CHAPTER

1

Cellular Components of Nervous Tissue

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Several types of cellular elements are integrated to constitute normally functioning brain tissue. The neuron is the communicating cell, and many neuronal subtypes are connected to one another via complex circuitries, usually involving multiple synaptic connections. Neuronal physiology is supported and maintained by neuroglial cells, which have highly diverse and incompletely understood functions. These include myelination, secretion of trophic factors, maintenance of the extracellular milieu, and scavenging of molecular and cellular debris from it. Neuroglial cells also participate in the formation and maintenance of the blood–brain barrier, a multicomponent structure that is interposed between the circulatory system and the brain substance and that serves as the molecular gateway to brain tissue.

NEURONS

The neuron is a highly specialized cell type and is the essential cellular element in the central nervous system (CNS). All neurological processes are dependent on complex cell–cell interactions between single neurons and/or groups of related neurons. Neurons can be categorized according to their size, shape, neurochemical characteristics, location, and connectivity, which are important determinants of that particular functional role of the neuron in the brain. More importantly, neurons form circuits, and these circuits constitute the structural basis for brain function. *Macrocircuits* involve a population of neurons projecting from one brain region to another region, and *microcircuits* reflect the local cell–cell interactions within a brain region. The detailed analysis of these macro- and microcircuits is an essential step in understanding the neuronal basis of a given cortical function in the healthy and the diseased brain. Thus, these cellular characteristics allow us to appreciate the special structural and biochemical qualities of a neuron in relation to its neighbors and to place it in the context of a specific neuronal subset, circuit, or function.

Broadly speaking, therefore, there are five general categories of neurons: inhibitory neurons that make local contacts (e.g., GABAergic interneurons in the cerebral and cerebellar cortex), inhibitory neurons that make distant contacts (e.g., medium spiny neurons of the basal ganglia or Purkinje cells of the cerebellar cortex), excitatory neurons that make local contacts (e.g., spiny stellate cells of the cerebral cortex), excitatory neurons that make distant contacts (eg., pyramidal neurons in the cerebral cortex), and neuromodulatory neurons that influence neurotransmission, often at large distances. Within these general classes, the structural variation of neurons is systematic, and careful analyses of the anatomic features of neurons have led to various categorizations and to the development of the concept of cell type. The grouping of neurons into descriptive cell types (such as chandelier, double bouquet, or bipolar cells) allows the analysis of populations of neurons and the linking of specified cellular characteristics with certain functional roles.

General Features of Neuronal Morphology

Neurons are highly polarized cells, meaning that they develop distinct subcellular domains that subserve different functions. Morphologically, in a typical

neuron, three major regions can be defined: (1) the cell body (*soma* or *perikaryon*), which contains the nucleus and the major cytoplasmic organelles; (2) a variable number of dendrites, which emanate from the perikaryon and ramify over a certain volume of gray matter and which differ in size and shape, depending on the neuronal type; and (3) a single axon, which extends, in most cases, much farther from the cell body than the dendritic arbor (Fig. 1.1). Dendrites may be spiny (as in pyramidal cells) or nonspiny (as in most interneurons), whereas the axon is generally smooth and emits a variable number of branches (collaterals). In vertebrates, many axons are surrounded by an insulating myelin sheath, which facilitates rapid impulse conduction. The axon terminal region, where contacts with other cells are made, displays a wide range of morphological specializations, depending on its target area in the central or peripheral nervous system.

The cell body and dendrites are the two major domains of the cell that receive inputs, and dendrites play a critically important role in providing a massive receptive area on the neuronal surface. In addition, there is a characteristic shape for each dendritic arbor, which can be used to classify neurons into morphological types. Both the structure of the dendritic arbor and the distribution of axonal terminal ramifications confer a high level of subcellular specificity in the localization of particular synaptic contacts on a given neuron. The three-dimensional distribution of dendritic arborization is also important with respect to the type of information transferred to the neuron. A neuron with a dendritic tree restricted to a particular cortical layer may receive a very limited pool of afferents, whereas the widely expanded dendritic arborizations of a large pyramidal neuron will receive highly diversified inputs within the different cortical layers in which segments of the dendritic tree are present (Fig. 1.2) (Mountcastle, 1978). The structure of the dendritic tree is maintained by surface interactions between adhesion molecules and, intracellularly, by an array of cytoskeletal components (microtubules, neurofilaments, and associated proteins), which also take part in the movement of organelles within the dendritic cytoplasm.

An important specialization of the dendritic arbor of certain neurons is the presence of large numbers of dendritic spines, which are membranous protrusions. They are abundant in large pyramidal neurons and are much sparser on the dendrites of interneurons (see following text).

The perikaryon contains the nucleus and a variety of cytoplasmic organelles. Stacks of rough endoplasmic reticulum are conspicuous in large neurons and, when interposed with arrays of free polyribosomes, are referred to as *Nissl substance*. Another feature of the perikaryal cytoplasm is the presence of a rich cytoskeleton composed primarily of neurofilaments and microtubules. These cytoskeletal elements are dispersed in bundles that extend from the soma into the axon and dendrites.

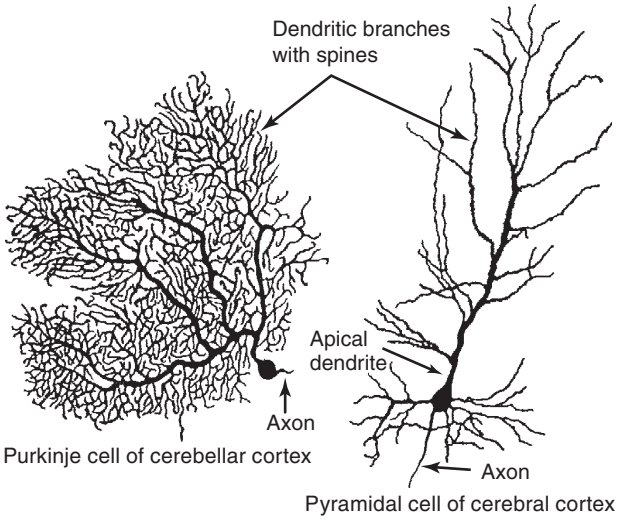

FIGURE 1.1 Typical morphology of projection neurons. (Left) A Purkinje cell of the cerebellar cortex and (right) a pyramidal neuron of the neocortex. These neurons are highly polarized. Each has an extensively branched, spiny apical dendrite, shorter basal dendrites, and a single axon emerging from the basal pole of the cell.

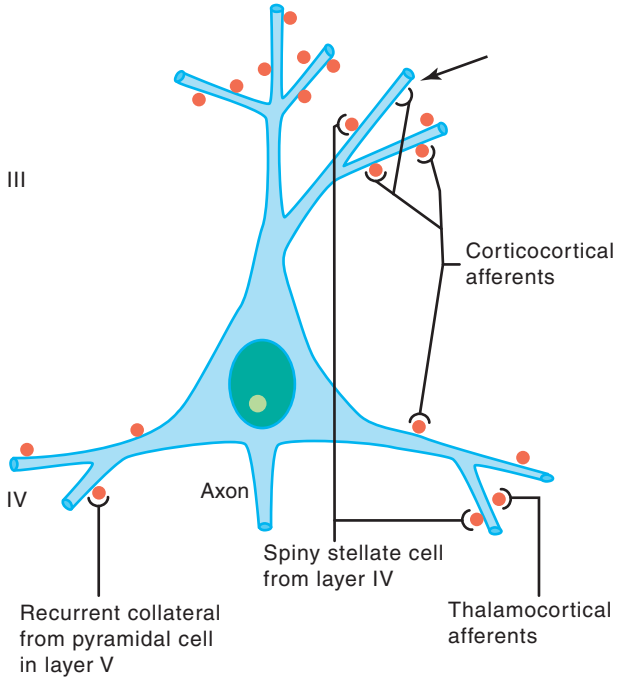

FIGURE 1.2 Schematic representation of four major excitatory inputs to pyramidal neurons. A pyramidal neuron in layer III is shown as an example. Note the preferential distribution of synaptic contacts on spines. Spines are labeled in red. Arrow shows a contact directly on the dendritic shaft.

Whereas dendrites and the cell body can be characterized as domains of the neuron that receive afferents, the axon, at the other pole of the neuron, is responsible for transmitting neural information. This information may be primary, in the case of a sensory receptor, or processed information that has already been modified through a series of integrative steps. The morphology of the axon and its course through the nervous system are correlated with the type of information processed by the particular neuron and by its connectivity patterns with other neurons. The axon leaves the cell body from a small swelling called the *axon hillock*. This structure is particularly apparent in large pyramidal neurons; in other cell types, the axon sometimes emerges from one of the main dendrites. At the axon hillock, microtubules are packed into bundles that enter the axon as parallel fascicles. The axon hillock is the part of the neuron where the action potential is generated. The axon is generally unmyelinated in local circuit neurons (such as inhibitory interneurons), but it is myelinated in neurons that furnish connections between different parts of the nervous system. Axons usually have higher numbers of neurofilaments than dendrites, although this distinction can be difficult to make in small elements that contain fewer neurofilaments. In addition, the axon may be extremely ramified, as in certain local circuit neurons; it may give out a large number of recurrent collaterals, as in neurons connecting different cortical regions, or it may be relatively straight in the case of projections to subcortical centers, as in cortical motor neurons that send their very long axons to the ventral horn of the spinal cord. At the interface of axon terminals with target cells are the synapses, which represent specialized zones of contact consisting of a presynaptic (axonal) element, a narrow synaptic cleft, and a postsynaptic element on a dendrite or perikaryon.

