurons. (Adapted from Mears and Frank 1997.)

A. Spindle afferents are activated by tapping the muscle. The resulting synaptic potential in the peripheral nerve is detected by intracellular recording from motor neurons in the spinal cord.

B. Although sensory neurons arborize in areas where dendrites from many types of motor neurons overlap, they preferentially synapse on motor neurons that project to their own muscles. In the example here, the quadriceps and obturator afferents make their most powerful synapses on quadriceps and obturator motor neurons, respectively. This selectivity is seen early in development and occurs even in the absence of activity.

Lapping territories (Figure 55-16). The selectivity of these connections has also been demonstrated by reinnervation and transplantation experiments.

Another common type of synaptic selectivity is the preference of some nerve terminals for specific portions of the target cell's surface. The best-studied example of this type of selectivity is reinnervation of the original synaptic site in adult muscle, described earlier in this chapter. Similar cases abound in the brain. For example, several distinct types of axons terminate on distinct domains of the Purkinje cells in the cerebellum: granule cell axons on dendritic spines, climbing fiber axons on dendritic shafts, basket cell axons on cell bodies, and so on (see Chapter 42). This specificity is likely to be based...
Figure 55-17 Synaptic input can profoundly influence the properties of the postsynaptic cell.

A. Motor neurons and muscles both have characteristic electrical properties that identify them as tonic (often called "slow") or phasic ("fast"). Fast and slow motor neurons connect exclusively with fast and slow muscle fibers, respectively. Thus muscles with a predominance of fast fibers are innervated mostly by fast motor neurons.

B. Cross-innervation can be produced by surgically connecting the fast axons to the predominantly slow muscle and vice versa. The properties of the motor neurons are essentially unchanged, but the properties of the muscle change profoundly: The fast nerve induces fast properties in the originally slow muscle and vice versa. (Adapted from Salmons and Sreter 1976.)

C. The effects of innervation by fast and slow nerves on muscle are mediated largely by their distinct patterns of activity. When a fast nerve is tonically stimulated in a slow pattern, the muscle acquires slow electrical and molecular properties. The selection of muscle fiber type is thought to involve the regulation of a Ca²⁺-regulated protein phosphatase, calcineurin, and a transcription factor, NFAT. In this view, under conditions of tonic activity, when steady-state Ca²⁺ levels are high, calcineurin is active and the dephosphorylated form of NFAT enters the nucleus and activates the slow muscle fiber transcriptional program. Under conditions of phasic activity, when steady-state Ca²⁺ levels are low, NFAT fails to enter the nucleus permitting expression of the fast fiber program. (Adapted from Chin et al. 1998.)
on molecular cues, either on the postsynaptic cell surface or in its immediate environment, that the approaching axon discerns. Likewise, axons terminating in layered structures often confine their synapses to dendrites in one layer, even though the dendritic tree of the postsynaptic cell traverses numerous layers.

Finally, synaptic selectivity can arise when one synaptic partner induces new properties in the other. This mechanism has been most clearly demonstrated in muscle. In general, mammalian muscle fibers can be divided into several categories such as fast-twitch and slow-twitch, according to their contractile characteristics. Fibers of each type express genes for distinctive isoforms of the main contractile proteins—myosins, troponins, and so on. A few muscles are composed exclusively of a single type of fiber, but most muscles have fibers of both (or all) types. The branches of an individual motor neuron innervate exclusively muscle fibers of a single type, even in “mixed” muscles.

This matching does not come about solely because each motor axon recognizes fibers of the appropriate type. Instead, the motor axon converts muscle fibers to the appropriate type. This was demonstrated in an experiment by John Eccles and colleagues, in which muscles were cross-reinnervated at birth, before the fiber properties were fully established. In this way a nerve that would normally innervate a predominantly slow muscle came to innervate a muscle destined to become predominantly fast and vice versa. Under these conditions the contractile properties of the muscle were partially transformed in a direction determined by the nerve. Other studies have shown that the pattern of neural activity is at least partially responsible for the switch in muscle properties (Figure 55-17).

New Neural Connections Can Reform Following Nerve Injury

One reason that clinicians are interested in the development of synaptic connections is their hope that developmental principles may shed light on the mechanisms underlying the regeneration of connections following injury. Understanding developmental mechanisms may eventually help us restore synaptic function by improving regenerative capacity. Here we outline how neural cells respond to injury, discuss why their regeneration is limited, and consider some strategies for improving regenerative capacity.

Both Neurons and Cells Around Them Are Affected by Damage to the Axon

Because neurons have long axons and small cell bodies, most injuries to the central or peripheral nervous system involve damage to axons. Transection of the axon, either acutely by cutting or more slowly by crushing, is called axotomy, and its consequences are numerous (Figure 55-18).

