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Cell Biology of the Nervous System

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OUTLINE

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Diverse cell types comprising the nervous system interact to create a functioning brain

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Although neurons share common elements with other cells, each component has specialized features
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The Synapse is a specialized junctional complex by which axons and dendrites emerging from different neurons intercommunicate

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OVERVIEW

More than 100 years since the idea of a nervous system made of distinct cell populations gained acceptance, we are beginning to understand how these different cells are produced and how they relate to each other. More importantly, many aspects of the molecular and biochemical basis for these relationships, i.e., the basic neurochemistry of the nervous system, have been defined. The molecular specialization of cells in the nervous system defines function and interactions. In this chapter, we begin by considering the cells and microanatomy of the nervous system as a way of providing a foundation for detailed considerations of the cellular, molecular, and biochemical properties of the nervous system.

CELLULAR NEUROSCIENCE IS THE FOUNDATION OF MODERN NEUROSCIENCE

Diverse cell types comprising the nervous system interact to create a functioning brain

Modern neurobiology emerged at the turn of the last century out of the demonstration that the brain represented a complex network of distinct cells interacting in precise ways, rather than a syncytium (Ramon y Cajal, 1967). Cells of the nervous system exhibit an extraordinary diversity in shape, size and number of unique interactions with other cells. In the first fifty years, the focus was on identifying and describing these cells, establishing a rich database of information on the anatomy of the nervous system. As our knowledge of neuroanatomy and histology deepened, scientists began to appreciate the specialized

biochemistry of the brain and neurochemistry emerged as a distinct field of investigation. Diverse cell types are organized into assemblies and patterns such that specialized components are integrated into a physiology of the whole organ (Fig. 1-1). Brain development and the origins and differentiation of these diverse cell types are discussed in Chapters 28, 30, and 31.

NEURONS: COMMON ELEMENTS AND DIVERSITY

The classic image of a neuron includes a perikaryon, multiple dendrites and an axon

The stereotypical image of a neuron is that of a stellate cell body, the perikaryon or soma, with broad dendrites emerging from one pole and a single axon emerging from the opposite pole (Fig. 1-2). Although this image is near universal in textbooks, neuroanatomists have long recognized the remarkable diversity of neuronal sizes and morphologies (Ramon y Cajal, 1909). The neuron is the most polymorphic cell in the body and defies formal classification on the basis of shape, location, function, fine structure or transmitter substance. Despite this diversity, homologous neurons are often easily recognized across considerable phylogenetic distance. Thus, a Purkinje cell from lamprey shares many recognizable features with those of humans (Bullock et al., 1977). Before the work of Deiters and Ramón y Cajal more than 100 years ago, neurons and neuroglia were believed to form syncytia, with no intervening membranes. The demonstration of neurons and glia as discrete cells proved to be the foundation of modern neuroscience.

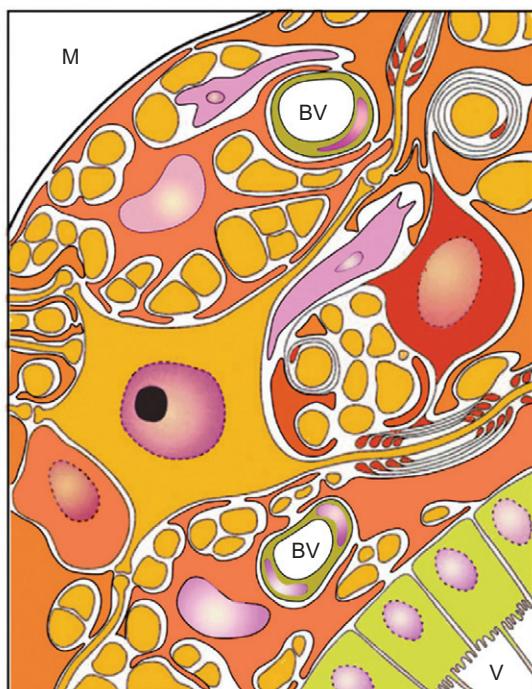
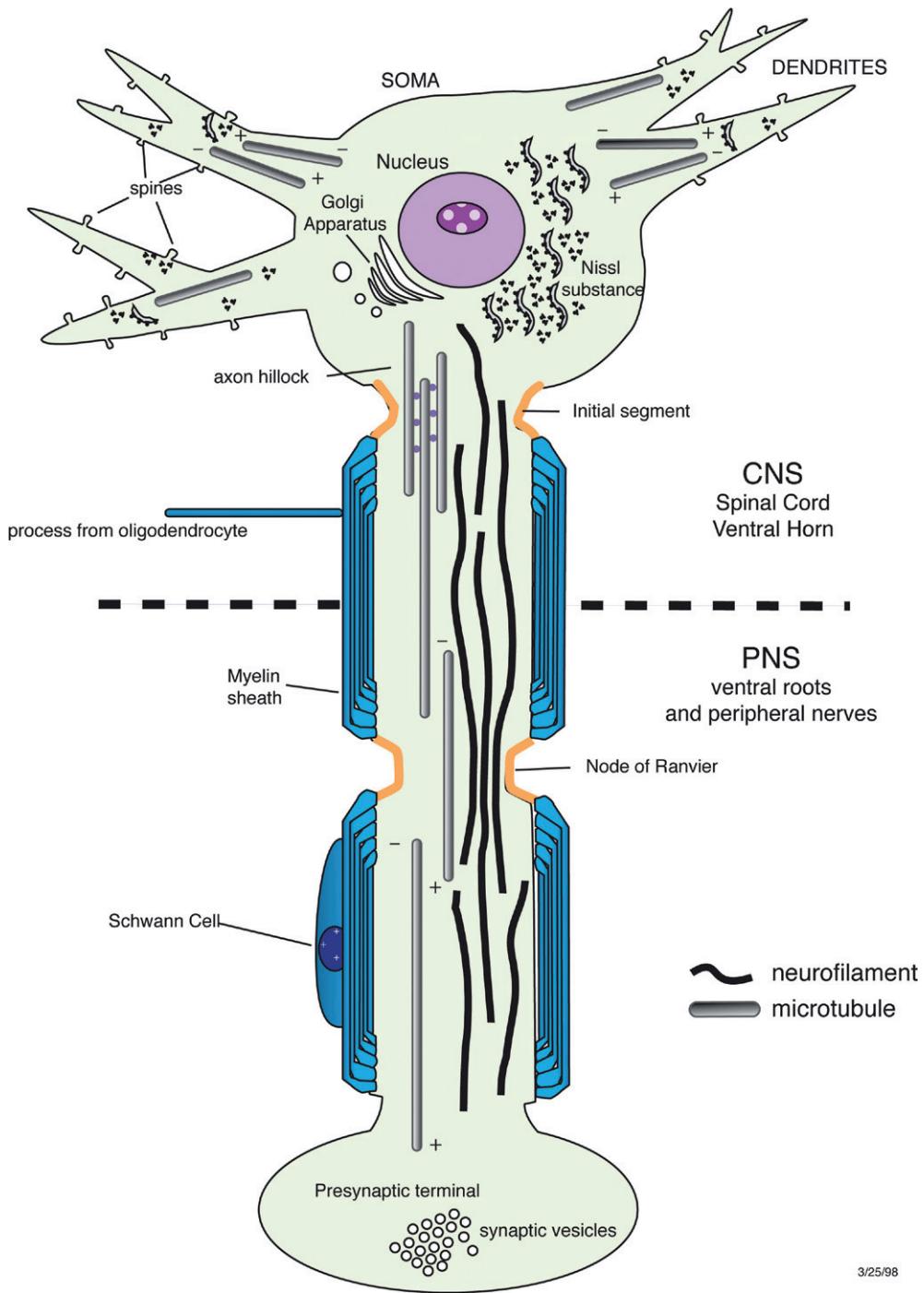


FIGURE 1.1 The major components of the CNS and their interrelationships. Microglia are depicted in light purple. In this simplified schema, the CNS extends from its meningeal surface (M), through the basal lamina (solid black line) overlying the subpial astrocyte layer of the CNS parenchyma, and across the CNS parenchyma proper (containing neurons and glia) and subependymal astrocytes to ciliated ependymal cells lining the ventricular space (V). Note how the astrocyte also invests blood vessels (BV), neurons and cell processes. The pia-astroglia (glia limitans) provides the barrier between the exterior (dura and blood vessels) and the CNS parenchyma. One neuron is seen (center), with synaptic contacts on its soma and dendrites. Its axon emerges to the right and is myelinated by an oligodendrocyte (above). Other axons are shown in transverse section, some of which are myelinated. The ventricles (V) and the subarachnoid space of the meninges (M) contain cerebrospinal fluid.