Synapses and Spines

Synapses

Each synapse is a complex of several components: (1) a *presynaptic element*, (2) a *cleft*, and (3) a *postsynaptic element*. The presynaptic element is a specialized part of the presynaptic neuron's axon, the postsynaptic element is a specialized part of the postsynaptic somatodendritic membrane, and the space between these two closely apposed elements is the cleft. The portion of the axon that participates in the axon is the *bouton*, and it is identified by the presence of synaptic vesicles and a presynaptic thickening at the active zone (Fig. 1.3). The postsynaptic element is marked by a postsynaptic thickening opposite the presynaptic thickening. When both sides are equally thick, the synapse is referred to as *symmetric*. When the postsynaptic thickening is greater, the synapse is *asymmetric*. Edward George Gray noticed this difference, and divided synapses into two types: *Gray's type 1* synapses are symmetric and have variably

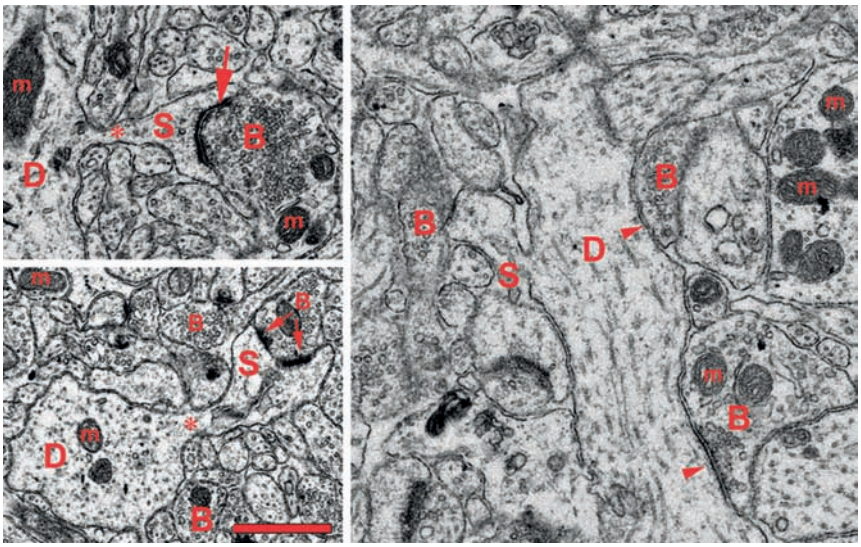

FIGURE 1.3 Ultrastructure of dendritic spines (S) and synapses in the human brain. Note the narrow spine necks (asterisks) emanating from the main dendritic shaft (D) and the spine head containing filamentous material, and the cisterns of the spine apparatus particularly visible in the lower panel spine. The arrows on the left panels point to postsynaptic densities of asymmetric excitatory synapses (arrows). The apposed axonal boutons (B) are characterized by round synaptic vesicles. A perforated synapse is shown on the lower left panel. The panel at right shows two symmetric inhibitory synapses (arrowheads) on a large dendritic shaft (D). In this case the axonal boutons (B) contain some ovoid vesicles compared to the ones in asymmetric synapses. The dendrites and axons contain numerous mitochondria (m). Scale bar = 1 μm. Electron micrographs courtesy of Drs. S.A. Kirov and M. Witcher (Medical College of Georgia), and K.M. Harris (University of Texas – Austin).

shaped, or pleomorphic, vesicles. *Gray's type 2* synapses are asymmetric and have clear, round vesicles. The significance of this distinction is that research has shown that, in general, Gray's type 1 synapses tend to be inhibitory, while Gray's type 2 synapses tend to be excitatory. This correlation greatly enhanced the usefulness of electron microscopy in neuroscience.

In cross section on electron micrographs, a synapse looks like two parallel lines separated by a very narrow space (Fig. 1.3). Viewed from the inside of the axon or dendrite, it looks like a patch of variable shape. Some synapses are a simple patch, or *macule.* Macular synapses can grow fairly large, reaching diameters over 1 μm. The largest synapses have discontinuities or holes within the macule and are called *perforated synapses* (Fig. 1.3). In cross section, a perforated synapse may resemble a simple macular synapse or several closely spaced smaller macules.

The portion of the presynaptic element that is apposed to the postsynaptic element is the *active zone.* This is the region where the synaptic vesicles are concentrated and where, at any time, a small number of vesicles are docked and presumably ready for fusion. The active zone is also enriched with voltage gated calcium channels, which are necessary to permit activity-dependent fusion and neurotransmitter release.

The synaptic cleft is truly a space, but its properties are essential. The width of the cleft (~20 μm) is critical because it defines the volume in which each vesicle releases its contents, and therefore, the peak concentration of neurotransmitter upon release. On the flanks of the synapse, the cleft is spanned by adhesion molecules, which are believed to stabilize the cleft.

The postsynaptic element may be a portion of a soma or a dendrite, or rarely, part of an axon. In the cerebral cortex, most Gray's type 1 synapses are located on somata or dendritic shafts, while most Gray's type 2 synapses are located on dendritic spines, which are specialized protrusions of the dendrite. A similar segregation is seen in cerebellar cortex. In nonspiny neurons, symmetric and asymmetric synapses are often less well separated. Irrespective of location, a postsynaptic thickening marks the postsynaptic element. In Gray's type 2 synapses, the postsynaptic thickening (or postsynaptic density, PSD), is greatly enhanced. Among the molecules that are associated with the PSD are neurotransmitter receptors (e.g., NMDA receptors) and molecules with less obvious function, such as PSD-95.

Spines

Spines are protrusions on the dendritic shafts of some types of neurons and are the sites of synaptic contacts, usually excitatory. Use of the silver impregnation techniques of Golgi or of the methylene blue used by Ehrlich in the late nineteenth century led to the discovery of spiny appendages on dendrites of a variety of neurons. The best known are those on pyramidal neurons and Purkinje cells, although spines occur on neuron types at all levels of the central nervous system. In 1896, Berkley observed that terminal axonal boutons were closely apposed to spines and suggested that spines may be involved in conducting impulses from neuron to neuron. In 1904, Santiago Ramón y Cajal suggested that spines could collect the electrical charge resulting from neuronal activity. He also noted that spines substantially increase the receptive surface of the dendritic arbor, which may represent an important factor in receiving the contacts made by the axonal terminals of other neurons. It has been calculated that the approximately 20,000 spines of a pyramidal neuron account for more than 40% of its total surface area (Peters *et al.*, 1991).

More recent analyses of spine electrical properties have demonstrated that spines are dynamic structures that can regulate many neurochemical events related to synaptic transmission and modulate synaptic efficacy. Spines are also known to undergo pathologic alterations and have a reduced density in a number of experimental manipulations (such as deprivation of a sensory input) and in many developmental, neurologic, and psychiatric conditions (such as dementing illnesses, chronic alcoholism, schizophrenia, trisomy 21). Morphologically, spines are characterized by a narrower portion emanating from the dendritic shaft, the neck, and an ovoid bulb or head, although spine morphology may vary from large mushroom-shaped bulbs to small bulges barely discernable on the surface of the dendrite. Spines have an average length of ~2 μm, but there is considerable variability in their dimensions. At the ultrastructural level (Fig. 1.3), spines are characterized by the presence of asymmetric synapses and contain fine and quite indistinct filaments. These filaments most likely consist of actin and α- and β-tubulins. Microtubules and neurofilaments present in dendritic shafts do not enter spines. Mitochondria and free ribosomes are infrequent, although many spines contain polyribosomes in their neck. Interestingly, most polyribosomes in dendrites are located at the bases of spines, where they are associated with endoplasmic reticulum, indicating that spines possess the machinery necessary for the local synthesis of proteins. Another feature of the spine is the presence of confluent tubular cisterns in the spine head that represent an extension of the dendritic smooth endoplasmic reticulum. Those cisterns are referred to as the *spine apparatus*. The function of the spine apparatus is not fully understood but may be related to the storage of calcium ions during synaptic transmission.

Specific Examples of Different Neuronal Types

Inhibitory Local Circuit Neurons

Inhibitory Interneurons of the Cerebral Cortex A large variety of inhibitory interneuron types is present in the cerebral cortex and in subcortical structures. These neurons contain the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and exert strong local inhibitory effects. Their dendritic and axonal arborizations offer important clues as to their role in the regulation of pyramidal cell function. In addition, for several GABAergic interneurons, a subtype of a given morphologic class can be defined further by a particular set of neurochemical characteristics. Interneurons have been extensively characterized in the neocortex and hippocampus of rodents and primates, but they are present throughout the cerebral gray matter and exhibit a rich variety of morphologies, depending on the brain region as well as on the species studied.

In the neocortex and hippocampus, the targets and morphologies of interneuron axons are most usefully classified into morphological and functional groups. For example, *basket cells* have axonal endings surrounding pyramidal cell somata (Somogyi *et al.*, 1983) and provide most of the inhibitory GABAergic synapses to the somas and proximal dendrites of pyramidal cells. These cells are also characterized by certain biochemical features in that the majority of them contain the calcium-binding protein parvalbumin, and cholecystokinin appears to be the most likely neuropeptide in large basket cells.

Chandelier cells have spatially restricted axon terminals that look like vertically oriented "cartridges," each consisting of a series of axonal boutons, or swellings, linked together by thin connecting pieces. These neurons synapse exclusively on the axon initial segment of pyramidal cells (this cell is also known as *axoaxonic cell*), and because the strength of the synaptic input is correlated directly with its proximity to the axon initial segment, there can be no more powerful inhibitory input to a pyramidal cell than that of the chandelier cell (Freund *et al.*, 1983; DeFelipe *et al.*, 1989).