First, axotomy divides the axon into a proximal segment that remains attached to the cell body and a distal segment that has lost that attachment. Because the capacity for protein synthesis is largely restricted to the cell body, axotomy dooms the distal segment. Generally, transmission fails rapidly at the terminals of the distal segment; physical degeneration of the axon, in contrast, may be a slow and gradual process but inevitably proceeds to completion. As this happens glial cells that ensheathe the distal segment are also affected. The myelin sheath, which requires axonal contact for its maintenance as well as genesis becomes fragmented and is eventually enveloped, along with axonal debris, by phagocytic cells. This pattern of changes is called Wallerian degeneration.

The proximal portion of the neuron also suffers. In some cases the neuron dies by apoptosis, probably because axotomy cuts it off from its supply of target-derived trophic factors. Even when this does not occur the cell body may undergo a series of changes called the chromatolytic reaction: The cell body swells, the nucleus moves to an eccentric position, and the rough endoplasmic reticulum becomes fragmented (Figure 55-18B). Metabolic changes also accompany chromatolysis, including overall increases in protein and RNA synthesis as well as changes in the pattern of genes that the neuron expresses. These changes are reversible if regeneration is successful; if not, many neurons eventually die.

Axotomy also affects postsynaptic neurons. When axotomy disrupts the major inputs to a cell—as happens in denervated muscle, for example, or to neurons in the lateral geniculate 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 subtle.

Axotomy affects not only the targets of but also inputs to the injured neuron. Frequently, synaptic terminals withdraw from the neuronal cell bodies 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 (see Figure 55-18B). This process, called synaptic stripping, depresses synaptic function and can impair recovery of function. The mechanism of synaptic stripping remains unclear, but two possibilities have been suggested. One is that axon terminals lose their adhesive to synaptic sites as a consequence of postsynaptic injury and are subsequently enwrapped by
Figures 55-18. Axotomy affects not only the injured neuron but also its synaptic partners and neighboring cells.

A. A normal neuron with an intact functional axon.

B. (1) After axotomy, the nerve terminals of the injured neuron fail rapidly. (2) The distal stump, separated from the cell body, undergoes Wallerian degeneration. (3) Myelin degenerates and is phagocytosed by microglia. (4) Phagocytic cells invade. (5) The cell body undergoes chromatolysis, in which the nucleus moves to an eccentric position. (6) Presynaptic terminals on the chromatolytic neuron withdraw and are engulfed by glial processes. (7, 8) The inputs to and targets of the injured neuron can atrophy and even degenerate.

Regenerative Capacity Is Greater in the Peripheral Than in the Central Nervous System

Damage to peripheral nerves can often be repaired. Although distal segments of axons degenerate, connective tissue elements of the so-called distal stump generally survive. Axonal sprouts grow from the proximal stump, enter the distal stump, and grow toward the nerve's end-organs. The mechanisms involved are related to those that guide embryonic axons: Chemotactic 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 perineurium prevent the regenerating axons from going astray (see Chapter 54).

Once they return to their targets the regenerated axons can form new functional nerve endings. Motor axons, for example, form new neuromuscular junctions (see Figure 55-9). Likewise, autonomic axons can successfully reinnervate glands, blood vessels, and viscera, and sensory axons can reinnervate muscle spindles. Finally, those axons that were demyelinated become remyelinated, and chromatolytic somata regain their original appearance. Thus in all three divisions of the peripheral nervous system (motor, sensory, and autonomic) the effects of axotomy are reversible.
This is not to say that regeneration is perfect. In the motor system recovery of strength may be substantial but recovery of fine movements is impaired because some motor axons are guided to and form synapses on inappropriate muscle fibers. Some axons may never find a target at all, and some neurons die. Nonetheless, regenerative capacities in the peripheral nervous system are impressive.

In contrast, little regeneration occurs in the central nervous system following injury. The proximal stumps of damaged axons often form short sprouts, but long-distance regeneration is rare and the damaged axons make few new synapses. This failure of regeneration has led to the dismal view that injuries to the brain and spinal cord are largely irreversible, and that therapy must be restricted to rehabilitative measures. For some time, however, neurobiologists have been systematically seeking the reasons why regenerative capacity differs so dramatically between the central and peripheral nervous systems. The aim of this work is to identify the crucial barriers to regeneration so that they can be overcome. In addition, neurobiologists have asked why regeneration of central nerves is much greater in lower vertebrates such as fish and frogs than in mammals. These studies have begun to bear fruit, and there is now cautious optimism that the injured human brain and spinal cord have a latent regenerative capacity that can be exploited.

Therapeutic Interventions May Promote Axonal Regeneration in the Injured Central Nervous System

At least four factors may contribute to the superior regenerative capacities of the peripheral nervous system. First, peripheral nerves appear to provide a more favorable environment for regeneration than do central axons. In a seminal experiment performed a century ago, Tello transplanted a segment of peripheral nerve into the brain. The central axons were capable of growing into the peripheral nerve even though they were incapable of regeneration in their normal central environment. This result implied that Schwann cells provide growth-promoting factors, normally absent from the brain, to the injured areas.