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FIGURE 1.2 Diagram of a motor neuron with myelinated axon. The traditional view of a neuron includes a perikaryon, multiple dendrites and an axon. The perikaryon contains the machinery for transcription and translation of proteins as well as their processing. These proteins must be targeted to somal, dendritic or axonal domains as appropriate. The dendrites typically contain postsynaptic specializations, particularly on spines. Some dendritic proteins are locally translated and processed in response to activity. Axonal domains typically contain presynaptic terminals and machinery for release of neurotransmitters. Large axons are myelinated by glia in both the CNS and PNS. The action potential is initiated at the initial segment and saltatory conduction is possible because of concentration of sodium channels at the nodes of Ranvier. Neuronal processes are maintained through the presence of cytoskeletal structures: neurofilaments (axons) and microtubules (axons and dendrites). However, there may be no neurons with this simple structure.

Nerve cell shapes and sizes range from the small, globular cerebellar granule cells, with a perikaryal diameter of approximately 6–8 µm, to the distinctive, pear-shaped Purkinje cells and star-shaped anterior horn cells, with perikarya that may reach diameters of 60–80 µm in humans. Perikaryal size is generally a poor index of total cell volume or surface area. The dendritic and axonal processes of a neuron may represent the overwhelming bulk of neuronal volume and surface, approaching 95–99% of the total cell volume in some cases.

Both axons and dendrites typically exhibit extensive branching with a cell type-specific pattern (see Fig. 1-3, for example). The extent of the branching displayed by the dendrites is a useful index of their functional importance. Dendritic trees represent the expression of the receptive fields, and large fields can receive inputs from multiple origins. A cell with a less-developed dendritic ramification, such as the cerebellar granule cell, has synapses with a more homogeneous population of afferent sources.

The axon emerges from a neuron as a slender thread and frequently does not branch until it nears its target. In contrast to the dendrite and the soma, the axon is frequently myelinated, thus increasing its efficiency as a conducting unit. Myelin, a spirally wrapped membrane (see Ch. 4), is laid down in segments, or internodes, by oligodendrocytes in the CNS and by Schwann cells in the PNS. The naked regions of axon between adjacent myelin internodes are known as nodes of Ranvier (Fig. 1-2).

Although neurons share common elements with other cells, each component has specialized features

Neurons contain the morphological features of other cell types, particularly with regard to the cell soma. The major

structures are similarly distributed and some of the most common, such as the Golgi apparatus, Nissl substance and mitochondria, for example, were described first in neurons. However, neurons are distinctive for their size, metabolic activity, and unusual degree of polarization.

The large, pale nucleus and prominent nucleolus of neurons helps identify neurons in histological sections and are consistent with the high level of transcription characteristic of neurons (Fig. 1-4). The nucleolus is vesiculated and easily visualized in the background of pale euchromatin with sparse heterochromatin. The nucleolus usually contains two textures: the pars fibrosa, which are fine bundles of filaments composed of newly transcribed ribosomal RNA, and the pars granulosa, with dense granules consisting of ribonuclear proteins that form ribosomes in the cytoplasm. As with other cells, the nucleus is enclosed by the nuclear envelope, made up of nuclear lamins with a cytoplasmic side membrane, which is in continuity with the endoplasmic reticulum and a more regular membrane on the inner, or nuclear, aspect of the envelope. Periodically, the inner and outer membranes of the envelope come together to form a single diaphragm, forming a 70 nm nuclear pore. In some neurons, as in Purkinje cells, that segment of the nuclear envelope that faces the dendritic pole is deeply invaginated.

The perikaryon (i.e., soma or cell body) of the neuron tends to be larger than other cells of the nervous system and is rich in organelles (Fig. 1-4) including components of the translational machinery, mitochondria, endoplasmic reticulum (ER), lysosomes and peroxisomes, Golgi complex, intermediate components, tubulovesicular organelles, endosomes and cytoskeletal structures. Various membranous cisternae are abundant, divisible into rough ER (rER), which forms part of the Nissl substance; smooth ER (sER); subsurface cisternae; and the Golgi apparatus, with some degree of interconnectivity. Despite these

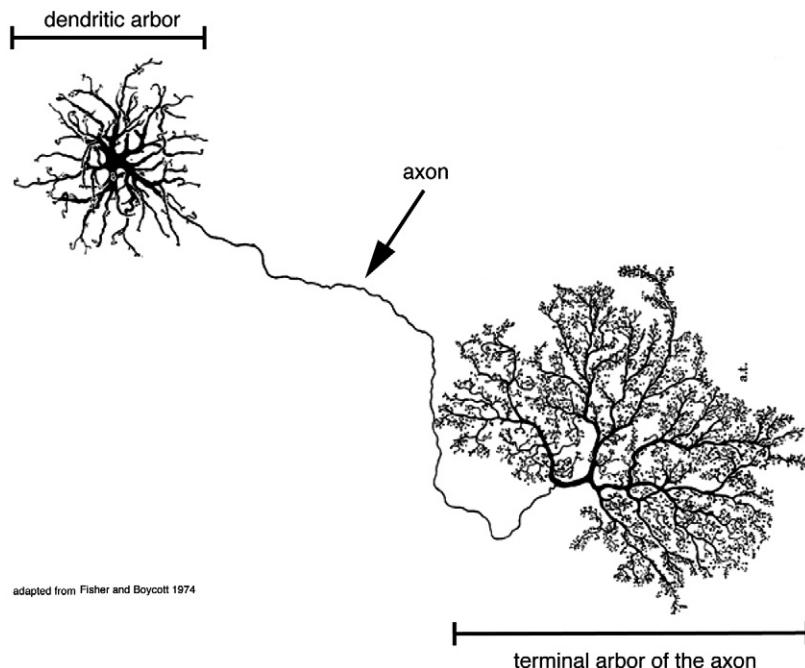


FIGURE 1.3 Real neurons have much more complex morphologies with elaborate branched arbors for both dendrites and axons. Individual neurons may have thousands of presynaptic terminals on their axons and thousands of postsynaptic specializations on their dendrites. Image is adapted from (Fisher & Boycott, 1974) and shows an example of a horizontal cell in the retina of the cat.

structural connections, each possesses distinct protein composition and enzymatic activities. In addition, lipofuscin granules, which also are termed *aging pigment*, are often seen in mature neurons.

Nissl substance, identified by staining for ribonucleic acid, comprises the various components of the translational machinery, including both rER, where membrane associated proteins

are synthesized, and cytoplasmic or free polysomes for cytoplasmic proteins, which are actually anchored to the cytoskeleton (Fig. 1-5). Histologically, Nissl substance is seen as cytoplasmic basophilic masses that ramify loosely throughout the cytoplasm and were first described in the nervous system. The distinctive Nissl staining of neurons reflects the high levels of protein synthesis in neurons needed to supply the large volume and surface area of neurons. Nissl substance appears excluded from axons at the axon hillock, but can be seen at lower levels in dendrites. The abundance and distribution of Nissl substance in certain neurons are characteristic and can be used as criteria for identification. In the electron microscope (EM), Nissl substance appears as arrays of flattened cisternae of the rER surrounded by clouds of free polyribosomes. The membranes of the rER are studded with rows of ribosomes, which produce the granular appearance of the rER. A space of 20–40 nm is maintained within cisternae. The rER in neurons produces some secretory components like neuropeptides, but must also generate the wide range of membrane proteins used throughout the neuron, a feature imposed by the extraordinary functional demands placed on the neuron.

Smooth ER is also abundant in neurons (see Ch. 7), although differentiating sER and rER can be problematic given the proximity and abundance of free polysomes. Ribosomes are not associated with sER, and the cisternae usually assume a meandering, branching course throughout the cytoplasm. In some neurons, i.e., Purkinje cells, the smooth ER is quite prominent. Individual cisternae of the smooth ER extend along axons and dendrites (Ch. 7). The cisternae of sER are metabolically active, representing the site of synthesis for lipids and steroids, as well for processing of proteins by glycosylation, formation and rearrangement of disulfide bonds, and conversion of pro forms of proteins or peptide hormones. The sER is also a site for metabolism of drugs, some carbohydrates, and steroids. Given the diverse functions of sER, there is likely to be regional segregation of specific functions and protein complements within the broad category of sER.