The double bouquet cells are characterized by a vertical bitufted dendritic tree and a tight bundle of vertically oriented varicose axon collaterals (Somogyi and Cowey, 1981). There are several subclasses of double bouquet cells based on the complement of calcium-binding protein and neuropeptide they contain. Their axons contact spines and dendritic shafts of pyramidal cells, as well as dendrites from non-pyramidal neurons.

Inhibitory Projection Neurons

Medium-Sized Spiny Cells These neurons are unique to the striatum, a part of the basal ganglia that comprises the caudate nucleus and putamen. Medium-sized spiny cells are scattered throughout the caudate nucleus, and putamen and are recognized by their relatively large size compared with other cellular elements of the basal ganglia, and by the fact that they are generally isolated neurons. They differ from all others in the striatum in that they have a highly ramified dendritic arborization radiating in all directions and densely covered with spines. They furnish a major output from the caudate nucleus and putamen and receive a highly diverse input from, among other sources, the cerebral cortex, thalamus, and certain dopaminergic neurons of the substantia nigra. These neurons are neurochemically quite heterogeneous, contain GABA, and may contain several neuropeptides and the calcium-binding protein calbindin. In Huntington disease, a neurodegenerative disorder of the striatum characterized by involuntary movements and progressive dementia, an early and dramatic loss of medium-sized spiny cells occurs.

Purkinje Cells Purkinje cells are the most salient cellular elements of the cerebellar cortex. They are arranged in a single row throughout the entire cerebellar cortex between the molecular (outer) layer and the granular (inner) layer. They are among the largest neurons and have a round perikaryon, classically described as shaped "like a chianti bottle," with a highly branched dendritic tree shaped like a candelabrum and extending into the molecular layer, where they are contacted by incoming systems of afferent fibers from granule neurons and the brainstem. The apical dendrites of Purkinje cells have an enormous number of spines (more than 80,000 per cell). A particular feature of the dendritic tree of the Purkinje cell is that it is distributed in one plane, perpendicular to the longitudinal axes of the cerebellar folds, and each dendritic arbor determines a separate domain of cerebellar cortex (Fig. 1.1). The axons of Purkinje neurons course through the cerebellar white matter and contact deep cerebellar nuclei or vestibular nuclei. These neurons contain the inhibitory neurotransmitter GABA and the calcium-binding protein calbindin. Spinocerebellar ataxia, a severe disorder combining ataxic gait and impairment of fine hand movements, accompanied by dysarthria and tremor, has been documented in some families and is related directly to Purkinje cell degeneration.

Excitatory Local Circuit Neurons

Spiny Stellate Cells Spiny stellate cells are small multipolar neurons with local dendritic and axonal arborizations. These neurons resemble pyramidal cells in that they are the only other cortical neurons with large numbers of dendritic spines, but they differ from pyramidal neurons in that they lack an elaborate apical dendrite. The relatively restricted dendritic arbor of

these neurons is presumably a manifestation of the fact that they are high-resolution neurons that gather afferents to a very restricted region of the cortex. Dendrites rarely leave the layer in which the cell body resides. The spiny stellate cell also resembles the pyramidal cell in that it provides asymmetric synapses that are presumed to be excitatory, and is thought to use glutamate as its neurotransmitter (Peters and Jones, 1984).

The axons of spiny stellate neurons have primarily intracortical targets and a radial orientation, and appear to play an important role in forming links among layer IV, the major thalamorecipient layer, and layers III, V, and VI, the major projection layers. The spiny stellate neuron appears to function as a high-fidelity relay of thalamic inputs, maintaining strict topographic organization and setting up initial vertical links of information transfer within a given cortical area (Peters and Jones, 1984).

Excitatory Projection Neurons

Pyramidal Cells All cortical output is carried by pyramidal neurons, and the intrinsic activity of the neocortex can be viewed simply as a means of finely tuning their output. A pyramidal cell is a highly polarized neuron, with a major orientation axis perpendicular (or orthogonal) to the pial surface of the cerebral cortex. In cross section, the cell body is roughly triangular (Fig. 1.1), although a large variety of morphologic types exist with elongate, horizontal, or vertical fusiform, or inverted perikaryal shapes. Pyramidal cells are the major excitatory type of neurons and use glutamate as their neurotransmitter. A pyramidal neuron typically has a large number of dendrites that emanate from the apex and form the base of the cell body. The span of the dendritic tree depends on the laminar localization of the cell body, but it may, as in giant pyramidal neurons, spread over several millimeters. The cell body and dendritic arborization may be restricted to a few layers or, in some cases, may span the entire cortical thickness (Jones, 1984).

In most cases, the axon of a large pyramidal cell extends from the base of the perikaryon and courses toward the subcortical white matter, giving off several collateral branches that are directed to cortical domains generally located within the vicinity of the cell of origin (as explained later). Typically, a pyramidal cell has a large nucleus, and a cytoplasmic rim that contains, particularly in large pyramidal cells, a collection of granular material chiefly composed of *lipofuscin*. Although all pyramidal cells possess these general features, they can also be subdivided into numerous classes based on their morphology, laminar location, and connectivity with cortical and subcortical regions (Fig. 1.4) (Jones, 1975).

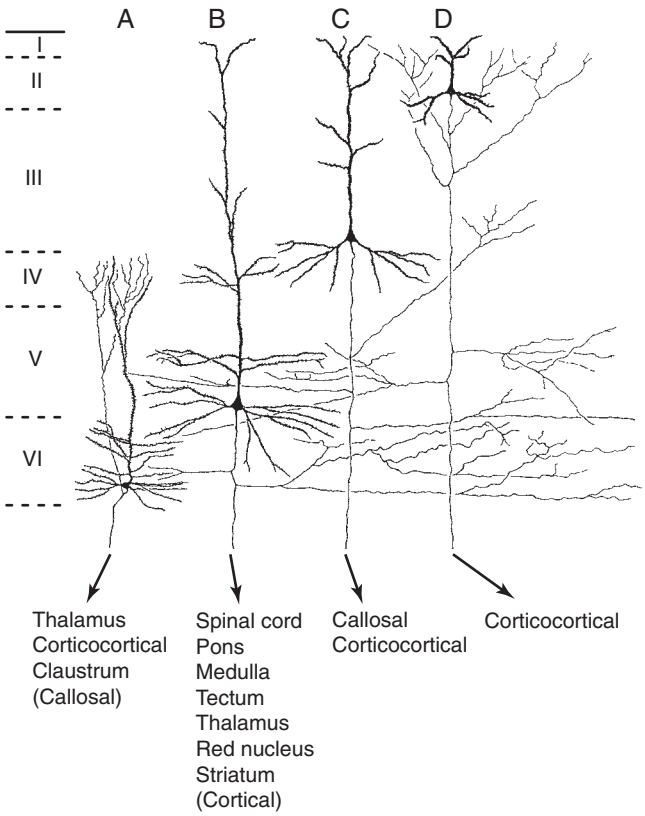

FIGURE 1.4 Morphology and distribution of neocortical pyramidal neurons. Note the variability in cell size and dendritic arborization, as well as the presence of axon collaterals, depending on the laminar localization (I–VI) of the neuron. Also, different types of pyramidal neurons with a precise laminar distribution project to different regions of the brain. Adapted from Jones (1984).

Spinal Motor Neurons Motor cells of the ventral horns of the spinal cord, also called α motoneurons, have their cell bodies within the spinal cord and send their axons outside the central nervous system to innervate the muscles. Different types of motor neurons are distinguished by their targets. The α motoneurons innervate skeletal muscles, but smaller motor neurons (the γ motoneurons, forming about 30% of the motor neurons) innervate the spindle organs of the muscles. The α motor neurons are some of the largest neurons in the entire central nervous system and are characterized by a multipolar perikaryon and a very rich cytoplasm that renders them very conspicuous on histological preparations. They have a large number of spiny dendrites that arborize locally within the ventral horn. The α motoneuron axon leaves the central nervous system through the ventral root of the peripheral nerves. Their distribution in the ventral horn is not random and corresponds to a somatotopic representation of the muscle groups of the limbs and axial musculature (Brodal, 1981). Spinal motor neurons use

acetylcholine as their neurotransmitter. Large motor neurons are severely affected in lower motor neuron disease, a neurodegenerative disorder characterized by progressive muscular weakness that affects, at first, one or two limbs but involves more and more of the body musculature, which shows signs of wasting as a result of denervation.

Neuromodulatory Neurons

Dopaminergic Neurons of the Substantia Nigra

Dopaminergic neurons are large neurons that reside mostly within the pars compacta of the substantia nigra and in the ventral tegmental area. A distinctive feature of these cells is the presence of a pigment, *neuromelanin*, in compact granules in the cytoplasm. These neurons are medium-sized to large, fusiform, and frequently elongated. They have several large radiating dendrites. The axon emerges from the cell body or from one of the dendrites and projects to large expanses of cerebral cortex and to the basal ganglia. These neurons contain the catecholamine-synthesizing enzyme *tyrosine hydroxylase*, as well as the monoamine dopamine as their neurotransmitter. Some of them contain both calbindin and calretinin. These neurons are affected severely and selectively in Parkinson disease—a movement disorder different from Huntington disease and characterized by resting tremor and rigidity—and their specific loss is the neuropathologic hallmark of this disorder.

NEUROGLIA

The term *neuroglia*, or "nerve glue," was coined in 1859 by Rudolph Virchow, who erroneously conceived of the neuroglia as an inactive "connective tissue" holding neurons together in the central nervous system. The metallic staining techniques developed by Ramón y Cajal and del Rio-Hortega allowed these two great pioneers to distinguish, in addition to the ependyma lining the ventricles and central canal, three types of supporting cells in the CNS: oligodendrocytes, astrocytes, and microglia. In the peripheral nervous system (PNS), the Schwann cell is the major neuroglial component.

