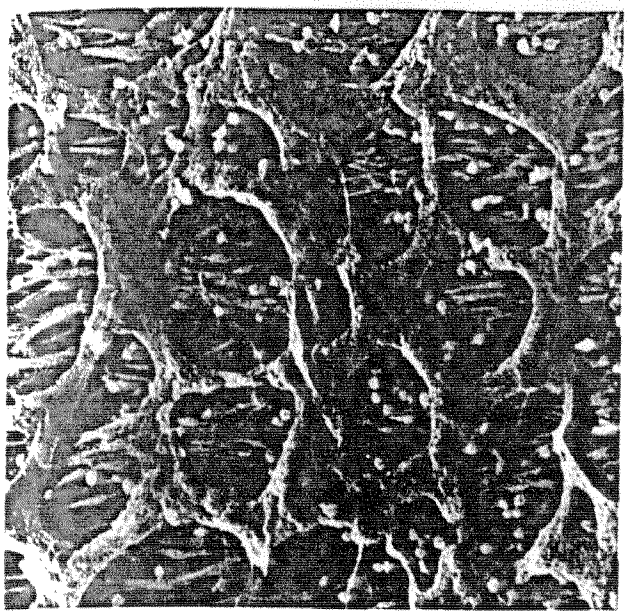

FIGURE 3.12 Scanning electron micrograph of the myenteric plexus in the intestine. Note the dense axonal bundles and synaptic boutons (pseudocolored green) and the network of large multipolar neurons with relatively short extensions contacting neighboring cells (pseudocolored red).

The neurochemistry of the enteric system is extremely complex and still poorly understood. A large number of classical neurotransmitters, such as acetylcholine, GABA, and noradrenaline, have been identified in enteric nerve fibers and ganglionic neurons. In addition, enteric neurons in both the myenteric and the submucosal plexuses contain a variety of neuropeptides. Neurons in the submucosal plexus are enriched in somatostatin, substance P, and vasoactive intestinal peptide but do not seem to contain Leu-enkephalin, which is observed in the myenteric nerve cells. It is not clear, however, whether these neuropeptides are the principal transmitters of subclasses of enteric neurons or are colocalized compounds that act as local neuromodulators.[33]

Summary

The neuron is one of the more highly specialized cell types and is the critical cellular element in the brain. All neurological processes are dependent on complex cell–cell interactions between single neurons and/or groups of related neurons. Neurons can be described according to their size, shape, neurochemical characteristics, location, and connectivity.

A neuron's size, shape, and neurochemistry are important determinants of that neuron's particular functional role in the brain. In this respect, there are three general classes of neurons: the inhibitory GABAergic interneurons that make local contacts, the local excitatory spiny stellate cells in the cerebral cortex, and the excitatory glutamatergic efferent neurons, exemplified by the cortical pyramidal neurons. 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. The relevant characteristics may include morphology, location, connectivity, and biochemistry.

Also, 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 a distant 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 that neuron in relation to its neighbors and to place it in the context of a specific neuronal subset, circuit, or function.

THE NEUROGLIA

The term neuroglia, or "nerve glue," was coined in 1859 by Rudolph Virchow, who conceived of the neuroglia as an inactive "connective tissue" holding neurons together in the central nervous system (CNS). The metallic staining techniques developed by Santiago Ramón y Cajal and Pio del Río-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

The more complex the brain, the more interconnections must be formed and maintained. As we will see in depth later, there is a practical limit to how fast an individual bare axon can conduct an action potential. Thus, neurons and their associated processes cannot communicate with each other extremely rapidly

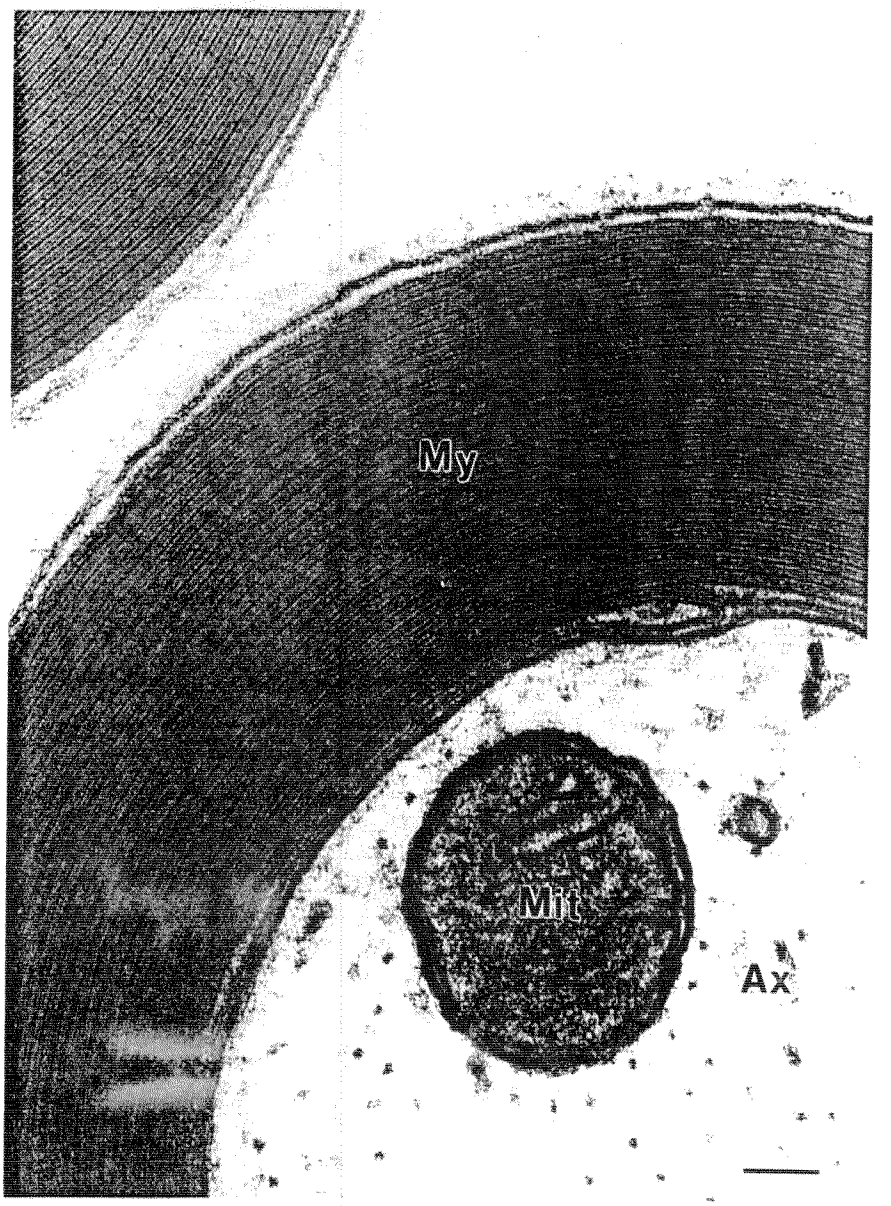

FIGURE 3.13 An electron micrograph of a transverse section through part of a myelinated axon from the sciatic nerve of a rat. The tightly compacted multilayer myelin sheath (My) surrounds and insulates the axon (Ax). Mit, mitochondria. Scale bar = 75 nm.

through the action potential without some help. Organisms have developed two kinds of solutions for enhancing rapid communication between neurons and their effector organs. In invertebrates, the diameters of individual axons that must conduct rapidly are enlarged. In vertebrates, the myelin sheath (Fig. 3.13) has evolved to permit rapid nerve conduction.