For example, a subsurface cisternal system that is often classified as sER plays a critical role in regulation of cytoplasmic Ca^{2+} (Ch. 24). These are membrane-bound, flattened

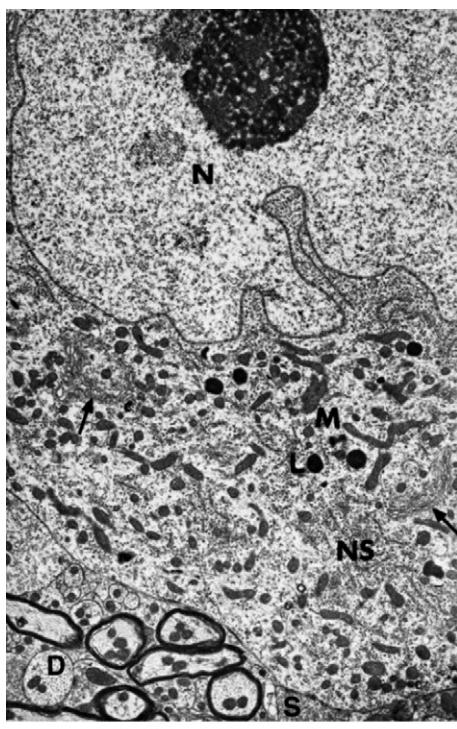
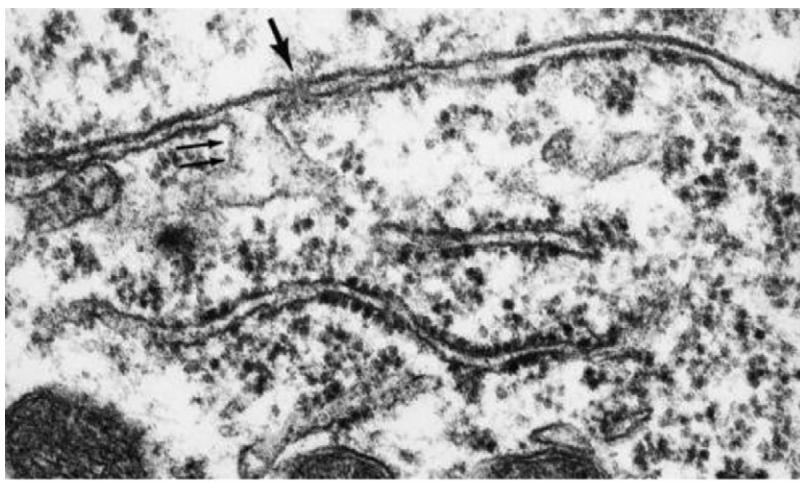
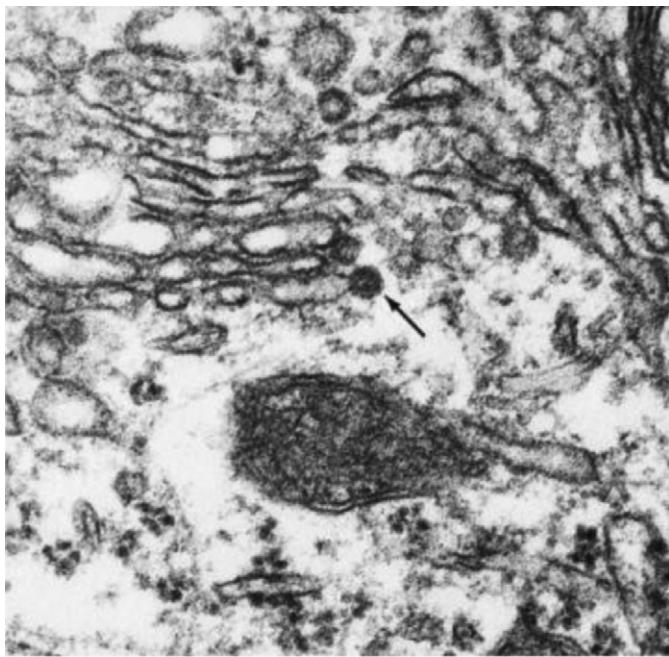


FIGURE 1.4 A motor neuron from the spinal cord of an adult rat shows a nucleus (N) containing a nucleolus, clearly divisible into a pars fibrosa and a pars granulosa, and a perikaryon filled with organelles. Among these, Golgi apparatus (arrows), Nissl substance (NS), mitochondria (M) and lysosomes (L) can be seen. An axosomatic synapse (S) occurs below, and two axodendritic synapses abut a dendrite (D). $\times 8,000$.



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FIGURE 1.5 Detail of the nuclear envelope showing a nuclear pore (single arrow) and the outer leaflet connected to the smooth endoplasmic reticulum (ER) (double arrows). Two cisternae of the rough ER with associated ribosomes are also present. $\times 80,000$.



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FIGURE 1.6 A portion of a Golgi apparatus. The smooth-membraned cisternae appear beaded. The many circular profiles represent tangentially sectioned fenestrations and alveolate vesicles (primary lysosomes). Two of the latter can be seen budding from Golgi saccules (arrows). $\times 60,000$.

cisternae that can be found in many neurons, bearing some elements in common with the sarcoplasmic reticulum in muscle. These structures abut the plasmalemma of the neuron and constitute a secondary membranous boundary within the cell. The distance between these cisternae and the plasmalemma is usually 10–12 nm and, in some neurons, such as the Purkinje cells, a mitochondrion may be found in close association with the innermost leaflet. Similar cisternae have been described beneath synaptic complexes, presumably playing a role in Ca^{2+} homeostasis in the presynaptic terminal. Membrane structures are described in Chapter 2.

The *Golgi apparatus* is another highly specialized agranular membranous structure (Fig. 1-6). Ultrastructurally, the Golgi apparatus consists of stacks of smooth-walled cisternae and a variety of vesicles (see Ch. 7). The Golgi complex is located near the cell center, adjacent to the nucleus and the centrosome. The neuronal Golgi is particularly well developed, consistent with the large amount of membrane protein synthesis and processing. In many neurons, the Golgi apparatus encompasses the nucleus and extends into dendrites but is absent from axons. A three-dimensional analysis of the system reveals that the stacks of cisternae are pierced periodically by fenestrations. Tangential sections of these fenestrations show them to be circular profiles. A multitude of vesicles is associated with each segment of the Golgi apparatus, particularly coated vesicles that are generated from the lateral margins of flattened cisternae (Fig. 1-6) (see Ch. 7).

Histochemical staining reveals that some of these membrane organelles in the vicinity of the Golgi are rich in acid

hydrolases, and they are believed to represent primary lysosomes (see Ch. 7). The *lysosome* is the principal organelle responsible for the degradation of cellular waste. It is a common constituent of all cell types of the nervous system and is particularly prominent in neurons, where it can be seen at various stages of development (Fig. 1-4). It ranges in size from 0.1 to 2 μm in diameter. The primary lysosome is elaborated from Golgi saccules as a small, vesicular structure (Fig. 1-6). Its function is to fuse with the membrane of waste-containing vacuoles, termed phagosomes or late endosomes, into which it releases hydrolytic enzymes (see Ch. 43 for inherited diseases of lysosomal enzymes). The sequestered material is then degraded within the vacuole, and the organelle becomes a secondary lysosome, which is typically electron dense and large. The matrix of this organelle will give a positive reaction when tested histochemically for acid phosphatase. Residual bodies containing nondegradable material are considered to be tertiary lysosomes, which may include lipofuscin granules. These granules contain brown pigment and lamellar stacks of membrane material, and become increasingly common in the aging brain.

Curiously, primary and secondary lysosomes are largely absent from axonal domains, although prelysosomal structures such as endosomes and phagosomes are prominent. These prelysosomal structures may take the form of *multivesicular bodies*, which profiles are commonly seen in retrograde axonal transport (Ch. 8). They contain several minute, spherical profiles, sometimes arranged about the periphery of the sphere. A variant of these structures consists of larger elements derived from autophagy and may include degenerating mitochondria (Ch. 7). In the cell soma, they are believed to belong to the lysosome series prior to secondary lysosomes because some contain acid hydrolases and apparently are derived from primary lysosomes.

Mitochondria are the centers for oxidative phosphorylation and the respiratory centers of all eukaryotic cells (see in Ch. 43). These organelles occur ubiquitously in the neuron and its processes. Their overall shape may change from one type of neuron to another but their basic morphology is identical to that in other cell types. Mitochondria consist morphologically of double-membrane sacs surrounded by protuberances, or cristae, extending from the inner membrane into the matrix space. Mitochondrial membranes have a distinctive lipid composition, including the mitochondrial-specific lipid cardiolipin. Mitochondria are primarily considered as the source of ATP from aerobic metabolism of pyruvate or fatty acids, but may have other functions as well. In particular, they play a critical role in regulation of cell death pathways (Chipuk, et al., 2010) (Ch. 37).