Oligodendrocytes and Schwann Cells Synthesize Myelin

Most brain functions depend on rapid communication between circuits of neurons. As shown in depth later, there is a practical limit to how fast an individual bare axon can conduct an action potential. Organisms developed two solutions for enhancing rapid communication between neurons and their effector organs. In invertebrates, the diameters of axons are enlarged. In vertebrates, the myelin sheath (Fig. 1.5) evolved to permit rapid nerve conduction.

Axon enlargement accelerates the rate of conduction of the action potential in proportion to the square root of axonal diameter. Thus small axons conduct at slower rates than larger ones. The largest axon in the invertebrate kingdom is the squid giant axon, which is about the thickness of a mechanical pencil lead. This axon conducts the action potential at speeds of 10–20 m/s. As the axon mediates an escape reflex, firing must be rapid if the animal is to survive. Bare axons and continuous conduction obviously provide sufficient rates of signal propagation for even very large invertebrates, such as the giant squid, and many human axons also remain bare. However, in the human brain with 10 billion neurons, axons cannot be as thick as pencil lead, otherwise human heads would weigh 100 pounds or more.

Thus, along the invertebrate evolutionary line, the use of bare axons imposes a natural, insurmountable limit— a constraint of axonal size—to increasing the processing capacity of the nervous system. Vertebrates, however, get around this problem through evolution of the myelin sheath, which allows 10- to 100-fold increases in conduction of the nerve impulse along axons with fairly minute diameters.

In the central nervous system, myelin sheaths (Fig. 1.6) are elaborated by oligodendrocytes. During brain development, these glial cells send out a few cytoplasmic processes that engage adjacent axons and form myelin around them (Bunge, 1968). Myelin consists of a long sheet of oligodendrocyte plasma membrane, which is spirally wrapped around an axonal segment. At the end of each myelin segment, there is a bare portion of the axon, the node of Ranvier. Myelin segments are thus called "internodes." Physiologically, myelin has insulating properties such that the action potential can "leap" from node to node and therefore does not have to be regenerated continually along the axonal segment that is covered by the myelin membrane sheath. This leaping of the action potential from node to node allows axons with fairly small diameters to conduct extremely rapidly (Ritchie, 1984) and is called "saltatory" conduction.

Because the brain and spinal cord are encased in the bony skull and vertebrae, CNS evolution has promoted compactness among the supporting cells of the CNS. Each oligodendrocyte cell body is responsible for the construction and maintenance of several myelin sheaths (Fig. 1.6), thus reducing the number of glial cells required. In both PNS and CNS myelin, cytoplasm is removed between each turn of the

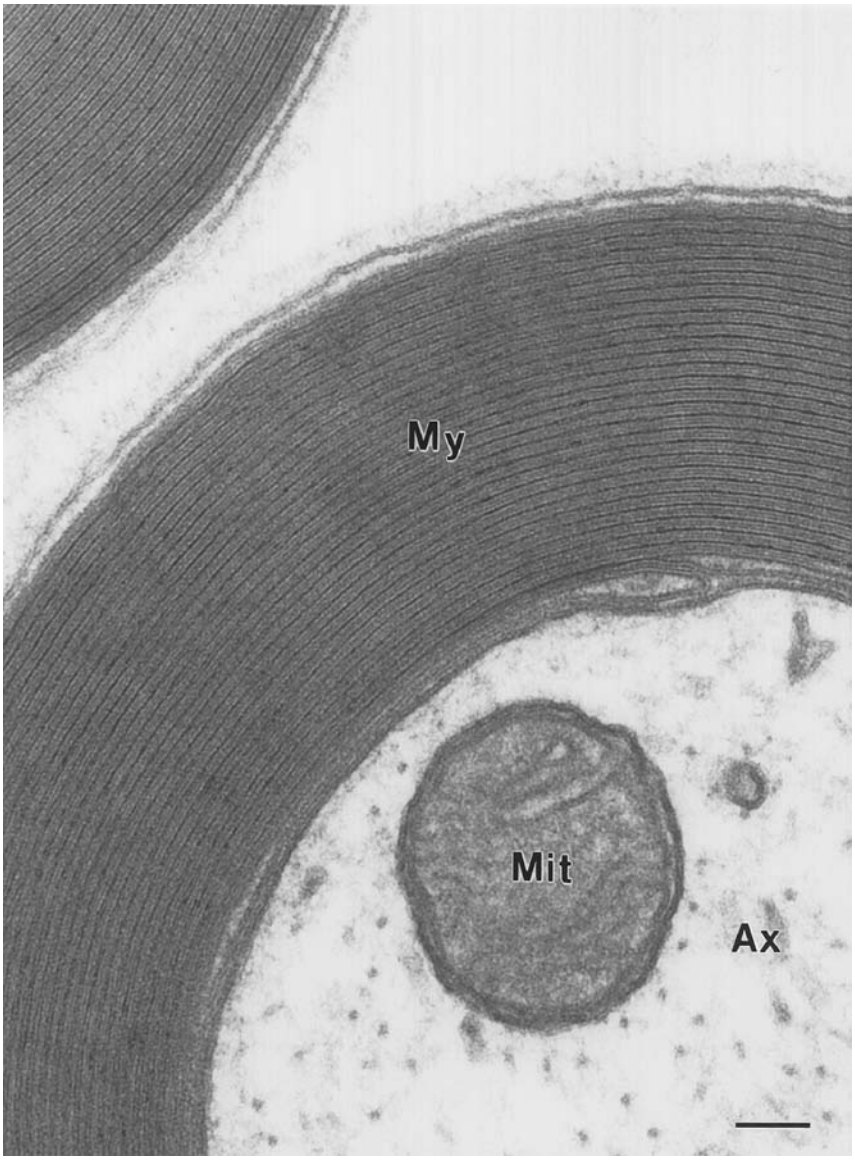

FIGURE 1.5 An electron micrograph of a transverse-section through part of a myelinated axon from the sciatic nerve of a rat. The tightly compacted multilayer myelin sheath (My) surrounds and insulates the axon (Ax). Mit, mitochondria. Scale bar: 75 nm.

myelin, leaving only the thinnest layer of plasma membrane. Due to protein composition differences, CNS lamellae are approximately 30% thinner than in PNS myelin. In addition, there is little or no extracellular space or extracellular matrix between the myelinated axons passing through CNS white matter. Brain volume is thus reserved for further expansion of neuronal populations.

Peripheral nerves pass between moving muscles and around major joints and are routinely exposed to physical trauma. A hard tackle, slipping on an icy sidewalk, or even just occupying the same uncomfortable seating posture for too long can painfully compress peripheral nerves and potentially damage them. Thus, evolutionary pressures shaping the PNS favor robustness and regeneration rather than conservation of space. Myelin in the PNS is generated by Schwann cells (Fig. 1.7), which are different from oligodendrocytes in several ways. Individual myelinating Schwann cells form a single internode. The biochemical composition of PNS and CNS myelin differs, as discussed in following text. Unlike oligodendrocytes, Schwann cells secrete copious extracellular matrix components and produce a basal lamina "sleeve" that runs the entire length of myelinated axons. Schwann cell and fibroblast-derived collagens prevent normal wear-and-tear compression damage. Schwann cells also respond vigorously to injury, in common with astrocytes but unlike oligodendrocytes. Schwann cell growth factor secretion, debris removal by Schwann cells after injury,

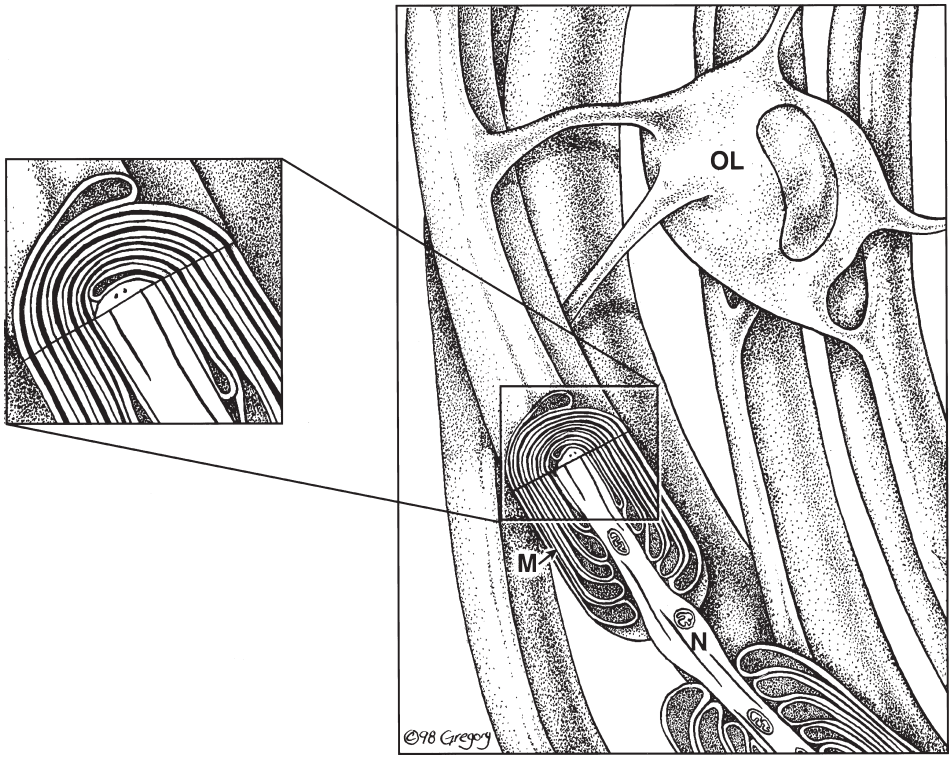

FIGURE 1.6 An oligodendrocyte (OL) in the central nervous system is depicted myelinating several axon segments. A cutaway view of the myelin sheath is shown (M). Note that the internode of myelin terminates in paranodal loops that flank the node of Ranvier (N). (Inset) An enlargement of compact myelin with alternating dark and light electron-dense lines that represent intracellular (major dense lines) and extracellular (intraperiod line) plasma membrane appositions, respectively.