Axon enlargement greatly accelerates the rate of conduction of the action potential, which increases with axonal diameter. The net effect, therefore, is that small axons conduct at a much slower rate 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. It conducts the action potential extremely rapidly, and the axon itself mediates an escape reflex, which must be rapid if the animal is to survive. An obvious trade-off in a nervous system with 10^{11} neurons, as in the human brain, is that all axons cannot be as thick as pencil lead, or each human head would be very large indeed.

Thus, along the invertebrate evolutionary line, there is a natural, insurmountable limit—a constraint imposed by axonal size—to increasing the processing capacity of the nervous system beyond a certain point. Vertebrates, however, devised a way to get around this problem through the evolution of the myelin sheath,

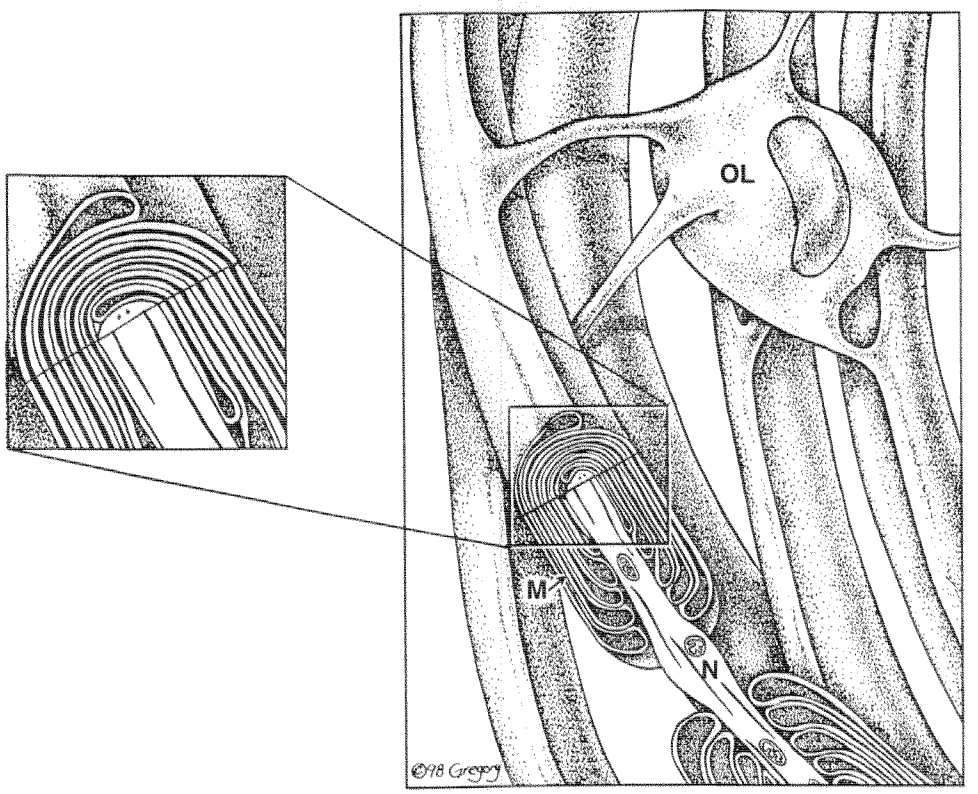

FIGURE 3.14 An oligodendrocyte (OL) in the central nervous system is depicted myelinating several axon segments. A cut away view of the myelin sheath is shown (M). Note that the internode of myelin terminates in paranodal loops that flank the node of Ranvier (N). The inset shows an enlargement of compact myelin with alternating dark and light electron-dense lines that represent the intracellular (major dense lines) and extracellular (intraperiod line) plasma membrane appositions, respectively.

allowing the tremendous evolutionary advantage of increased rapidity of conduction of the nerve impulse along axons with fairly minute diameters.[34] As we know, neurons interact in complex ways with the other cell types that exist within the nervous system. Virtually all axons, for example, are wrapped or ensheathed by cells that subserve what is vaguely termed a "supportive" or "trophic" function. This is true in invertebrate as well as vertebrate nervous systems. Along the vertebrate lineage, however, certain ensheathing cells have become highly specialized to generate vast quantities of plasma membrane that is compacted to form the myelin sheath, which supports rapid nerve conduction.

Not all axons in central or peripheral nervous systems are myelinated, and one of the puzzles is to determine why some are selected for myelination and others remain unmyelinated. It is believed that early in the nervous system development of an organism, signals relayed between the axon and the myelinating cell determine whether the "myelination program" is triggered in that cell. These signals have not yet been identified.

In the central nervous system, the myelin sheath (Fig. 3.14) is elaborated by **oligodendrocytes,** nonneuronal glial cells that, during brain development, send out a few cytoplasmic processes that engage adjacent axons, some of which go on to become myelinated.[35] Myelin itself consists of a single sheet of oligodendrocyte plasma membrane, which is wrapped tightly around an axonal segment.[36] Each myelinated segment of an axon is termed an **internode** because, at the end of each segment, there is a bare portion of the axon, the **node of Ranvier,** that is flanked by another internode. 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.[37] The jumping of the action potential from node to node along a given axon is called **saltatory conduction** (from the Latin *saltare*, to dance). The same Latin root can be found in the words sauté, somersault, and assault.

The evolution of a system in which a single oligo-

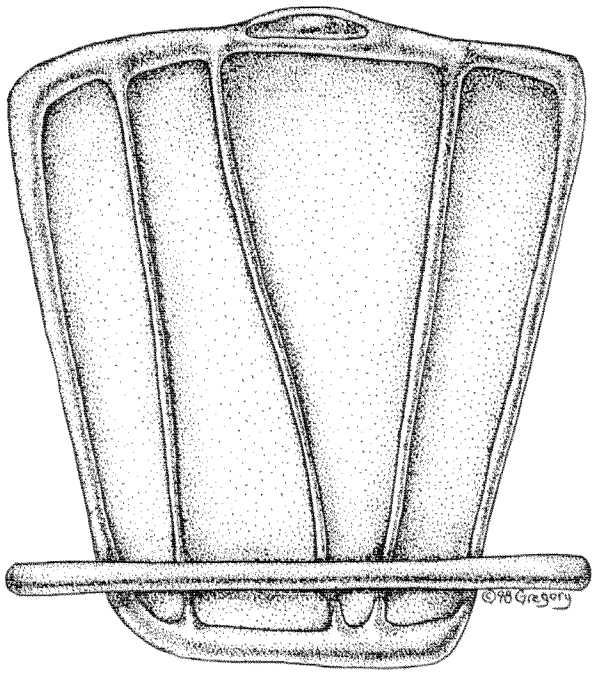

FIGURE 3.15 An "unrolled" Schwann cell in the PNS is illustrated in relation to the single axon segment that it myelinates. The broad stippled region is compact myelin surrounded by cytoplasmic channels that remain open even after compact myelin has formed, allowing exchange of materials among the myelin sheath, the Schwann cell cytoplasm, and perhaps the axon as well.