Mitochondria and plant chloroplasts are unique among organelles in containing their own genetic complement and machinery for protein synthesis. There are more than twenty mitochondrial genes encoding polypeptides having a mitochondrial function, along with tRNA and ribosomal RNA genes (Szibor & Holtz, 2003). Multiple copies of these genes serve to protect against DNA damage. Protein synthesis in mitochondria shares many features with prokaryotic protein synthesis, including sensitivity to antibiotics that inhibit bacterial protein synthesis. However, mitochondria age and must be renewed on a regular basis (Szibor & Holtz, 2003). As

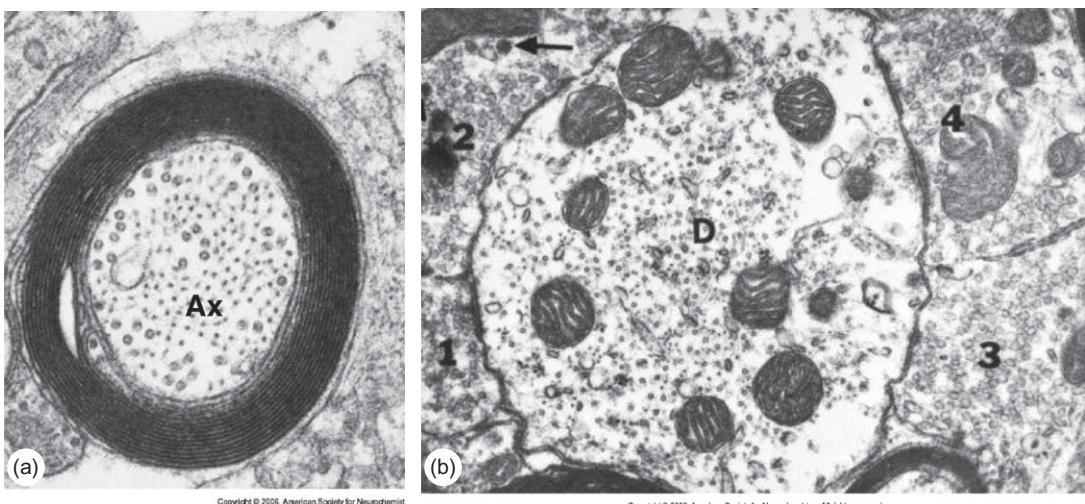


FIGURE 1.7 Axons and dendrites are distinguished morphologically. **Left panel:** Transverse section of a small myelinated axon in dog spinal cord. The axon contains scattered neurotubules and loosely packed neurofilaments interconnected by side-arm material. $\times 60,000$. **Right panel:** A dendrite (D) emerging from a motor neuron in the anterior horn of a rat spinal cord is contacted by four axonal terminals: terminal 1 contains clear, spherical synaptic vesicles; terminals 2 and 3 contain both clear, spherical and dense-core vesicles (arrow); and terminal 4 contains many clear, flattened (inhibitory) synaptic vesicles. Note also the synaptic thickenings and, within the dendrite, the mitochondria, neurofilaments and neurotubules. $\times 33,000$.

nuclear genes encode the majority of mitochondrial proteins, both cellular and mitochondrial protein synthesis are needed for generation of new mitochondria (Ch. 43).

The axon compartment comprises the axon hillock, initial segment, shaft and terminal arbor

These regions differ ultrastructurally in membrane morphology and cytoskeletal organization. The axon hillock may contain fragments of Nissl substance, including abundant ribosomes, which diminish as the hillock continues into the initial segment. Here, the various axoplasmic components begin to align longitudinally. A few ribosomes and the smooth ER persist, and some axoaxonic synapses occur. The axolemma of the initial segment where the action potential originates exhibits a dense granular layer similar to that seen at the nodes of Ranvier, consistent with a specialized membrane cytoskeleton. Also present in this region are microtubules, neurofilaments and mitochondria. The arrangement of the microtubules in the initial segment is distinctive in forming fascicles interconnected by side arms. Beyond the initial segment, the axon maintains a relatively uniform caliber even after branching with little or no diminution until the very terminal arbors (Fig. 1-7). One exception is a reduction of caliber for myelinated axons at the peripheral node of Ranvier (Hsieh et al., 1994) (see Fig. 1-2 and below). Myelinated axons show granular densities on the axolemma at nodes of Ranvier (Raine, 1982) that correspond to adhesion molecules and high densities of sodium channels. In myelinated fibers, there is a concentration of sodium channels at the nodal axon, a feature underlying the rapid, saltatory conduction of such fibers (Ch. 4).

Microtubules are a prominent feature of all axons. Axonal microtubules are aligned with the long axis of the axon and

have a uniform polarity with plus ends distal to the soma (Ch. 6). Microtubules are present in loose groupings rather than bundles and vary in their spacing (Fig. 1-7A). Vesicles and mitochondria are typically seen in association with these microtubule domains, consistent with their movement in fast axonal transport (Ch. 8). In axons less than a micron in diameter, which are usually unmyelinated, microtubules are the primary structural cytoskeletal elements, with sparse neurofilaments and gaps in the neurofilament cytoskeleton. As axons get larger, the number of neurofilaments increases dramatically, becoming the primary determinant of axonal caliber. For large, myelinated axons, neurofilaments occupy the bulk of an axon cross-section (Ch. 6) with microtubules found in small groups along with membrane profiles.

Although neuroscientists typically draw neurons with a single unbranched axon and one presynaptic terminal, most axons are extensively branched into terminal arbors, often producing hundreds or thousands of presynaptic terminals (Fig. 1-3). In addition, many axons in the CNS have *en passant* presynaptic specializations (Peters et al., 1991) that allow a single axon to have many presynaptic specializations in series. Parallel fibers in the cerebellar cortex may have thousands of these specializations. When *en passant* synapses occur on myelinated fibers, these synaptic specializations are seen at the nodes of Ranvier. The terminal portion of the axon arborizes and enlarges to form presynaptic specializations at sites of synaptic contact (Chs. 7 and 12).

Dendrites are the afferent components of neurons

In some neurons, they may arise from a single trunk, while other neurons have multiple dendritic trunks emerging from the cell soma. Unlike the axon, dendritic processes taper

distally and each successive branch is reduced in diameter. The extensive branching into a dendritic tree gave rise to the name dendrite. Dendrites are typically rich in microtubules and microfilaments, but largely lack neurofilaments. Unlike in axons, the microtubules in proximal dendrites are distinctive in having a mixed polarity and a distinctive microtubule associated protein, MAP2. Proximal dendrites generally contain Nissl substance and components of the Golgi complex. A subset of neuronal mRNAs is transported into the dendrites, where local synthesis and processing of proteins occur in response to synaptic activity (Martin & Zukin, 2006).

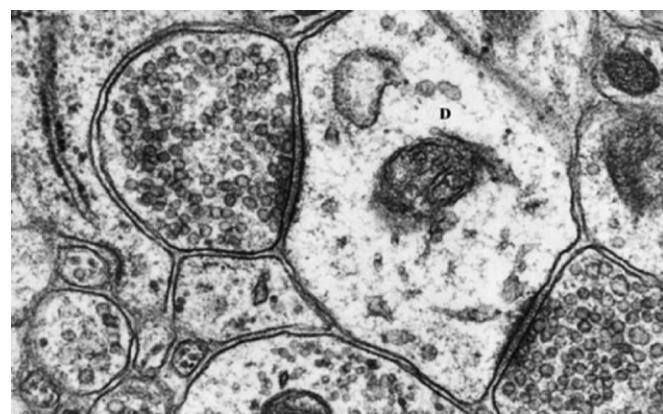
Some difficulty may be encountered in distinguishing small unmyelinated axons or terminal segments of axons from similar-sized dendrites. In the absence of morphologically identifiable synaptic structures, they can often be assessed by the content of neurofilaments, which are more typical of axons. The postsynaptic regions of dendrites occur either along the main stems (Fig. 1-8) or more commonly at small protuberances known as dendritic spines (Luscher et al., 2000). Axon presynaptic terminals abut these spines, whose number and detailed structure may be highly dynamic, changing with activity (Bhatt et al., 2009). Spine dynamics are thought to reflect altered synaptic function and may be a substrate for learning and memory. Considerable insights into spine function have been obtained through imaging of spines in intact brain (Bhatt et al., 2009).

The synapse is a specialized junctional complex by which axons and dendrites emerging from different neurons intercommunicate

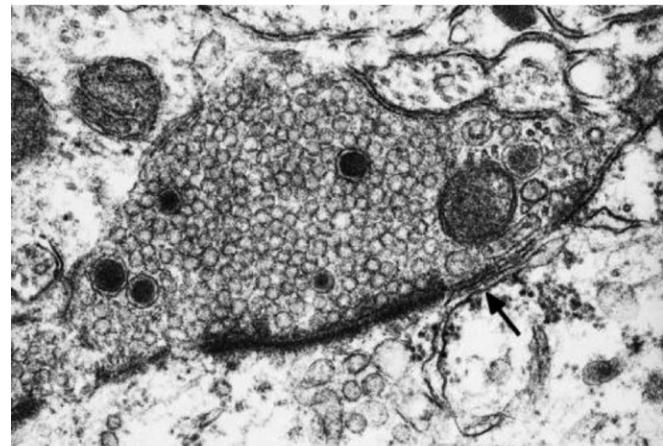
This was proposed first in 1897 by Sherrington, who also coined the term 'synapse'. The existence of synapses was immediately demonstrable by EM and can be recognized today in a dynamic fashion by Nomarski optics (differential interference microscopy), confocal and light microscopy, and scanning EM.