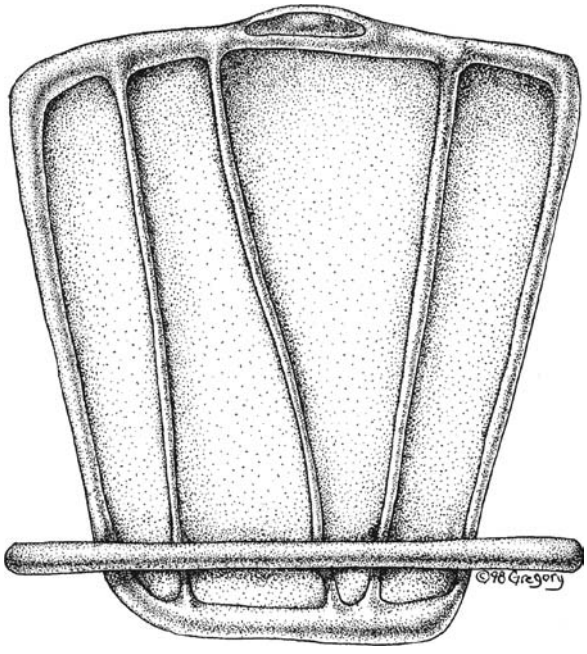

FIGURE 1.7 An "unrolled" Schwann cell in the PNS is illustrated in relation to the single axon segment that it myelinates. The broad stippled region is compact myelin surrounded by cytoplasmic channels that remain open even after compact myelin has formed, allowing an exchange of materials among the myelin sheath, the Schwann cell cytoplasm, and perhaps the axon as well.

and the axonal guidance function of the basal lamina are responsible for the exceptional regenerative capacity of the PNS compared with the CNS.

The major integral membrane protein of peripheral nerve myelin is protein zero (P0), a member of a very large family of proteins termed the *immunoglobulin gene superfamily.* This protein makes up about 80% of the protein complement of PNS myelin. Interactions between the extracellular domains of P0 molecules expressed on one layer of the myelin sheath with those of the apposing layer yield a characteristic regular periodicity that can be seen by thin-section electron microscopy (Fig. 1.5). This zone, called the *intraperiod line,* represents the extracellular apposition of the myelin bilayer as it wraps around itself. On the other side of the bilayer, the cytoplasmic side, the highly charged P0 cytoplasmic domain probably functions to neutralize the negative charges on the polar head groups of the phospholipids that make up the plasma membrane itself, allowing the membranes of the myelin sheath to come into close apposition with one another. In electron microscopy, this cytoplasmic apposition is a bit darker than the intraperiod line and is termed the *major dense line.* In peripheral nerves, although other molecules are

present in small quantities in compact myelin and may have important functions, compaction (i.e., the close apposition of membrane surfaces without intervening cytoplasm) is accomplished solely by P0–P0 interactions at both extracellular and intracellular (cytoplasmic) surfaces.

Curiously, P0 is present in the CNS of lower vertebrates such as sharks and bony fish, but in terrestrial vertebrates (reptiles, birds, and mammals), P0 is limited to the PNS. CNS myelin compaction in these higher organisms is subserved by proteolipid protein (PLP) and its alternate splice form, DM-20. These two proteins are generated from the same gene, both span the plasma membrane four times, and they differ only in that PLP has a small, positively charged segment exposed on the cytoplasmic surface. Why did PLP–DM-20 replace P0 in CNS myelin? Manipulation of PLP and P0 content of CNS myelin established an axonotrophic function for PLP in CNS myelin. Removal of PLP from rodent CNS myelin altered the periodicity of compact myelin and produced a late-onset axonal degeneration (Griffiths *et al.*, 1998). Replacing PLP with P0 in rodent CNS myelin stabilized compact myelin but enhanced the axonal degeneration (Yin *et al.*, 2006). These and other observations in primary demyelination and inherited myelin diseases have established axonal degeneration as the major cause of permanent disability in diseases such as multiple sclerosis.

Myelin membranes also contain a number of other proteins such as the myelin basic protein, which is a major CNS myelin component, and PMP-22, a protein that involved in a form of peripheral nerve disease. A large number of naturally occurring gene mutations can affect the proteins specific to the myelin sheath and cause neurological disease. In animals, these mutations have been named according to the phenotype that is produced: the shiverer mouse, the shaking pup, the rumpshaker mouse, the jimpy mouse, the myelin-deficient rat, the quaking mouse, and so forth. Many of these mutations are well characterized and have provided valuable insights into the role of individual proteins in myelin formation and axonal survival.

Astrocytes Play Important Roles in CNS Homeostasis

As the name suggests, astrocytes are star-shaped, process-bearing cells distributed throughout the central nervous system. They constitute from 20 to 50% of the volume of most brain areas. Astrocytes come in many shapes and forms. The two main forms, protoplasmic and fibrous astrocytes, predominate in gray and white matter, respectively (Fig. 1.8). Embryonically, astrocytes develop from radial glial cells, which transversely compartmentalize the neural tube. Radial glial cells serve as scaffolding for the migration of neurons and play a critical role in defining the cytoarchitecture of the CNS (Fig. 1.9). As the CNS matures, radial glia retract their processes and serve as progenitors of astrocytes. However, some specialized astrocytes of a radial nature are still found in the adult cerebellum and the retina and are known as *Bergmann glial cells* and *Müller cells,* respectively.

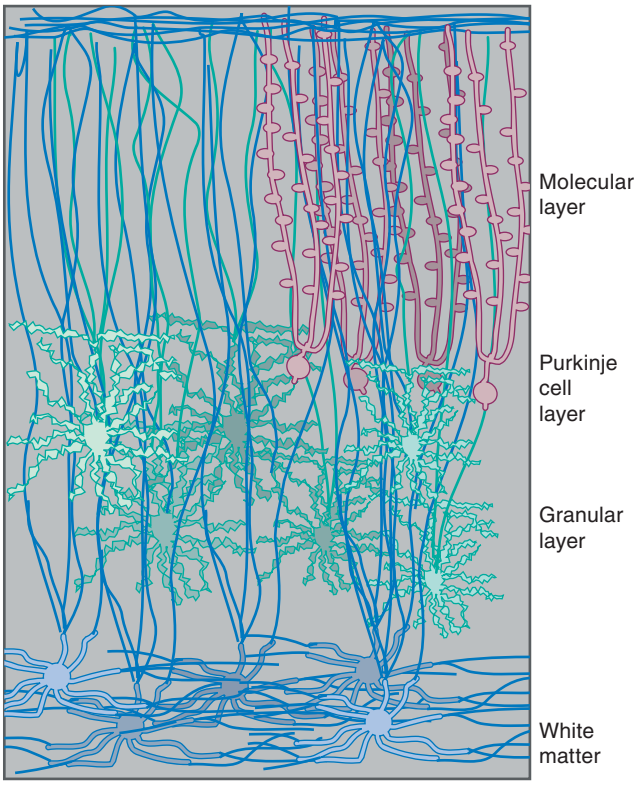

FIGURE 1.8 The arrangement of astrocytes in human cerebellar cortex. Bergmann glial cells are in red, protoplasmic astrocytes are in green, and fibrous astrocytes are in blue.

Astrocytes "fence in" neurons and oligodendrocytes. Astrocytes achieve this isolation of the brain parenchyma by extending long processes projecting to the pia mater and the ependyma to form the glia limitans, by covering the surface of capillaries and by making a cuff around the nodes of Ranvier. They also ensheath synapses and dendrites and project processes to cell somas (Fig. 1.10). Astrocytes are connected to each other by gap junctions, forming a syncytium that allows ions and small molecules to diffuse across the brain parenchyma. Astrocytes have in common unique cytological and immunological properties that make them easy to identify, including their star shape, the glial end feet on capillaries, and a

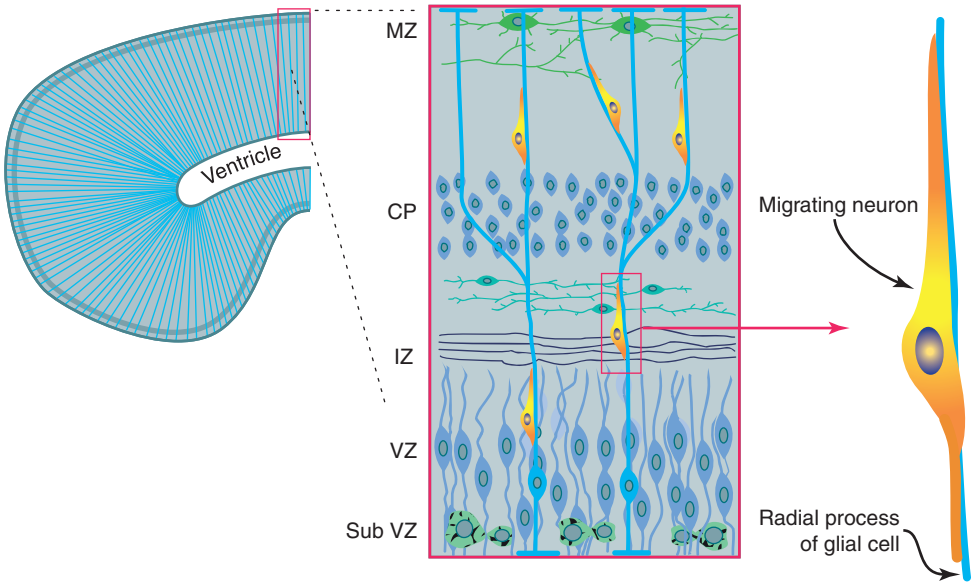

FIGURE 1.9 Radial glia perform support and guidance functions for migrating neurons. In early development, radial glia span the thickness of the expanding brain parenchyma. (Inset) Defined layers of the neural tube from the ventricular to the outer surface: VZ, ventricular zone; IZ, intermediate zone; CP, cortical plate; MZ, marginal zone. The radial process of the glial cell is indicated in blue, and a single attached migrating neuron is depicted at the right.

unique population of large bundles of intermediate filaments. These filaments are composed of an astroglial-specific protein commonly referred to as *glial fibrillary acidic protein* (GFAP). S-100, a calcium-binding protein, and glutamine synthetase are also astrocyte markers. Ultrastructurally, gap junctions (connexins), desmosomes, glycogen granules, and membrane orthogonal arrays are distinct features used by morphologists to identify astrocytic cellular processes in the complex cytoarchitecture of the nervous system.