dendrocyte cell body is responsible for the construction and maintenance of several myelin sheaths (Fig. 3.14) and the removal of the cytoplasm between each turn of the myelin lamellae so that only the thinnest layer of plasma membrane is left have resulted in saving a huge amount of space. Brain volume is thus reserved for further expansion of neuronal populations.[38]

Conservation of space in the peripheral nervous system does not seem to have presented such a pressing problem. Myelin in the PNS is generated by **Schwann cells** (Fig. 3.15), each of which wraps only a single axonal segment. The biochemical composition of the myelin derived in the CNS and the composition of that derived in the PNS differ somewhat, although there are common proteins found in each nervous system subdivision. Myelin has a high lipid-to-protein ratio, and the lipids are specialized. The myelin sheath has become an excellent model system for studying the generation or formation of specialized plasma membrane and membrane adhesion, because each layer of the multilayered myelin sheath must adhere to the adjacent layers. This adhesion is largely accomplished by protein–protein interactions, which have been best studied in the PNS.

The major integral membrane protein of peripheral nerve myelin is Protein zero (P_0), a member of a very large family of proteins termed the immunoglobulin gene superfamily. These proteins have in common recognition or adhesion functions or both and, although the primary amino acid sequences differ among the members of this family, all members are related to one another by certain common structural motifs. Members of the immunoglobulin (Ig) gene superfamily have one or more Ig-like domains that contain cysteines placed about 100 amino acids or so apart. These cysteines are linked to one another by disulfide bridges. Most of these Ig domains are displayed on the extracellular surfaces of cells, where they can act as ligands or receptors. Protein zero is relatively simple in primary structure, consisting of a single Ig-like domain, a transmembrane segment, and a highly charged (basic) cytoplasmic domain.[39] This protein makes up about 80% of the protein complement of peripheral nerve myelin. The interactions between the extracellular domains of P_0 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. 3.13). 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 P_0 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 P_0–P_0 interactions at both extracellular and intracellular (cytoplasmic) surfaces.[40]

Protein zero is a "perfect" plasma-membrane compactor,[41] allowing the close apposition of adjacent bilayers such that the space between them effectively prevents the passage of anything but small ions and water along the compacted bilayer surfaces. It is in effect a "streamlined" Ig superfamily molecule that probably arose *de novo* with the development of the myelin sheath.[38] Curiously, P_0 is not present in all myelin sheaths in the central nervous system of every species—an evolutionary paradox that has attracted much attention. In fish, P_0 is present in both the central and the peripheral nervous systems, where it performs its compaction function, as the major integral membrane protein. However, in terrestrial vertebrates (rep-

tiles, birds, and mammals), P_0 is limited to the PNS, and so is not found in the central nervous system. Instead, the compaction function is probably subserved by totally unrelated molecules, the **DM-20** protein and its insertion isoform, the myelin **proteolipid protein** (PLP).[42] These two proteins are generated from the same gene and are identical to each other with the exception that the proteolipid protein has, in addition, a positively charged segment exposed on the cytoplasmic aspect of the bilayer.[43,44] Both PLP and DM20 are extremely hydrophobic and traverse the bilayer four times, and so have hydrophilic segments exposed on both cytoplasmic and extracellular surfaces of the bilayer. In this respect, the topology of these molecules is very similar to that of connexins and other polypeptides (see Chapters 9 and 11) that are known to function in channel or pore formation.

A large number of naturally occurring neurological mutations can affect the proteins specific to the myelin sheath. 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 have been well characterized, and their analyses have allowed us to begin to understand at a molecular level what the proteins affected by each mutation actually do in the formation and maintenance of the myelin sheath (see Box 3.2).[45]

The first neurologic mutation that was studied in this respect was the *shiverer* mouse, in which the gene that encodes a major set of peripheral membrane proteins, the myelin basic proteins (MBPs), is damaged. Normally, these proteins serve to seal the cytoplasmic aspects of the myelin bilayer, possibly by charge neutralization similar in function to the cytoplasmic tail of P_0. When the gene is functionally deleted, as in the *shiverer*, the cytoplasmic aspects fail to appose and do not fuse, and a mouse that exhibits tremors and convulsions ("shivers") as it walks is produced. This is a naturally occurring mutation and was the first neurological mutation whose effects were cured by gene transfer. This was accomplished by the introduction of an intact MBP gene into the *shiverer* genome.[46] The *shiverer* mutation is an autosomal recessive, but even a single allele of the gene (i.e., the heterozygote MBP^+/MBP^-) produces sufficient myelin to phenotypically at least "cure" the *shiverer* of its overtly abnormal behavior. These heterozygotes myelinate to somewhat less extent than normal, but the fact that the *shiverer* phenotype is eliminated in the heterozygote even though the number of myelin sheaths around each axon is reduced indicates that there is a built-in safety factor in the normal situation.

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. 3.16). 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. 3.17). 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.

Astrocytes "fence in" neurons and oligodendrocytes.[47] The 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. 3.18). 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 unique population of large bundles of intermediate filaments. These filaments are composed of an astroglial-specific protein commonly referred to as GFAP (glial fibrillary acidic protein). S-100, a calcium-binding protein, and glutamine synthetase also are 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, the astrocytes are responsible for inducing and maintaining the tight junctions in endothelial cells that effectively form the barrier.[48,49] Astrocytes also take part in angiogenesis, which may be important in the development and repair of the

BOX 3.2

INHERITED PERIPHERAL NEUROPATHIES

The peripheral myelin protein-22 (PMP22) is a very hydrophobic glycoprotein and is highly expressed in compact PNS myelin. It has been mapped to the previously defined *Tr* locus on mouse chromosome 11. Comparison of marker genes on mouse chromosome 11 and human chromosome 17 revealed that PMP22 was also a candidate gene for the most common form of autosomal-dominant demyelinating hereditary peripheral neuropathy in humans, Charcot–Marie–Tooth disease type 1A (CMT1A). Indeed, the entire PMP22 gene is contained within a 1.5-Mb intrachromosomal duplication on chromosome 17p11.2, a genetic abnormality that had been linked to CMT1A by human molecular genetics. Consistent with these results, PMP22 is overexpressed in CMT1A patients who carry the characteristic duplication. The crucial role of PMP22 in the etiology of CMT1A was confirmed by generating transgenic mice and rats with increased PMP22 gene dosage, which resulted in severe PNS myelin deficits.

CMT is one of the more frequent hereditary diseases of the nervous system, with an overall prevalence of approximately 1 in 4000, and the CMT1A duplication accounts for around 70% of all cases. Why is this chromosomal abnormality so common? Detailed analysis of the CMT1A locus suggests that the duplication is due to crossing over involving repetitive sequences that flank the monomeric region. If correct, such a mechanism should also generate an allele carrying the reciprocal deletion of the same region. Indeed, the expected deletion is associated with the relatively mild recurrent neuropathy hereditary neuropathy with liability to pressure palsy (HNPP). Thus, overexpression and underexpression of the myelin protein PMP22 are associated with myelin deficiencies in distinct human diseases. Although one might speculate from these data that correct stochiometry of myelin protein expression is crucial for a myelinating Schwann cell, the exact disease mechanism remains to be clarified.