Synaptic structures are diverse in morphology and function (Fig. 1-8). Some are polarized or asymmetrical, due to the unequal distribution of electron-dense material on the apposing membranes of the junctional complex and heavier accumulation of organelles within the presynaptic component. The closely applied membranes constituting the synaptic site are overlaid on the presynaptic and postsynaptic aspects by an electron-dense material similar to that seen in desmosomes and separated by a gap or cleft of 15–20 nm. The classic presynaptic terminal of a chemical synapse contains a collection of clear, 40–50 nm synaptic vesicles. The morphology of synaptic vesicles in the terminal may exhibit subtle differences depending on the neurotransmitter being released (Peters & Palay, 1996).

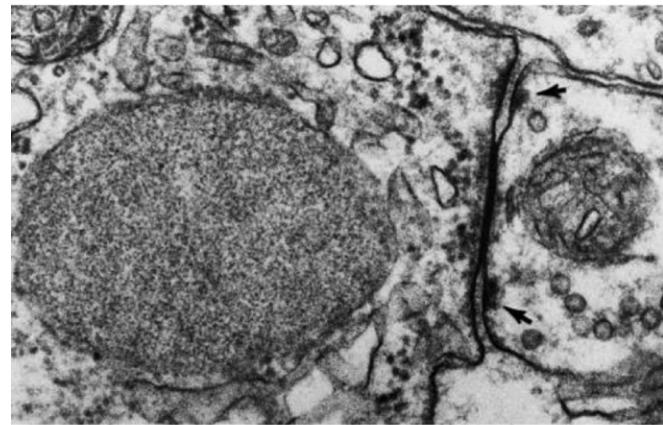
Synaptic vesicles are important in packaging, transport and release of neurotransmitters and, after their discharge into the synaptic cleft, they are recycled within the axon terminal (Ch. 7). Also present are small mitochondria approximately 0.2–0.5 μm in diameter (Fig. 1-8). Microtubules, coated vesicles and cisternae of the smooth ER may be found in the presynaptic compartment. On the postsynaptic side is a density referred to as the subsynaptic web. Aside from relatively



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(Photograph courtesy of Drs. G. D. Pappas and J. S. Keeter.)

FIGURE 1.8 Presynaptic morphologies reflect differences in synaptic function. **Top panel:** A dendrite (D) is flanked by two axon terminals packed with clear, spherical synaptic vesicles. Details of the synaptic region are clearly shown. $\times 75,000$. **Middle panel:** An axonal terminal at the surface of a neuron from the dorsal horn of a rabbit spinal cord contains both dense-core and clear, spherical synaptic vesicles lying above the membrane thickenings. A subsurface cisterna (arrow) is also seen. $\times 68,000$. **Bottom panel:** An electrotonic synapse is seen at the surface of a motor neuron from the spinal cord of a toadfish. Between the neuronal soma (left) and the axonal termination (right), a gap junction flanked by desmosomes (arrows) is visible. $\times 80,000$. (Photograph courtesy of Drs. G. D. Pappas and J. S. Keeter.)

sparse profiles of smooth ER or subsurface cisternae and Golgi profiles, there are few aggregations of organelles in the dendrite. At the neuromuscular junction, the morphological organization is somewhat different. Here, the axon terminal is greatly enlarged and ensheathed by Schwann cells; the post-synaptic or sarcolemmal membrane displays less density and is infolded extensively.

Today, most neuroanatomists categorize synapses depending on the profiles between which the synapse is formed, such as axodendritic, axosomatic, axoaxonic, dendrodendritic, somatosomatic and somatodendritic synapses. However, such a classification does not specify whether the transmission is chemical or electrical nor does it address the neurotransmitter involved in chemical synapses. Alternatively, physiological typing of synapse defines three groups: excitatory, inhibitory and modulatory. Depending on the methods used, the synaptic vesicles can be distinctive (Peters & Palay, 1996). For example, excitatory synapses may have spherical synaptic vesicles, whereas inhibitory synapses contain a predominance of flattened vesicles (Fig. 1-8). However, some consider that the differences between flat and spherical vesicles may reflect an artifact of aldehyde fixation or a difference in physiological state at the time of sampling. Moreover, this classification does not hold true for all regions of the CNS.

The most extensively studied synapses *in situ* or in synaptosomes are cholinergic (Ch. 13). However, there is a wide range of chemical synapses that utilize biogenic amines (Chs. 14–16) as neurotransmitter substances, as well as other small molecules such as GABA (Ch. 18) and adenosine (Ch. 19). In addition to clear vesicles, slightly larger dense-core or granular vesicles of variable dimensions can be seen in the presynaptic terminal (Fig. 1-8). These larger, dense core vesicles contain neuropeptides (Ch. 20), whose secretion is regulated independently of classic neurotransmitters. Further, some synapses may be so-called silent synapses, which are observed in CNS tissue both *in vitro* and *in vivo*. These synapses are morphologically identical to functional synapses but are physiologically dormant.

Finally, there is the well-characterized electrical synapse, where current can pass from cell to cell across regions of membrane apposition that essentially lack the associated collections of organelles present at the chemical synapse. In the electrical synapse (Fig. 1-8 lower panel), the unit membranes are closely apposed, and the outer leaflets sometimes fuse to form a pentalaminar structure; however, in most places, a gap of approximately 20 nm exists, producing a so-called gap junction. Not infrequently, desmosome-like domains separate gap junctions. Sometimes, electrical synapses exist at terminals that also display typical chemical synapses; in such cases, the structure is referred to as a mixed synapse.

MACROGLIA: MORE THAN MEETS THE EYE

In 1846, Virchow recognized the existence of a fragile, non-nervous, interstitial component made up of stellate or spindle-shaped cells in the CNS. These cells were morphologically distinct from neurons, and were thought to hold the neurons

together, hence the name neuroglia, or ‘nerve glue’ (Peters et al., 1991). Today, we recognize three broad groups of glial cells in the CNS: (a) macroglia, such as astrocytes, radial glia and oligodendrocytes of ectodermal origin, like neurons; (b) microglia, of mesodermal origin; and (c) ependymal cells, also of ectodermal origin. Microglia invade the CNS via the pia mater, the walls of blood vessels and the tela choroidea at the time of vascularization. Glial cells are not electrically excitable and some types, such as astrocytes, retain the ability to proliferate, particularly in response to injury, while others may be replaced by differentiation from progenitors (see Ch. 30). The rough schema represented in Fig. 1-1 illustrates the interrelationships between glia and other CNS components.

Virtually nothing can enter or leave the central nervous system parenchyma without passing through an astrocytic interphase

The complex packing achieved by the processes and cell bodies of astrocytes reflects their critical role in brain metabolism (Sofroniew & Vinters, 2010). Astrocytes traditionally have been subdivided into stellate-shaped protoplasmic and fibrous astrocytes as well as the elongate radial and Bergman glia (Kimelberg & Nedergaard, 2010). The astrocyte lineage is increasingly recognized as more complex and dependent on the developmental context than previously recognized (Kimelberg & Nedergaard, 2010).

Although many structural components of fibrous and protoplasmic astrocytes are shared, their functions are diverse. Protoplasmic astrocytes range in size from 10–40 µm, are frequently located in gray matter in relation to capillaries and have a clearer cytoplasm than fibrous astrocytes (Fig. 1-9). Fibrous astrocytes are found in white matter and are typically smaller. All astrocytes have intermediate filaments containing glial fibrillary acidic protein (GFAP), which is a standard marker for

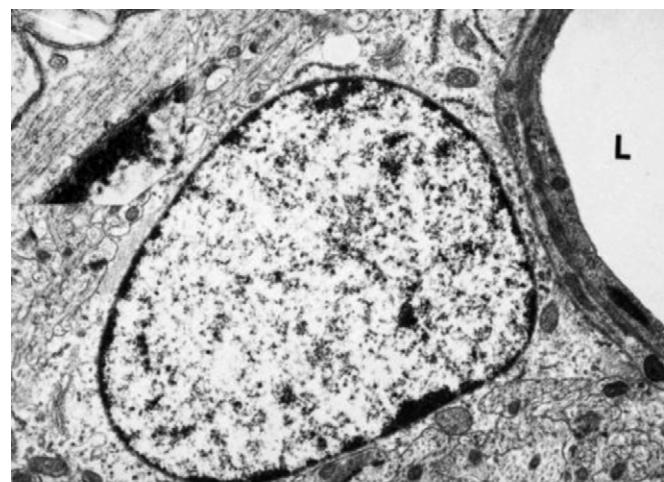
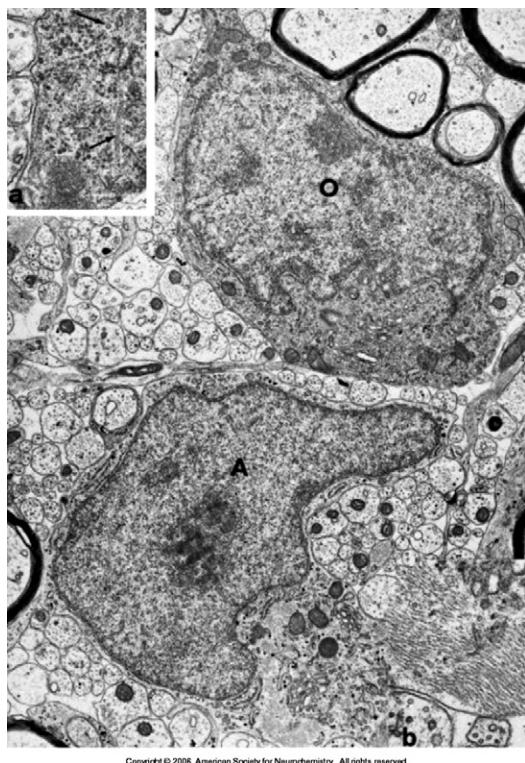


FIGURE 1.9 A protoplasmic astrocyte abuts a blood vessel (lumen at L) in rat cerebral cortex. The nucleus shows a rim of denser chromatin, and the cytoplasm contains many organelles, including Golgi and rough endoplasmic reticulum. ×10,000.