For a long time, astrocytes were thought to physically form the blood–brain barrier (considered later in this chapter), which prevents the entry of cells and diffusion of molecules into the CNS. In fact, astrocytes are indeed the blood–brain barrier in lower species. However, in higher species, astrocytes are responsible for inducing and maintaining the tight junctions in endothelial cells that effectively form the barrier. Astrocytes also take part in angiogenesis, which may be important in the development and repair of the CNS. However, their role in this important process is still poorly understood.

Astrocytes Have a Wide Range of Functions

There is strong evidence for the role of radial glia and astrocytes in the migration and guidance of neurons in early development. Astrocytes are a major source of extracellular matrix proteins and adhesion molecules in the CNS; examples are nerve cell–nerve cell adhesion molecule (N-CAM), laminin, fibronectin, cytotactin, and the J-1 family members janusin and tenascin. These molecules participate not only in the migration of neurons but also in the formation of neuronal aggregates, so-called nuclei, as well as networks.

Astrocytes produce, *in vivo* and *in vitro*, a very large number of growth factors. These factors act singly or in combination to selectively regulate the morphology, proliferation, differentiation, survival, or all four, of distinct neuronal subpopulations. Most of the growth factors also act in a specific manner on the development and functions of astrocytes and oligodendrocytes. The production of growth factors and cytokines by astrocytes and their responsiveness to these factors is a major mechanism underlying the developmental function and regenerative capacity of the CNS. During neurotransmission, neurotransmitters and ions are released at high concentration in the synaptic cleft. The rapid removal of these substances is important so that they do not interfere with future synaptic activity. The presence of astrocyte processes around synapses positions them well to regulate neurotransmitter uptake and inactivation (Kettenman and Ransom, 1995). These possibilities are consistent with the presence in astrocytes of transport systems for many neurotransmitters. For instance, glutamate reuptake is performed mostly by astrocytes, which convert glutamate into glutamine

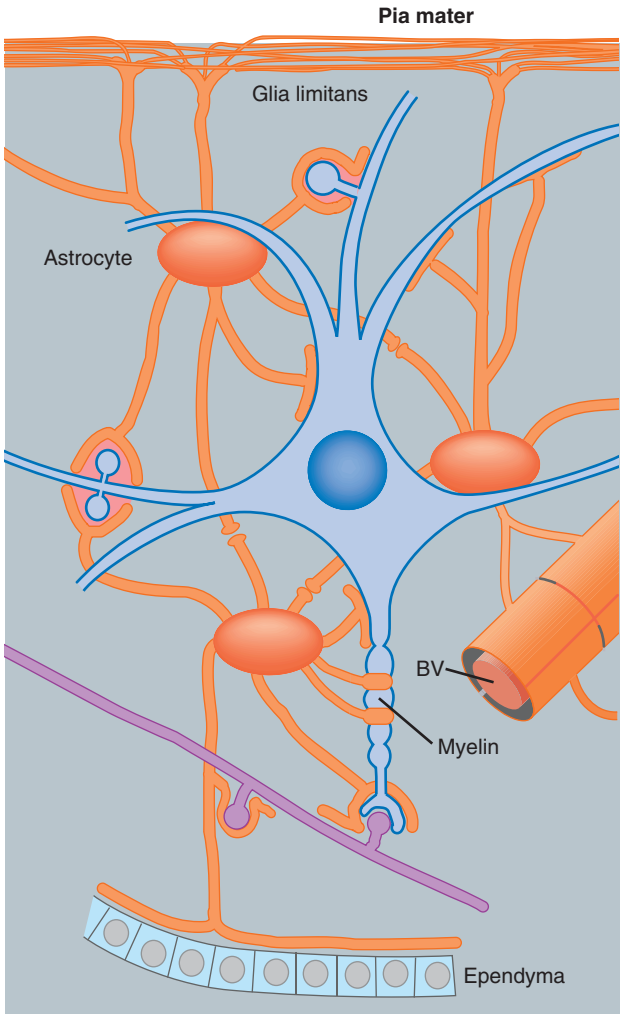

FIGURE 1.10 Astrocytes (in orange) are depicted *in situ* in schematic relationship with other cell types with which they are known to interact. Astrocytes send processes that surround neurons and synapses, blood vessels, and the region of the node of Ranvier and extend to the ependyma, as well as to the pia mater, where they form the glial limitans.

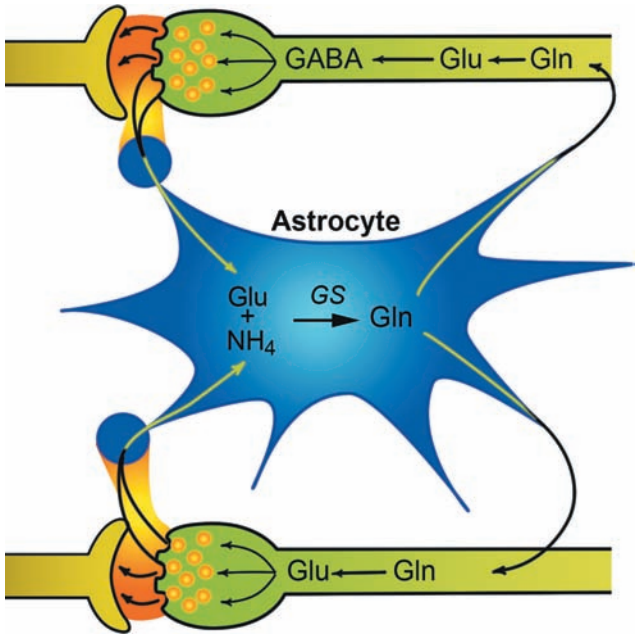

FIGURE 1.11 The glutamate–glutamine cycle is an example of a complex mechanism that involves an active coupling of neurotransmitter metabolism between neurons and astrocytes. The systems of exchange of glutamine, glutamate, GABA, and ammonia between neurons and astrocytes are highly integrated. The postulated detoxification of ammonia and the inactivation of glutamate and GABA by astrocytes are consistent with the exclusive localization of glutamine synthetase in the astroglial compartment.

and then release it into the extracellular space. Glutamine is taken up by neurons, which use it to generate glutamate and γ-aminobutyric acid, potent excitatory and inhibitory neurotransmitters, respectively (Fig. 1.11). Astrocytes contain ion channels for K^+, Na^+, Cl^-, HCO_3, and Ca^{2+}, as well as displaying a wide range of neurotransmitter receptors. K^+ ions released from neurons during neurotransmission are soaked up by astrocytes and moved away from the area through astrocyte gap junctions. This is known as "spatial buffering." Astrocytes play a major role in detoxification of the CNS by sequestering metals and a variety of neuroactive substances of endogenous and xenobiotic origin.

In response to stimuli, intracellular calcium waves are generated in astrocytes. Propagation of the Ca^{2+} wave can be visually observed as it moves across the cell soma and from astrocyte to astrocyte. The generation of Ca^{2+} waves from cell to cell is thought to be mediated by second messengers, diffusing through gap junctions. Because they develop postnatally in rodents, gap junctions may not play an important role in development. In the adult brain, gap junctions are present in all astrocytes. Some gap junctions have also been detected between astrocytes and neurons. Thus, they may participate, along with astroglial neurotransmitter receptors, in the coupling of astrocyte and neuron physiology.

In a variety of CNS disorders—neurotoxicity, viral infections, neurodegenerative disorders, HIV, AIDS, dementia, multiple sclerosis, inflammation, and trauma—astrocytes react by becoming hypertrophic and, in a few cases, hyperplastic. A rapid and huge upregulation of GFAP expression and filament formation is associated with astrogliosis. The formation of reactive astrocytes can spread very far from the site of origin. For instance, a localized trauma can recruit astrocytes from as far as the contralateral side, suggesting the existence of soluble factors in the

mediation process. Tumor necrosis factor (TNF) and ciliary neurotrophic factors (CNTF) have been identified as key factors in astrogliosis.

Microglia Are Mediators of Immune Responses in Nervous Tissue

The brain has traditionally been considered an "immunologically privileged site," mainly because the blood–brain barrier normally restricts the access of immune cells from the blood. However, it is now known that immunological reactions do take place in the central nervous system, particularly during cerebral inflammation. Microglial cells have been termed the *tissue macrophages* of the CNS, and they function as the resident representatives of the immune system in the brain. A rapidly expanding literature describes microglia as major players in CNS development and in the pathogenesis of CNS disease.