Interestingly, the finding that a myelin protein was responsible for CMT1A led to the discovery that two other components of PNS myelin are mutated in rare forms of CMT1. The adhesion protein P_0, which is largely responsible for PNS myelin compaction, is affected in CMT1B, and an X-linked form of CMT (CMTX) has been linked to mutations in the gap junction protein connexin-32. In contrast to PMP22 and P_0, connexin-32 is located in uncompacted lamellae of PNS myelin, where it is thought to facilitate the exchange of small molecules via reflexive gap junctions between adaxonal and abaxonal aspects of myelinating Schwann cells.

Finally, there is a striking correlation between the role of PMP22 in the PNS and that of PLP/DM20 in the CNS with respect to biology and involvement in disease; both genes can be affected by various genetic mechanisms, including gene duplication and gene deletion. However, despite our vast knowledge derived from human molecular genetics, the molecular functions of both proteins are largely unknown. Given the recent findings that PMP22 and PLP/DM20 are members of extended gene families and may be involved in the control of cell proliferation and cell death, these proteins may have broader functions than simply being stabilizing building blocks of compact myelin.

In summary, the combination of basic and clinical sciences has led to substantial progress in our current understanding of common hereditary neuropathies. Using clinical, genetic, and cell biology approaches in concert, we will continue to learn more about disease mechanisms involved in neuropathies to the benefit of the clinic as much as to our understanding of myelin biology.

Ueli Suter

CNS.[50] 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, or 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

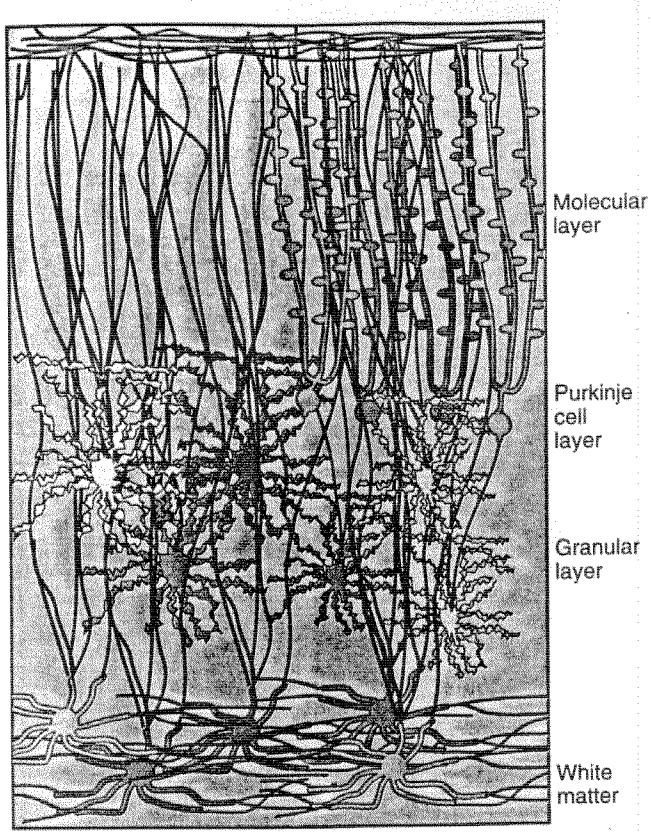

FIGURE 3.16 The arrangement of astrocytes in human cerebellar cortex. The Bergmann glial cells are in red, the protoplasmic astrocytes are in green, and the fibrous astrocytes are in blue.

production of growth factors and cytokines by astrocytes and their responsiveness to these factors are 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.[51] 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 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. 3.19). 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." The 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. The 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 (see Chapter 11). 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 α (TNFα) 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 (see below) 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. These cells are perhaps the least understood of the CNS cells. Although the function of microglia in the normal adult CNS remains to be clarified, a rapidly expanding literature describes microglia as major players in CNS development and in the pathogenesis of CNS disease. The notion that the CNS is an immune-privileged organ is no longer valid. A hallmark of microglia cells is their ability to become reactive and to respond to pathological challenges in a variety of ways.

The first description of microglia cells can be traced

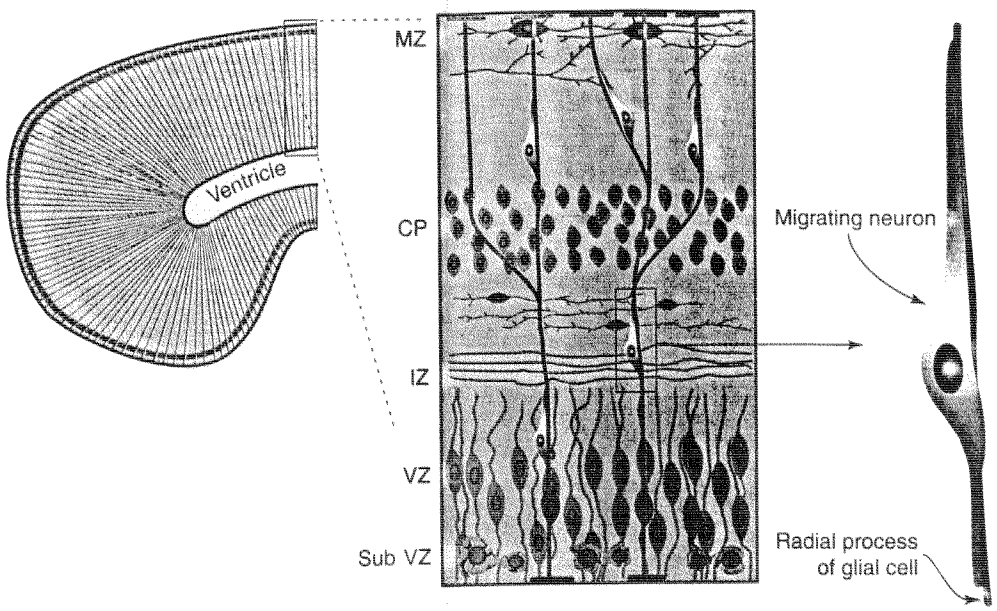

FIGURE 3.17 Radial glia perform support and guidance functions for migrating neurons. In early development, the radial glia span the thickness of the expanding brain parenchyma. The inset shows defined layers of the neural tube from the ventricular to the outer surface: VZ, ventricular zone; IZ, intermediate 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.

to Franz Nissl,[52] who used the term "rod cell" (Stäbchenzellen) 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.[53]

Del Río-Hortega[54] divided Cajal's third element into oligodendrocytes and microglia, two cell types with different morphology, function, and origin. 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. A fundamental question raised by del Río-Hortega's studies was the origin of microglial cells. Although he provided evidence that microglia originated from cells that migrate into the brain from the pial surface, he also raised the possibility that microglia originate from blood "mononuclears." Controversy over the lineage of microglia still exists today.