Indeed, numerous studies over the succeeding century have revealed that several constituents of peripheral nerves and Schwann cells 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, such as NgCAM/L1. In addition, denervated distal stumps upregulate production of neurotrophins and other trophic molecules. Central neurons are poor sources of these molecules. For example, they contain little laminin and typically produce only low amounts of trophic molecules. In the embryo both the central and peripheral nervous systems provide environments that
promote axon outgrowth, it may be that only the peripheral environment retains that capacity. In adulthood, the microenvironment surrounds the injured nerve fibers, and regeneration occurs only if the injured nerve fibers are placed in a favorable environment. To understand how this favors regeneration, we need to consider the factors that are present in the periphery and naturally favoring this in the central nervous system.

Chapter 25: The Formation and Regulation of Synapses

Promote axon outgrowth: It may be that only the peripheral environment retains that capacity in adulthood. 

In the 1980s, a radical interpretation was proposed that central nerves could be conditioned to grow by observing the behavior of other nerve fibers in the periphery. In this case, the central axon is thought to be critical for guiding growth elongation. 

Levels of GAP-43 remain high in mature peripheral neurons, but they do not change in the central nervous system. 

The activity of central axons can regenerate themselves in peripheral nerves, but the reason for this remains unknown. 

Kallikrein, a structural component of myelin, is present in the axonal outgrowth of Schwann cells. 

The presence of antibodies to N-35, a novel myelin (anti-35) or N-33, is novel myelin (anti-33) in culture fail to grow on cilia of myelin or oligodendrocytes. 

The two models describe above emphasize differences in the axon outgrowth: A third model emphasizes differences between peripheral and central axons. In peripheral axons, the central axon is thought to be critical for guiding growth elongation. 

In summary, analysis of axon outgrowth has led to a variety of possible ways to enhance elongation. Current studies are now aimed at perfecting and combining these methods. The prospect of clinical useful intervention is, for the first time, a very real one.
maturity their capacity to form synapses. For example, Albert Aguayo and his colleagues have carried out extensive studies on the regeneration of retinal axons that project to the superior colliculus. They first showed that a modest number of axons were capable of regenerating in a grafted peripheral nerve, thereby providing strong support for the theory that the central environment is inhospitable to regeneration (Figure 55-20A). They then recorded the electrical activity of neurons in the superior colliculus while flashing spots of light on the retina. Remarkably, at least some collicular neurons fired action potentials when the eye was illuminated, proving that functional synaptic connections had been reestablished (Figure 55-20B). Moreover, electron microscopic studies showed that the new synapses had formed on appropriate target cells in appropriate laminae. The number of new synapses was small and retinotopic arrangement of inputs to the superior colliculus was not restored, but these and other results show that mature neurons retain their synaptogenetic abilities.

An Overall View

The formation of synapses completes the hard wiring of the nervous system and enables it to function. The requirements for an adequate synapse are stringent: The nerve terminal must recognize the proper target cell and often even a specific portion of the target cell’s surface. The postsynaptic membrane must be highly responsive to the particular neurotransmitter that the nerve terminal releases. The apposition of pre- and postsynaptic elements must be spatially matched at a submicron level of precision, so that responses can occur on a time scale of milliseconds. And the whole structure must be sufficiently stable to last a lifetime yet sufficiently plastic to change with experience.

To meet these specifications, synaptogenesis is a highly interactive process. Although the pre- and postsynaptic cells can each synthesize synaptic components on their own, they exchange numerous signals to coordinate their activities in space and time. In this chapter we have used the neuromuscular junction to illustrate these interactions. Motor nerve terminals use a combination of electrical and chemical signals to sculpt the postsynaptic apparatus of the muscle fiber, and the muscle fiber in turn provides retrograde signals to organize synaptic specialization in the nerve terminal.

A key anterograde signal is agrin, which activates a tyrosine kinase called MuSK; MuSK in turn uses the cytoplasmic protein rapsyn to aggregate ACh receptors in the postsynaptic membrane. Simultaneously, neutrophilins and electric activity regulate the synthesis of ACh receptors. Retrograde signals include both soluble trophic factors and matrix-associated proteins such as the laminins. Some of these molecules, such as neurulins, also function at central synapses. In addition to the molecules that direct synaptic specialization, target recognition molecules must account for the selectivity of synapse formation, but few of these have been identified.

Finally, axons can regenerate and form new synapses following injury. This regeneration is far more effective in the peripheral than in the central nervous system. However, recent studies have identified several...
selected factors that limit regeneration of central axons. By manipulating these factors it should be possible to enhance regeneration following injury and thus provide restoration of function to many patients for whom there is currently little hope.

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Selected Readings


References