The first description of microglial cells can be traced to Franz Nissl (1899), who used the term *rod cell* to describe a population of glial cells that reacted to brain pathology. He postulated that rod-cell function was similar to that of leukocytes in other organs. Cajal described microglia as part of his "third element" of the CNS—cells that he considered to be of mesodermal origin and distinct from neurons and astrocytes (Ramón y Cajal, 1913).

Del Rio-Hortega (1932) distinguished this third element into microglia and oligodendrocytes. He used silver impregnation methods to visualize the ramified appearance of microglia in the adult brain, and he concluded that ramified microglia could transform into cells that were migratory, ameboid, and phagocytic. Indeed, a hallmark of microglial cells is their ability to become reactive and to respond to pathological challenges in a variety of ways. A fundamental question raised by del Rio-Hortega's studies was the origin of microglial cells. Some questions about this remain even today.

Microglia Have Diverse Functions in Developing and Mature Nervous Tissue

On the basis of current knowledge, it appears that most ramified microglial cells are derived from bone marrow–derived monocytes, which enter the brain parenchyma during early stages of brain development. These cells help phagocytose degenerating cells that undergo programmed cell death as part of normal development. They retain the ability to divide and have the immunophenotypic properties of monocytes and macrophages. In addition to their role in remodeling the CNS during early development, microglia secrete cytokines and growth factors that are important in fiber tract development, gliogenesis, and angiogenesis. They are also the major CNS cells involved in presenting antigens to T lymphocytes. After the early stages of development, ameboid microglia transform into the ramified microglia that persist throughout adulthood (Altman, 1994).

Little is known about microglial function in the healthy adult vertebrate CNS. Microglia constitute a formidable percentage (5–20%) of the total cells in the mouse brain. Microglia are found in all regions of the brain, and there are more in gray than in white matter. The neocortex and hippocampus have more microglia than regions like the brainstem or cerebellum. Species variations have also been noted, as human white matter has three times more microglia than rodent white matter.

Microglia usually have small rod-shaped somas from which numerous processes extend in a rather symmetrical fashion. Processes from different microglia rarely overlap or touch, and specialized contacts between microglia and other cells have not been described in the normal brain. Although each microglial cell occupies its own territory, microglia collectively form a network that covers much of the CNS parenchyma. Because of the numerous processes, microglia present extensive surface membrane to the CNS environment. Regional variation in the number and shape of microglia in the adult brain suggests that local environmental cues can affect microglial distribution and morphology. On the basis of these morphological observations, it is likely that microglia play a role in tissue homeostasis. The nature of this homeostasis remains to be elucidated. It is clear, however, that microglia can respond quickly and dramatically to alterations in the CNS microenvironment.

Microglia Become Activated in Pathological States

"Reactive" microglia can be distinguished from resting microglia by two criteria: (1) change in morphology and (2) upregulation of monocyte–macrophage molecules (Fig. 1.12). Although the two phenomena generally occur together, reactive responses of microglia can be diverse and restricted to subpopulations of cells within a microenvironment. Microglia not only respond to pathological conditions involving immune activation but also become activated in neurodegenerative conditions that are not considered immune mediated. This latter response is indicative of the phagocytic role of microglia. Microglia change their morphology and antigen expression in response to almost any form of CNS injury.

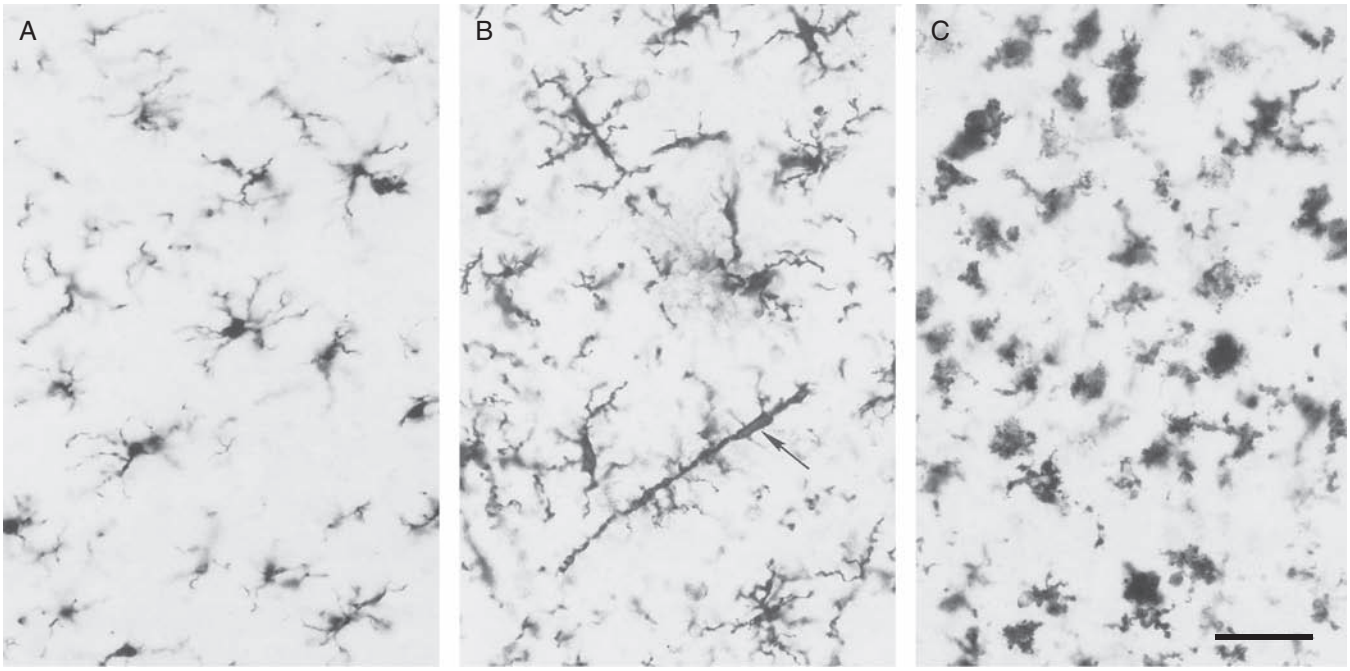

FIGURE 1.12 Activation of microglial cells in a tissue section from human brain. Resting microglia in normal brain (A). Activated microglia in diseased cerebral cortex (B) have thicker processes and larger cell bodies. In regions of frank pathology (C), microglia transform into phagocytic macrophages, which can also develop from circulating monocytes that enter the brain. Arrow in B indicates rod cell. Sections stained with antibody to ferritin. Scale bar = 40 μm.

CEREBRAL VASCULATURE

Blood vessels form an extremely rich network in the central nervous system, particularly in the cerebral cortex and subcortical gray masses, whereas the white matter is less densely vascularized (Fig. 1.13) (Duvernoy *et al*., 1981). There are distinct regional patterns of microvessel distribution in the brain. These patterns are particularly clear in certain subcortical structures that constitute discrete vascular territories and in the cerebral cortex, where regional and laminar patterns are striking. For example, layer IV of the primary visual cortex possesses an extremely rich capillary network in comparison with other layers and adjacent regions (Fig. 1.13). Interestingly, most of the inputs from the visual thalamus terminate in this particular layer. Capillary densities are higher in regions containing large numbers of neurons and where synaptic density is high. Progressive occlusion of a large arterial trunk, as seen in stroke, induces an ischemic injury that may eventually lead to necrosis of the brain tissue. The size of the resulting infarction is determined in part by the worsening of the blood circulation through the cerebral microvessels. Occlusion of a large arterial trunk results in rapid swelling of the capillary endothelium and surrounding astrocytes, which may reduce the capillary lumen to about one-third of its normal diameter, preventing red blood cell circulation and oxygen delivery to the tissue. The severity of these changes subsequently determines the time course of neuronal necrosis, as well as the possible recovery of the surrounding tissue and the neurological outcome of the patient. In addition, the presence of multiple microinfarcts caused by occlusive lesions of small cerebral arterioles may lead to a progressively dementing illness, referred to as *vascular dementia,* affecting elderly humans.

The Blood–Brain Barrier Maintains the Intracerebral Milieu

Capillaries of the central nervous system form a protective barrier that restricts the exchange of solutes between blood and brain. This distinct function of brain capillaries is called the *blood–brain barrier* (Fig. 1.14) (Bradbury, 1979). Capillaries of the retina have similar properties and are termed the *blood–retina barrier.* It is thought that the blood–brain and blood–retina barriers function to maintain a constant

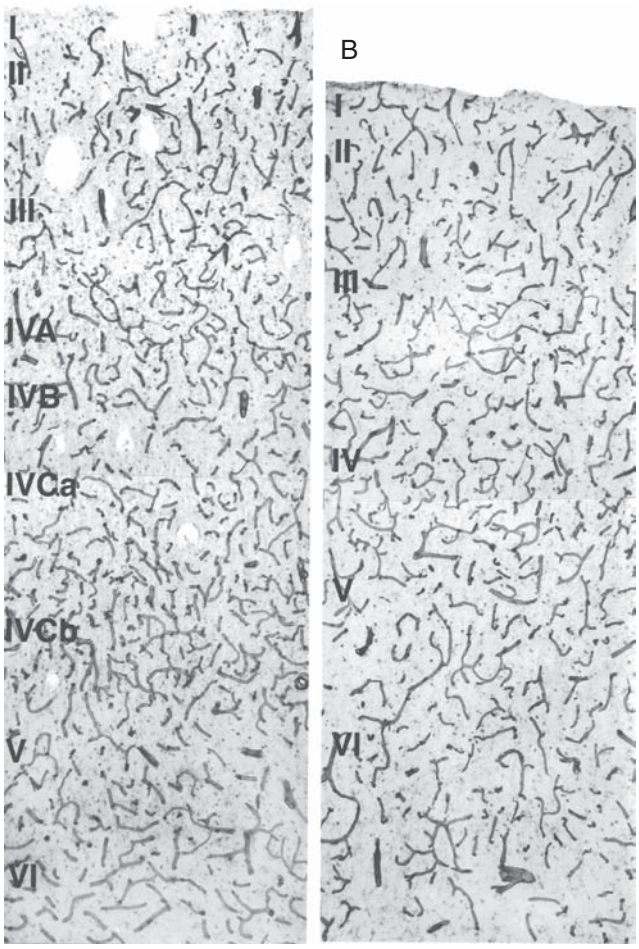

FIGURE 1.13 Microvasculature of the human neocortex. (A) The primary visual cortex (area 17). Note the presence of segments of deep penetrating arteries that have a larger diameter than the microvessels and run from the pial surface to the deep cortical layers, as well as the high density of microvessels in the middle layer (layers IVCa and IVCb). (B) The prefrontal cortex (area 9). Cortical layers are indicated by Roman numerals. Microvessels are stained using an antibody against heparan sulfate proteoglycan core protein, a component of the extracellular matrix.