Microglia Have Diverse Functions in Developing and Mature Nervous Tissue

Four different sources of microglia have been proposed[55]: (1) bone-marrow derived monocytes, (2) mesodermal pial elements, (3) neural epidermal cells, and (4) capillary-associated pericytes. On the basis of current knowledge, it appears that most ramified microglia 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 may secrete cytokines or growth factors that are important in fiber-tract development, gliogenesis, and angiogenesis. After the early stages of development, ameboid microglia cells transform into the ramified microglia cells that persist throughout adulthood.[56]

Little is known about microglial function in the normal 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 phylogenetically newer regions of the CNS (cerebral cortex, hippocampus) have more microglia than do older regions (brainstem, cerebellum).[57] Species variations also have been noted, as human white matter has three times more microglia than does 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

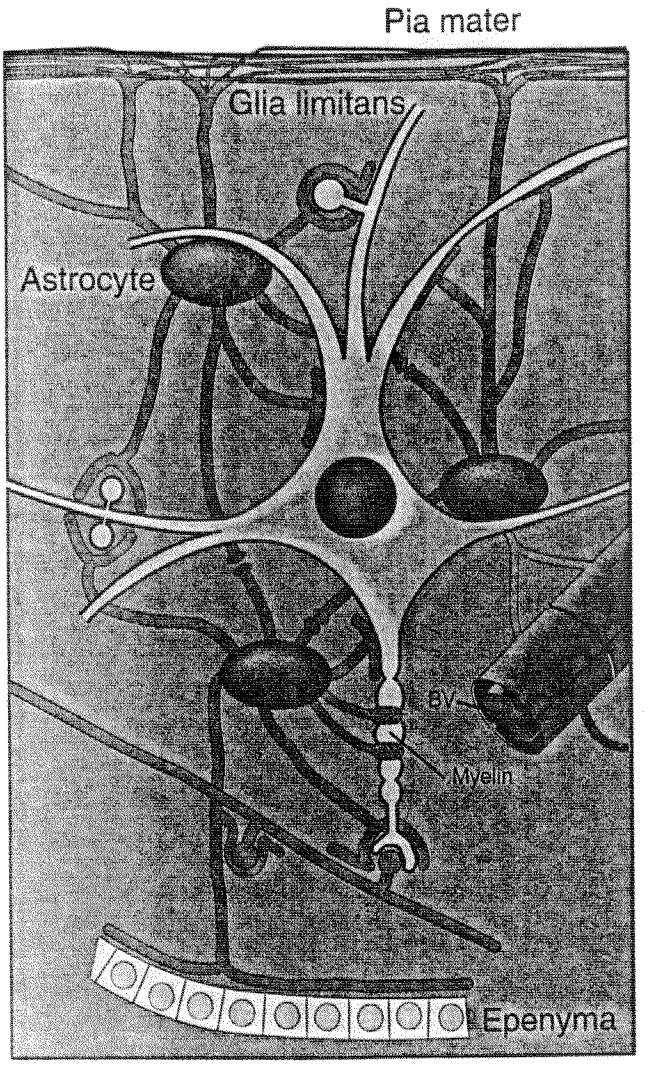

FIGURE 3.18 Astrocytes (in orange) are depicted *in situ* in schematic relationship with other cell types with which they are known to interact. Astrocytes send processes that surround neurons and synapses, blood vessels, and the region of the node of Ranvier and extend to the ependyma as well as to the pia mater, where they form the glial limitans.

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. 3.20). 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.[58] 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.

Summary

Neuroglia are a set of cell types that together subserve supportive and trophic roles critical for the normal functioning of nervous tissue. Certain glial cells—the myelinating cells, for example—have clearly shaped nervous system evolution and development in that they evolved to facilitate rapid conduction of the action potential along small-caliber axons. The coordinated integrative functions of the vertebrate brain therefore depend on a normal complement of myelinated axons. Astrocytes and microglial cells also have major and extremely important functions in development and in tissue injury, but these roles are not as well understood as yet. In pathological states of all kinds (autoimmune, toxic insult, trauma), these cells react to contain and limit tissue damage. They also contribute in a major way to repair mechanisms.

THE 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. 3.21).[59] The vascular bed is supplied by perforating arteries that arise from a relatively small number of large, peripheral arterial trunks. The main trunks give off smaller cerebral arteries whose branches penetrate the subarachnoidal space, where they divide into many subbranches before penetrating the brain tissue. Within the cerebral gray matter, these penetrating arteries divide into a large number of small arterioles that eventually form an extremely rich, highly anastomotic capillary bed. At the other end of the capillary network are veinules, draining into larger cerebral veins, which are