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FIGURE 1.10 A section of myelinating white matter from a kitten contains a fibrous astrocyte (A) and an oligodendrocyte (O). The nucleus of the astrocyte (A) has homogeneous chromatin with a denser rim and a central nucleolus. That of the oligodendrocyte (O) is denser and more heterogeneous. Note the denser oligodendrocytic cytoplasm and the prominent filaments within the astrocyte. $\times 15,000$. Inset: Detail of the oligodendrocyte, showing microtubules (arrows) and absence of filaments. $\times 45,000$.

astrocytic cells, and microtubules (Fig. 1-10), often extending together with loose bundles of filaments along cell processes. They also contain glycogen granules; lysosomes and lipofuscin-like bodies; isolated cisternae of the rough ER; a small Golgi apparatus opposite one pole of the nucleus; and small, elongated mitochondria. Characteristically, the nucleus is ovoid and nucleochromatin homogeneous, except for a narrow, continuous rim of dense chromatin and one or two poorly defined nucleoli, consistent with modest levels of transcription and translation. Another common feature of astrocytes is that they form tight junctions, particularly desmosomes (mediated by cadherins) and gap junctions (mediated by connexins) that occur between adjacent astrocytic processes.

Fibrous astrocyte (Fig. 1-10) processes appear twig-like, with large numbers of tightly bundled GFAP filaments, while GFAP filaments in protoplasmic astrocytes are less tightly bundled. Filaments within these astrocyte processes can be readily distinguished from neurofilaments by their close packing and the absence of side arms (see Ch. 6). GFAP staining is a standard marker for identification of astrocytes and has traditionally been used to estimate the extent of astrocytic processes. However, expression of green fluorescent protein (GFP) under astrocyte-specific promoters indicates that GFAP

staining significantly underestimates the size and extent of astrocytic processes. This is important because each astrocyte typically defines a domain based on the soma and processes, with little overlap between adjacent domains through peripheral processes that are largely invisible with GFAP staining (Nedergaard et al., 2003). Remarkably, the number, size and extent of astrocytes is species-dependent, so human astrocytes are 2.5 times larger than comparable mouse astrocytes (Kimelberg & Nedergaard, 2010) and the number of astrocytes per neuron is 3–4 times greater in human brain (Nedergaard et al., 2003).

In addition to protoplasmic and fibrous forms, a set of elongate cells is also derived from the astrocyte lineage, including Muller glia in the retina (Ch. 51) and Bergman glia in the cerebellum (Kimelberg & Nedergaard, 2010). In addition to providing structural support, these elongated astrocytes may have additional roles to play. For example, Müller cells may serve as light guides, analogous to fiber optics, channeling light to photoreceptors (Franze et al., 2007). Finally, regional specialization occurs among astrocytes. In addition to differences between white (fibrous) and grey matter (protoplasmic) astrocytes, there may be additional subtypes. For example, the outer membranes of astrocytes located in subpial zones and those facing blood vessels possess a specialized thickening, sometimes called hemidesmosomes, and there may be additional functional specializations.

New functions of astrocytes continue to be identified (Kimelberg & Nedergaard, 2010). Astrocytes ensheathe synaptic complexes and the soma of some neurons (i.e., Purkinje cells). This places them in a unique position to influence the environment of neurons and to modulate synaptic function. Astrocytes are not excitable cells, but have large negative membrane potentials. This allows them to buffer extracellular K^+ , so astrocytes play a significant role in K^+ homeostasis in the brain (Leis et al., 2005), particularly after injury (see also Aquaporin 4 in Ch. 3). Astrocytes similarly buffer extracellular pH in the brain and may modulate Na^+ levels as well (Deitmer & Rose, 2010). Recent studies have established that astrocytes express metabotropic glutamate receptors (Ch. 17) and purinergic receptors (Ch. 19). Activation of purinergic receptors may produce Ca^{2+} waves that affect groups of astrocytes by release of Ca^{2+} from intracellular stores and that may involve communication between astrocytes through gap junctions (Nedergaard et al., 2003). Complementary to these functions, astrocytes may play a role in regulation of cerebral blood flow and availability of both glucose and lactate for maintenance of neuronal metabolism. Further, even the entry of water into the brain may be modulated by the action of aquaporins on astrocytes (Kimelberg & Nedergaard, 2010).

Astrocytes may affect neuronal signaling in a variety of ways. Prolonged elevation of extracellular levels of the excitatory neurotransmitter glutamate can lead to excitotoxicity due to overactivation of glutamate receptors and excessive entry of Ca^{2+} into neurons. Astrocytes express both metabotropic glutamate receptors and glutamate transporters, which are responsible for glutamate uptake and limit the possibility of neuronal damage (Sattler & Rothstein, 2006). The astrocyte enzymatically converts glutamate to glutamine, which can then be recycled to the neuron. Astrocytes similarly provide glutathione to neurons through a uptake and conversion of

cysteine (McBean, 2011). Finally, GABA transporters on astrocytes may affect the balance between excitatory and inhibitory pathways.

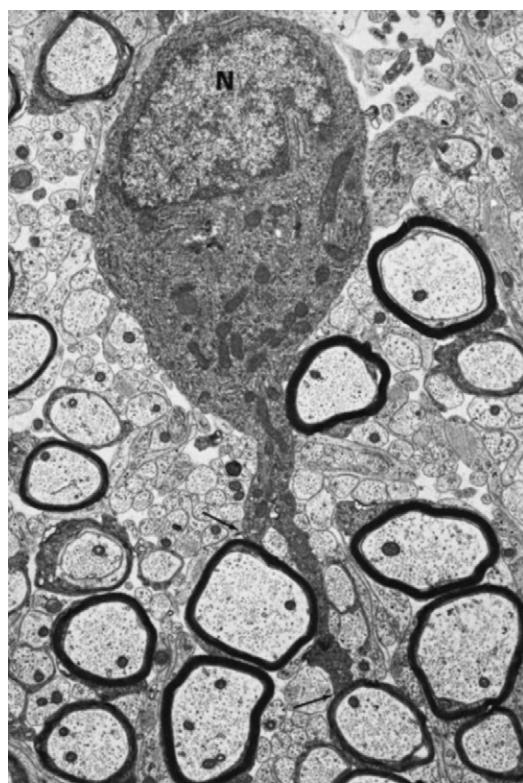
The role of astrocytes in injury and neuropathology is complex (Sofroniew & Vinters, 2010). Subsequent to trauma, astrocytes invariably proliferate, swell, accumulate glycogen and undergo fibrosis by the accumulation of GFAP filaments. This state of gliosis may be total, in which case all other elements are lost, leaving a glial scar, or it may be a generalized response occurring against a background of regenerated or normal CNS parenchyma. With age, both fibrous and protoplasmic astrocytes accumulate filaments. Mutations in GFAP are now known to be the cause of the childhood leukodystrophy called Alexander disease (Johnson, 2002) (Ch. 41).

Oligodendrocytes are myelin-producing cells in the central nervous system

Oligodendrocytes are definable by morphological criteria. The roughly globular cell soma ranges from 10–20 µm and is denser than that of an astrocyte. The margin of the cell is irregular and compressed against the adjacent neuropil. In contrast to astrocytes, few cell processes are seen. Within the cytoplasm, many organelles are found. Parallel cisternae of rough ER and a widely dispersed Golgi apparatus are common. Free ribosomes occur, scattered amid occasional multivesicular bodies, mitochondria and coated vesicles. Distinguishing the oligodendrocyte from the astrocyte is the absence of glial or any other intermediate filament, but abundant microtubules are present (Figs. 1-10 and 11). Microtubules are most common at the margins of the cell, in the occasional cell process and in cytoplasmic loops around myelin sheaths. Lamellar dense bodies, typical of oligodendrocytes, are also present. The nucleus is usually ovoid, but slight lobation is not uncommon. The nucleochromatin stains heavily and contains clumps of denser heterochromatin. Desmosomes and gap junctions occur between interfascicular oligodendrocytes.