intracerebral milieu, so that neuronal signaling can occur without interference from substances leaking in from the bloodstream. This function is important because of the nature of intercellular communication in the CNS, which includes chemical signals across intercellular spaces. Without a blood–brain barrier, circulating factors in the blood, such as certain hormones, which can also act as neurotransmitters, would interfere with synaptic communication. When the blood–brain barrier is disrupted, edema fluid accumulates in the brain. Increased permeability of the blood–brain barrier plays a central role in many neuropathological conditions, including multiple sclerosis, AIDS, and childhood lead poisoning, and may also play a role in Alzheimer disease. The cerebral capillary wall is composed of an endothelial cell surrounded by a very thin (about 30 nm) basement membrane or basal lamina. End feet of perivascular astrocytes are apposed against this continuous basal lamina. Around the capillary lies a virtual perivascular space occupied by another cell type, the pericyte, which surrounds the capillary walls. The endothelial cell forms a thin monolayer around the capillary lumen, and a single endothelial cell can completely surround the lumen of the capillary (Fig. 1.14). A fundamental difference between brain endothelial cells and those of the systemic circulation is the presence in the brain of interendothelial tight junctions, also known as *zonula occludens.* In the systemic circulation, the interendothelial space serves as a diffusion pathway that offers little resistance to most blood solutes entering the surrounding tissues. In contrast, blood–brain barrier tight junctions effectively restrict the intercellular route of solute transfer. The blood–brain barrier interendothelial junctions are not static seals; rather they are a series of active gates that can allow certain small molecules to penetrate. One such molecule is the lithium ion, used in the control of manic depression.

Another characteristic of endothelial cells of the brain is their low transcytotic activity. Brain endothelium, therefore, is by this index not very permeable. It is of interest that certain regions of the brain, such as the area postrema and periventricular organs, lack a blood–brain barrier. In these regions, the perivascular space is in direct contact with the nervous tissue, and endothelial cells are fenestrated and show many pinocytotic vesicles. In these brain regions, neurons are known to secrete hormones and other factors that require rapid and uninhibited access to the systemic circulation.

Because of the high metabolic requirements of the brain, blood–brain barrier endothelial cells must have transport mechanisms for the specific nutrients needed for proper brain function. One such mechanism is the glucose transporter isoform 1 (GLUT1), which is expressed asymmetrically on the surface of blood–brain barrier endothelial cells. In Alzheimer disease, the expression of GLUT1 on brain endothelial cells is reduced. This reduction may be due to a lower metabolic requirement of the brain after extensive neuronal loss. Other specific transport mechanisms on the cerebral endothelium include the large neutral amino acid carrier-mediated system that transports, among other amino acids, L-3,4-dihydroxyphenylalanine (L-dopa), used as a therapeutic agent in Parkinson disease. Also on the surface of blood–brain barrier endothelial cells are transferrin receptors that allow the transport of iron into specific areas of the brain. The amount of iron that is transported into the

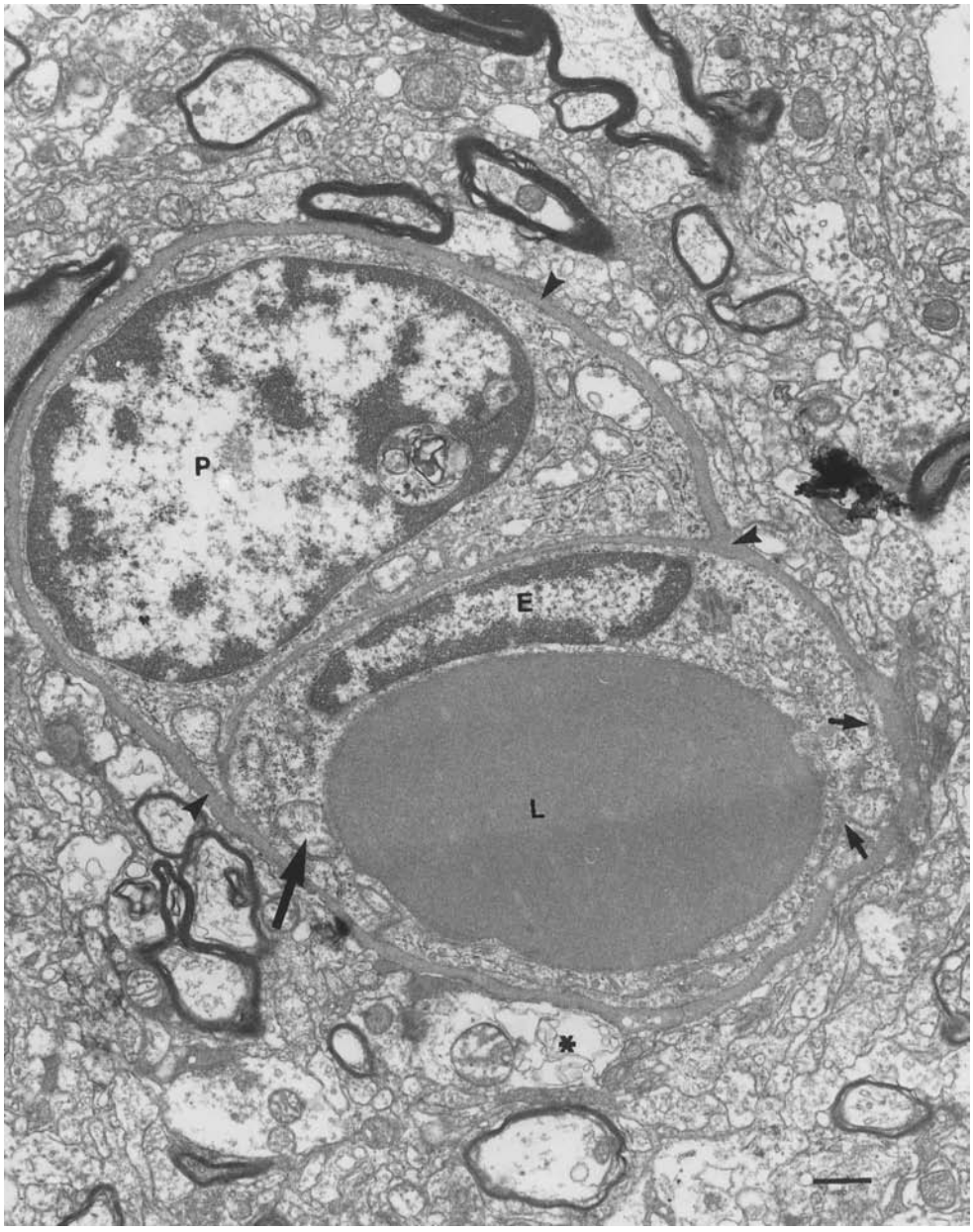

FIGURE 1.14 Human cerebral capillary obtained at biopsy. Blood–brain barrier (BBB) capillaries are characterized by the paucity of transcytotic vesicles in endothelial cells (E), a high mitochondrial content (large arrow), and the formation of tight junctions (small arrows) between endothelial cells that restrict the transport of solutes through the interendothelial space. The capillary endothelium is encased within a basement membrane (arrowheads), which also houses pericytes (P). Outside the basement membrane are astrocyte foot processes (asterisk), which may be responsible for the induction of BBB characteristics on the endothelial cells. L, lumen of the capillary. Scale bar = 1 μm. From Claudio *et al.* (1995).

various areas of the brain appears to depend on the concentration of transferrin receptors on the surface of endothelial cells of that region. Thus, the transport of specific nutrients into the brain is regulated during physiological and pathological conditions by blood–brain barrier transport proteins distributed according to the regional and metabolic requirements of brain tissue.

In general, disruption of the blood–brain barrier causes perivascular or vasogenic edema, which is the accumulation of fluids from the blood around the blood vessels of the brain. This is one of the main features of multiple sclerosis. In multiple sclerosis, inflammatory cells, primarily T cells and macrophages, invade the brain by migrating through the blood–brain barrier and attack cerebral elements as if these

elements were foreign antigens. It has been observed by many investigators that the degree of edema accumulation causes the neurological symptoms experienced by people suffering from multiple sclerosis.

Studying the regulation of blood–brain barrier permeability is important for several reasons. Therapeutic treatments for neurological disease need to be able to cross the barrier. Attempts to design drug delivery systems that take therapeutic drugs directly into the brain have been made by using chemically engineered carrier molecules that take advantage of receptors such as that for transferrin, which normally transports iron into the brain. Development of an *in vitro* test system of the blood–brain barrier is of importance in the creation of new neurotropic drugs that are targeted to the brain.

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