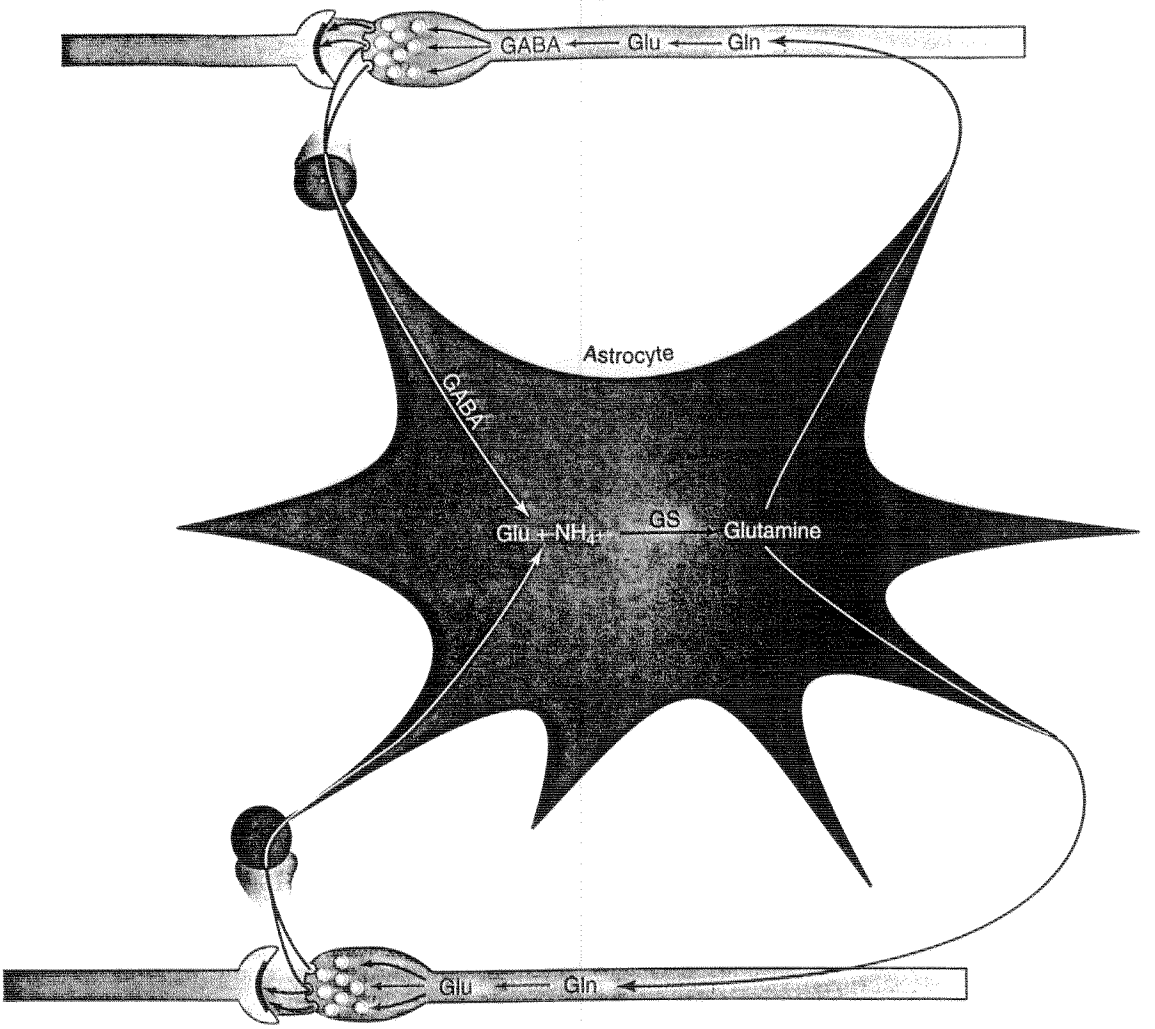

FIGURE 3.19 The glutamate–glutamine cycle is an example of a complex mechanism that involves an active coupling of neurotransmitter metabolism between neurons and astrocytes. The systems of exchange of glutamine, glutamate, GABA, and ammonia between neurons and astrocytes are highly integrated. The postulated detoxification of ammonia and inactivation of glutamate and GABA by astrocytes are consistent with the exclusive localization of glutamine synthetase in the astroglial compartment.

the tributaries of large venous sinuses responsible for returning blood to the general circulation. The brain vascular system has no end arteries, and there is a relatively free circulation throughout the central nervous system. There are, however, 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. 3.21). Interestingly, most of the inputs from the visual thalamus terminate in this particular layer. Whether similar functional correlations may be derived from comparable vascular patterns in other brain regions remains to be determined. Nonetheless, capillary densities are higher in regions containing large numbers of neurons and where synaptic density is high. Penetrating arteries and draining veins have well-defined, tree-shaped branching patterns.[59] With regard to cortical vessels, some arteries divide in the upper cortical layers, whereas others penetrate to the lower layers before dividing. The branches of penetrating arteries define local vascular fields of approximately similar size and shape around the vessel of origin, which cover the entire cortical mantle in a continuous network.

Pathologic factors that affect the patency of brain microvessels may result in the development of an ischemic injury localized to a variable amount of tissue, depending on the size and location of the affected arter-

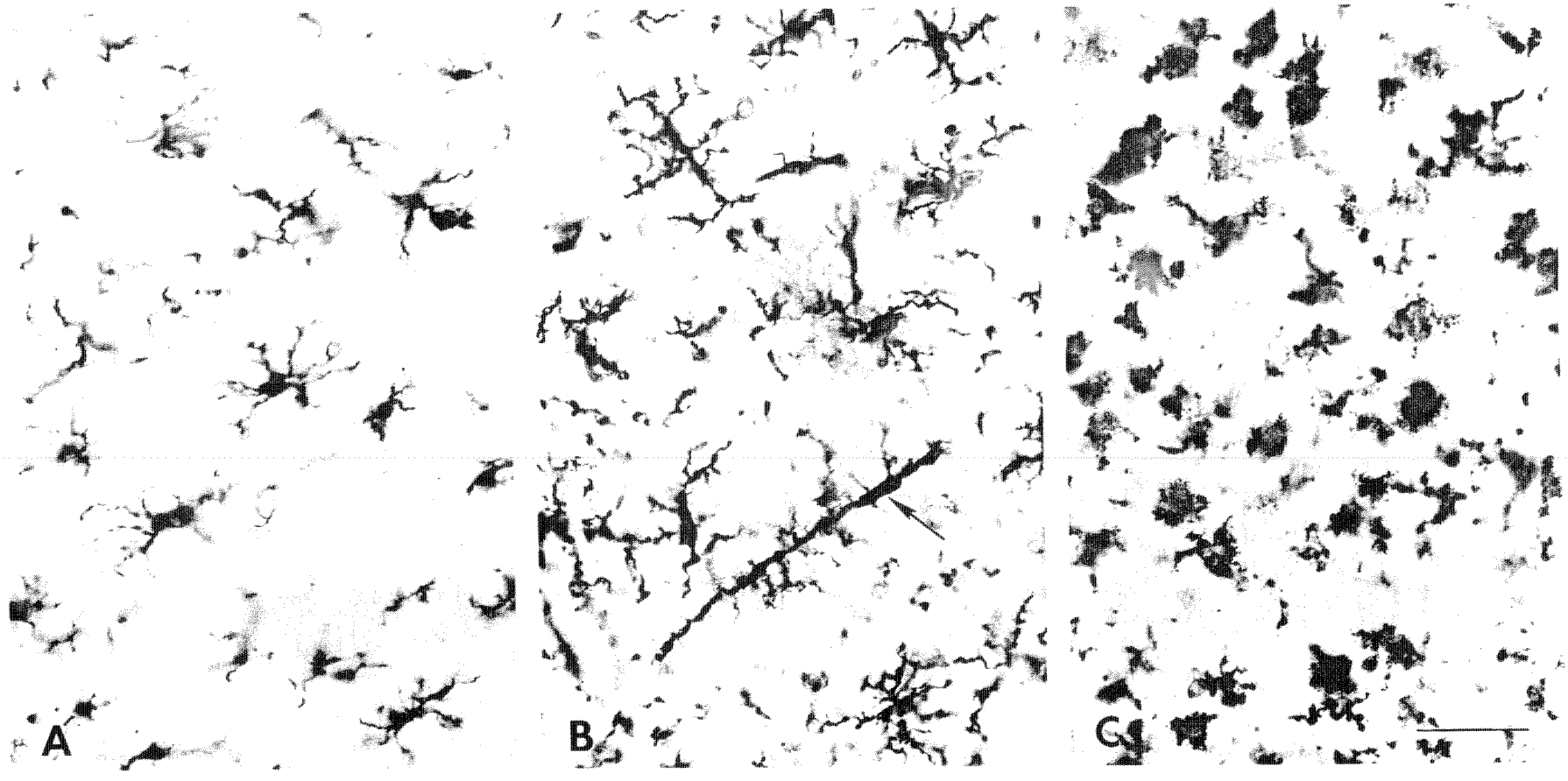

FIGURE 3.20 Activation of microglial cells in a tissue section from human brain. Resting microglia in normal brain (A). Activated microglia in diseased cerebral cortex (B) have thicker processes and larger cell bodies. In regions of frank pathology (C) microglia transform into phagocytic macrophages, 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.