Myelinating oligodendrocytes have been studied extensively (see Chs. 10 and 31). Examination of the CNS during myelinogenesis (Fig. 1-11) reveals connections between the cell body and the myelin sheath (Chs. 10 and 31). The oligodendrocyte is capable of producing many internodes of myelin simultaneously. It has been estimated that oligodendrocytes in the optic nerve produce between 30 and 50 internodes of myelin. Damage to only a few oligodendrocytes, therefore, can be expected to produce an appreciable area of primary demyelination. Oligodendrocytes are among the most vulnerable elements and the first to degenerate (Ch. 39). Like neurons, they lose their ability to proliferate once differentiated.

Analogous to a neuron, the relatively small oligodendrocyte soma produces and supports many times its own volume of membrane and cytoplasm. For example, an average oligodendrocyte produces 20 internodes of myelin. Each axon has a diameter of 3 µm and is covered by at least six lamellae of myelin, with each lamella representing two fused layers of unit membrane. Calculations based on the length of the myelin internode (which may exceed 500 µm) and the length of the cell processes connecting the sheaths to the cell body indicate



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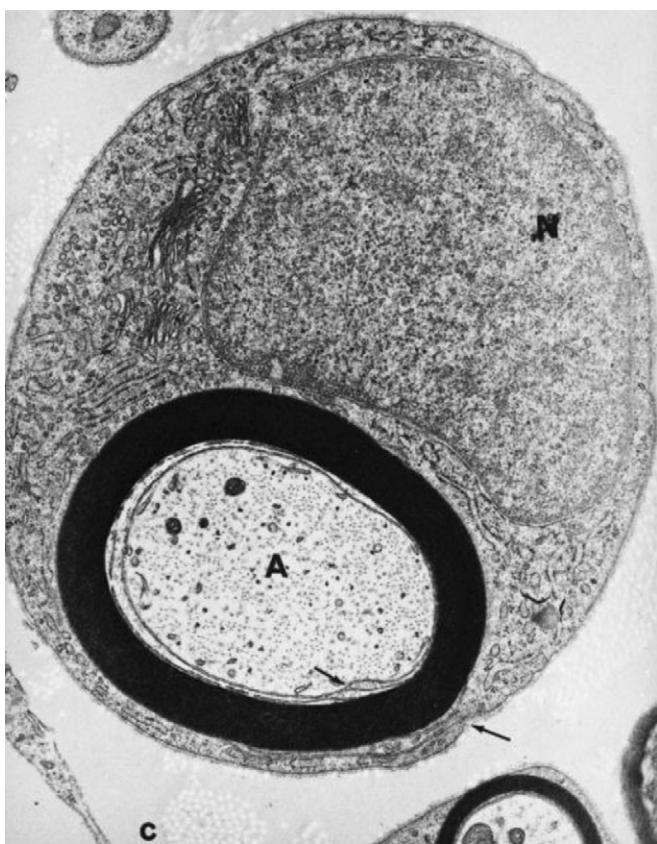
FIGURE 1.11 A myelinating oligodendrocyte, nucleus (N), from the spinal cord of a 2-day-old kitten extends cytoplasmic connections to at least two myelin sheaths (arrows). Other myelinated and unmyelinated fibers at various stages of development, as well as glial processes, are seen in the surrounding neuropil. $\times 12,750$.

that the ratio between the surface area for the cell soma and the myelin it sustains can be 1:1000 or greater.

The oligodendrocyte is a primary target in autoimmune diseases like multiple sclerosis and experimental autoimmune encephalopathy (Ch. 39). This vulnerability to immune mediated damage may reflect the presence in the myelin sheath of many molecules with known affinities to elicit specific T- and B-cell responses (Chs. 33 and 39). Many of these molecules, such as myelin basic protein, proteolipid protein, myelin-associated glycoprotein, galactocerebroside, myelin/oligodendrocyte protein, and myelin oligodendrocyte glycoprotein (MOG), among others, can also be used to generate specific antibodies for anatomical and pathological analyses of oligodendrocytes *in vivo* and *in vitro*.

The schwann cell is the myelin-producing cell of the peripheral nervous system

When axons leave the CNS, they lose their neuroglial interrelationships and traverse a short transitional zone where they are invested by an astroglial sheath enclosed in the basal lamina of the glia limitans. The basal lamina then becomes



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FIGURE 1.12 A myelinated PNS axon (A) is surrounded by a Schwann cell nucleus (N). Note the fuzzy basal lamina around the cell, the rich cytoplasm, the inner and outer mesaxons (arrows), the close proximity of the cell to its myelin sheath and the 1:1 (cell:myelin internode) relationship. A process of an endoneurial cell is seen (lower left), and unstained collagen (c) lies in the endoneurial space (white dots). $\times 20,000$.

continuous with that of axon-investing Schwann cells, at which point the astroglial covering terminates. Schwann cells, therefore, are the axon-ensheathing cells of the PNS, equivalent functionally to the oligodendrocyte of the CNS, but sharing some aspects of astrocyte function as well. Unlike the CNS, where an oligodendrocyte produces many internodes, each myelinating Schwann cell produces a single internode of myelin and interacts with a single neuron. Another difference is that the myelinating Schwann cell body remains in intimate contact with its myelin internode (Fig. 1-12), whereas the oligodendrocyte soma connects to internodes via long, attenuated processes. Periodically, myelin lamellae open up into ridges of Schwann cell cytoplasm, producing bands of cytoplasm around the fiber called Schmidt-Lanterman incisures, reputed to be the stretch points along PNS fibers. These incisures are not a common feature in the CNS. The PNS myelin period is 11.9 nm in preserved specimens, some 30% less than in the fresh state and in contrast to the 10.6 nm of central myelin. In addition to these structural differences, PNS

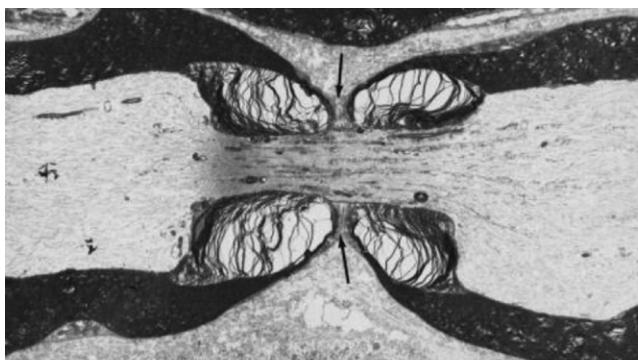
myelin differs biochemically and antigenically from that of the CNS (see Ch. 10).

Not all PNS fibers are myelinated but all PNS axons interact with Schwann cells. For small axons ($<1\text{ }\mu\text{m}$), nonmyelinating Schwann cells interact with multiple axons (Peters et al., 1991). Nonmyelinated fibers in the PNS are grouped into bundles surrounded by Schwann cell processes, in contrast to the situation in the CNS. Each axon is largely separated from adjacent axons by invaginations of Schwann cell membrane and cytoplasm. However, the axon connects to the extracellular space via a short channel, the mesaxon, formed by the invaginated Schwann cell plasmalemma.

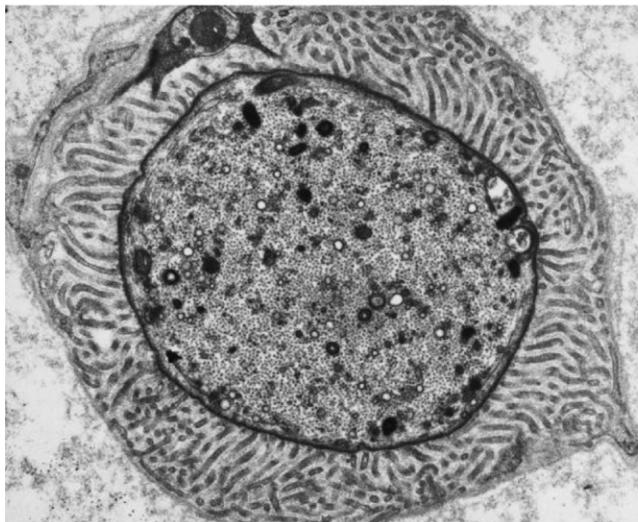
Ultrastructurally, the Schwann cell is unique and distinct from the oligodendrocyte. Each Schwann cell is surrounded by a basal lamina made up of a mucopolysaccharide approximately 20–30 nm thick that does not extend into the mesaxon (Fig. 1-12). The basal lamina of adjacent myelinating Schwann cells at the nodes of Ranvier is continuous, and Schwann cell processes interdigitate so that the PNS myelinated axon is never in direct contact with the extracellular space (Fig. 1-13). These nodal Schwann cell fingers display intimate relationships with the axolemma, and a similar arrangement between the nodal axon and the fingers of astroglial cells is seen in the CNS, but the specific function of these fingers is not well understood. The axon in the peripheral node of Ranvier is significantly restricted (Fig. 1-13) and the neurofilaments are dephosphorylated (Witt & Brady, 2000), which is thought to be related to targeting of proteins to the nodal membrane. However, changes in axon caliber and neurofilament density at CNS nodes of Ranvier are not as dramatic. The Schwann cells of nonmyelinated PNS fibers overlap, so there are no gaps and no nodes of Ranvier.