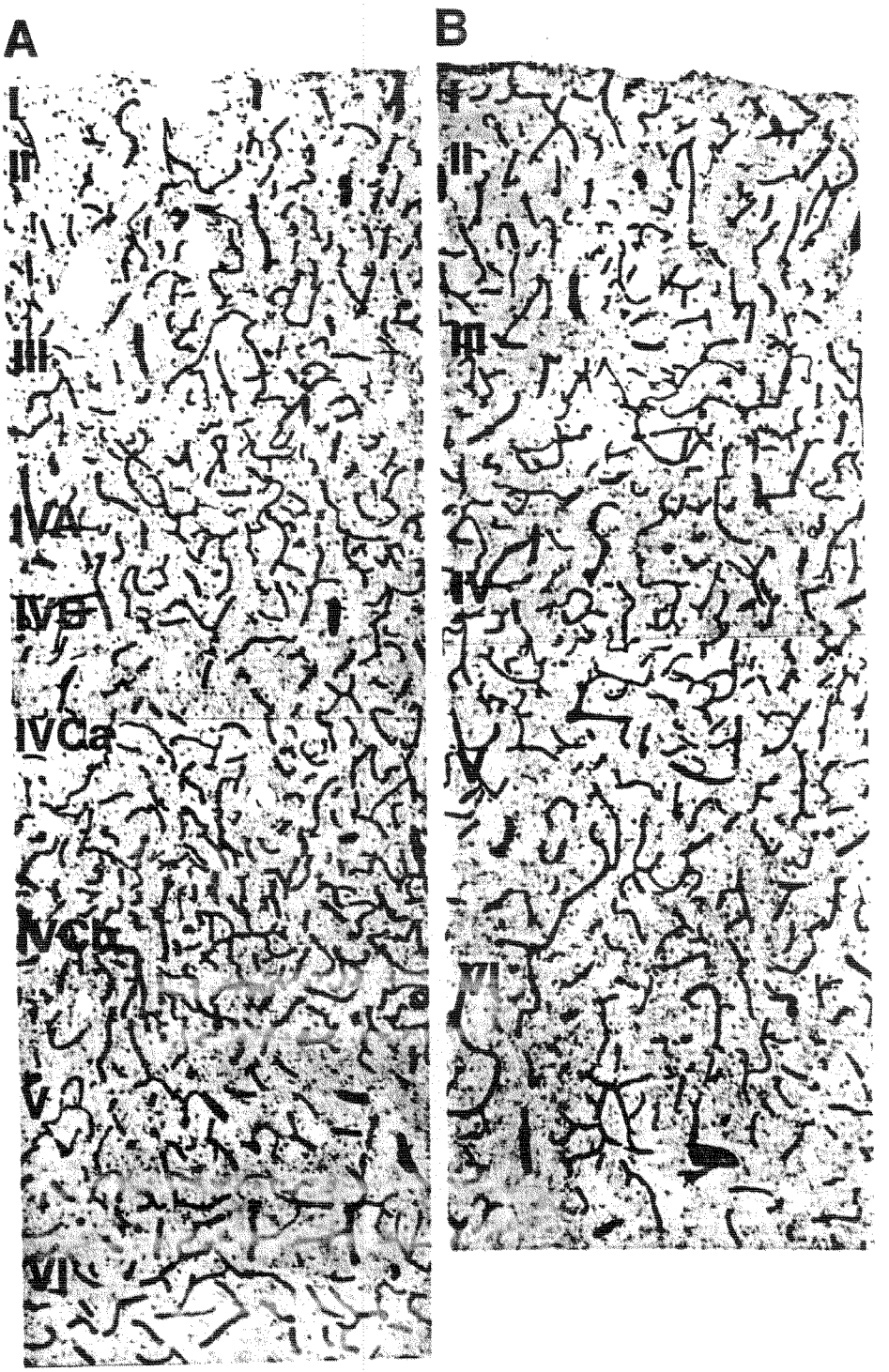

FIGURE 3.21 Microvasculature of the human neocortex. (A) The primary visual cortex (area 17). Note the presence of segments of deep penetrating arteries that have a larger diameter than the microvessels and run from the pial surface to the deep cortical layers, as well as the high density of microvessels in the middle layer (layers IVB and IVCb). (B) The prefrontal cortex (area 9). Cortical layers are indicated by Roman numerals. The microvessels are stained using an antibody against heparan sulfate proteoglycan core protein, a component of the extracellular matrix.

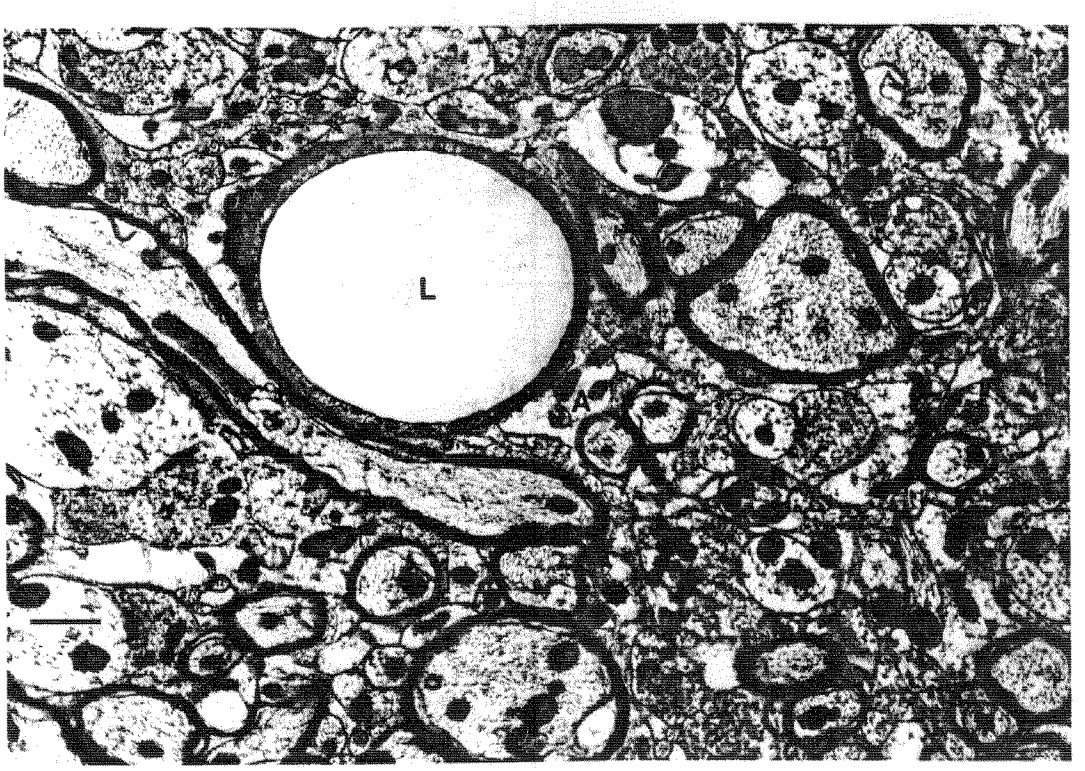

FIGURE 3.22 Electron micrograph of a blood–brain barrier capillary. Endothelial cells joined by tight junctions form continuous capillaries with no fenestrations and restrict the passage of solutes between blood and brain. Pericytes (P) are present within the basement membrane (arrowheads) of these capillaries, serve to control vascular tone, and can also be phagocytic in the brain. Astrocyte foot processes (A) surround the basement membrane and are responsible for the induction of BBB properties on endothelial cells. Bar = 2 μm.

ies. For instance, the 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. In fact, 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

The 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 (Figs. 3.22 and 3.23).[60] The 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 intracerebral milieu, critical for neuronal function. 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, leading to neurological impairments. 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.[61,62] The blood–brain barrier is composed of three cellular components—endothelial cells, pericytes, and astrocytes—and one noncellular component—the basement membrane. These components interact with each other to

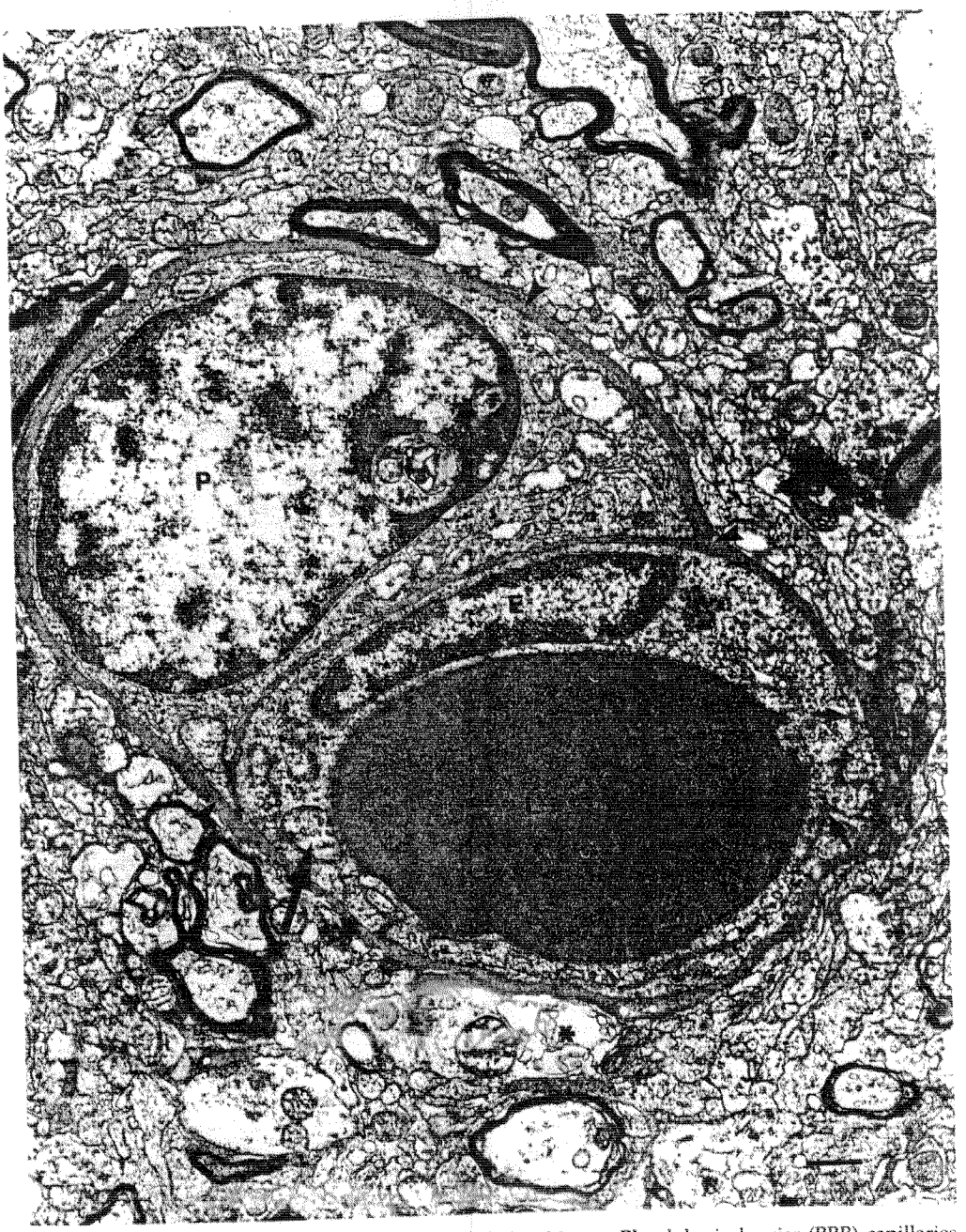

FIGURE 3.23 Human cerebral capillary obtained at biopsy. Blood–brain barrier (BBB) capillaries are characterized by the paucity of transcytotic vesicles in endothelial cells (E), a high mitochondrial content (large arrow), and the formation of tight junctions (small arrows) between endothelial cells 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 induction of BBB characteristics on the endothelial cells. L, lumen of the capillary. Bar = 1 μm. From Claudio *et al.*[61]

produce a highly selective and dynamic barrier system.

In general, the cerebral capillaries are comparable to those seen in other tissues.[1] The capillary wall is composed of an endothelial cell surrounded by a very thin (about 30 nm) basal lamina, similar to that seen in capillaries in peripheral tissues. 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. 3.23). The

cytoplasm is rich in actin filaments and contains an extensive Golgi apparatus and high numbers of mitochondria.

A fundamental difference between brain endothelial cells and those of the systemic circulation is the presence in brain of interendothelial tight junctions, also known as **zonula occludens.**[1] 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. This is illustrated by the paucity of transcytotic vesicles in the cytoplasm, compared with endothelial cells of the systemic circulation. The frequency of transcytotic vesicles tends to increase with increasing permeability of an endothelium. 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 the 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 glucose transporter isoform 1 (GLUT-1), which is asymmetrically expressed on the surface of blood–brain barrier endothelial cells. During Alzheimer disease, the expression of GLUT-1 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 transport of iron into specific areas of the brain. The amount of iron that is transported into the 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.

The Basement Membrane, Pericytes, and Astrocytes Are Also Blood–Brain Barrier Components

The basement membranes are not true membranes but are extracellular matrices with a width varying from 20 to 300 nm, composed mainly of collagens, glycoproteins, laminin, proteoglycans, and other proteins. In the cerebral microvasculature, the basement membrane surrounds the endothelium and the adjacent pericytes. The nature of the basement membrane surrounding the blood vessels varies with the type of vasculature and during pathological conditions. The composition and structure of the basement membrane affect the permeability of the vessel. For example, *in vitro* studies of endothelial monolayers in which the underlying matrix was composed primarily of collagen type I restricted passage of albumin, suggesting a role for the basement membrane in blood–brain barrier permeability.[63] Replacement of collagen type I with fibronectin resulted in increased permeability to albumin. This finding correlates with the lack of fibronectin around blood–brain barrier vessels.

Pericytes (Fig. 3.22) are present within the basement membrane of all vessels in the body, including the nervous system.[1] The functions of pericytes include the secretion of basement membrane components, the regulation of revascularization and repair, and the regulation of vascular tone in capillaries. In the central nervous system, pericytes may act as part of the vascular barrier by increased phagocytosis after blood–brain barrier injury.

The processes of astrocytes form a sheath around blood–brain barrier microvessels. These processes are termed astrocyte foot processes or end feet, and they assist in inducing the blood–brain barrier properties of brain endothelia.[64,65]

Disruption of the Blood–Brain Barrier Causes Edema

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.[61] It has been observed by many investigators that it is the degree of edema accumulation that 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. This could be especially useful in the treatment of neurodegenerative diseases and the AIDS dementia complex.

Summary

The hallmark of the brain vasculature is the blood–brain barrier, a multicomponent gateway between brain tissue and other organ systems. We can consider the blood–brain barrier the gateway to the brain because it restricts access to macromolecules present in the blood. It also serves as the interface between the immune and the nervous systems, acting as the "meeting site" for communication between the two.

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