The cytoplasm of the Schwann cell is rich in organelles (Fig. 1-12). A Golgi apparatus is located near the nucleus, and cisternae of the rough ER occur throughout the cell. Lysosomes, multivesicular bodies, glycogen granules and lipid granules, sometimes termed *pi* granules, also can be seen. The cell is rich in microtubules and filaments, in contrast to the oligodendrocyte. The plasmalemma frequently shows pinocytic vesicles. Small, round mitochondria are scattered throughout the soma. The nucleus, which stains intensely, is flattened and oriented longitudinally along the nerve fiber. Aggregates of dense heterochromatin are arranged peripherally.

In sharp contrast to the differentiated oligodendrocyte, the Schwann cell responds vigorously to most forms of injury (Ch. 39). An active phase of mitosis occurs following traumatic insult, and the cells are capable of local migration. Studies on their behavior after primary demyelination have shown that they phagocytose damaged myelin. They possess remarkable ability for regeneration and begin to lay down new myelin approximately one week after a fiber loses its myelin sheath. After primary demyelination, PNS fibers are remyelinated efficiently, whereas similarly affected areas in the CNS show relatively little proliferation of new myelin (see Ch. 39). After severe injury leading to transection of the axons, axons degenerate and the Schwann cells form tubes, termed *Büngner bands*, containing cell bodies and processes surrounded by a single basal lamina. These structures provide channels along which regenerating axons might later grow. The presence and integrity of the Schwann cell basal lamina is essential for



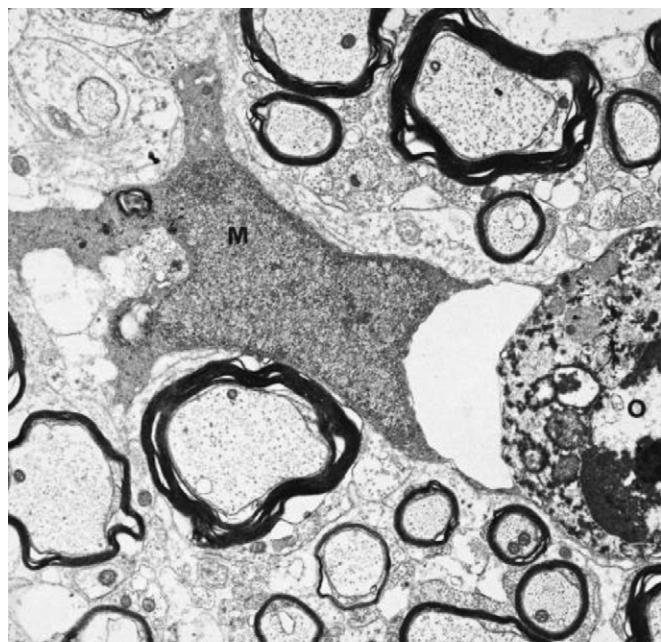
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FIGURE 1.13 The axon is constricted at the peripheral node of Ranvier. **Top panel:** Low-power electron micrograph of a node of Ranvier in longitudinal section. Note the abrupt decrease in axon diameter and the attendant condensation of axoplasmic constituents in the paranodal and nodal regions of the axon. Paranodal myelin is distorted artifactually, a common phenomenon in large-diameter fibers. The nodal gap substance (arrows) contains Schwann cell fingers, the nodal axon is bulbous and lysosomes lie beneath the axolemma within the bulge. Beaded smooth endoplasmic reticulum sacs are also seen. $\times 5,000$. **Bottom panel:** A transverse section of the node of Ranvier (7–8 nm across) of a large fiber shows a prominent complex of Schwann cell fingers around an axon highlighted by its subaxolemmal densification and closely packed organelles. The Schwann cell fingers arise from an outer collar of flattened cytoplasm and abut the axon at regular intervals of approximately 80 nm. The basal lamina of the nerve fiber encircles the entire complex. The nodal gap substance is granular and sometimes linear. Within the axoplasm, note the transversely sectioned sacs of beaded smooth endoplasmic reticulum (ER); mitochondria; dense lamellar bodies, which appear to maintain a peripheral location; flattened smooth ER sacs; dense-core vesicles; cross-bridged neurofilaments; and microtubules, which in places run parallel to the circumference of the axon (above left and lower right), perhaps in a spiral fashion. $\times 16,000$.

reinnervation, and transplantation of Schwann cells into the CNS environment can facilitate regeneration of CNS axons (Kocsis & Waxman, 2007) (Ch. 32). A number of pathologies have been identified that are associated with mutations in Schwann cell proteins, including many forms of Charcot-Marie-Tooth disease (Ch. 38).



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FIGURE 1.14 A microglial cell (M) has elaborated two cytoplasmic arms to encompass a degenerating apoptotic oligodendrocyte (O) in the spinal cord of a 3-day-old kitten. The microglial cell nucleus is difficult to distinguish from the narrow rim of densely staining cytoplasm, which also contains some membranous debris. $\times 10,000$.

MICROGLIA

The microglial cell plays a role in phagocytosis and inflammatory responses

Of the few remaining types of CNS cells, some of the most interesting and enigmatic cells are the microglia (Graeber, 2010). The microglia are of mesodermal origin, are located in normal brain in a resting state (Fig. 1-14), and convert to a mobile, active brain macrophage during disease or injury (van Rossum & Hanisch, 2004) (Ch. 34). Microglia sense pathological changes in the brain and are the major effector cell in immune-mediated damage in the CNS. However, they also express immunological molecules that have functions in the normal brain. Indeed, microglia in healthy tissue behave very differently from macrophages and should be considered a distinct cell type (Graeber, 2010).

Microglia are pleiotropic in form, being extensively ramified cells in quiescent state and converting to macrophage-like amoeboid cells with activation. The availability of new selective stains for different stages of activation has expanded our understanding of their number, location and properties (Graeber, 2010). Nonactivated microglia have a thin rim of densely staining cytoplasm that is difficult to distinguish from the nucleus. The nucleochromatin is homogeneously dense and the cytoplasm does not contain an abundance of organelles, although representatives of the usual components can be found. During normal wear and tear, some CNS elements degenerate and microglia phagocytose the debris (Fig. 1-14). Their identification and numbers, as determined by light microscopy, differ from species to

species. The CNS of rabbit is richly endowed. In a number of disease instances, such as trauma, microglia are stimulated and migrate to the area of injury, where they phagocytose debris. As our understanding of microglia expands, the number of functions and pathologies in which they play a role increases.

Ependymal cells line the brain ventricles and the spinal cord central canal

They typically extend cilia into the ventricular cavity and play numerous roles in development and maintenance of the nervous system (Del Bigio, 2010). One emerging aspect of ependymal cells is their role in supporting neurogenesis in subventricular zones. They express aquaporins and help regulate the fluid balance of the brain. Defects in ependymal cells can produce hydrocephalus, and these cells are particularly vulnerable to viral infections of the nervous system.

The presence of motile cilia is a hallmark of the ependymal cell. The cilia emerge from the apical pole of the cell, where they are attached to a blepharoplast, the basal body (Fig. 1-15), which is anchored in the cytoplasm by means of ciliary rootlets and a basal foot. The basal foot is the contractile component that determines the direction of the ciliary beat. Like all flagellar structures, the cilium contains the common microtubule arrangement of nine peripheral pairs around a central doublet (Fig. 1-15). In the vicinity of the basal body, the arrangement is one of nine triplets; at the tip of each cilium, the pattern is one of haphazardly organized single tubules. Also extending from the free surface of the cell are numerous microvilli containing actin microfilaments (Fig. 1-15). The cytoplasm of ependymal cells stains intensely, having an electron density comparable to oligodendrocyte, whereas the nucleus is more similar in density to that of the astrocyte. Microtubules, large whorls of filaments, coated vesicles, rough ER, Golgi apparatus, lysosomes and abundant small, dense mitochondria are also present in ependymal cells.

The base of the cell is composed of inviolated processes that interdigitate with the underlying neuropil (Fig. 1-15). Tight junctions between ependymal cells make them an effective component of the brain–cerebrospinal fluid barrier. The coordinate action of various cells to isolate the brain illustrates multiple elements of cell biology of the brain and will be considered in greater depth.

BLOOD–BRAIN BARRIERS AND THE NERVOUS SYSTEM

Homeostasis of the central nervous system (CNS) is vital to the preservation of neuronal function

In order to create a stable environment, the CNS milieu is separated from the vasculature by two main interfaces: the blood–brain barrier (BBB) and the blood–cerebral spinal (CSF) fluid barrier (BCSFB) (Fig. 1-16). In addition, the arachnoid epithelium underlying the dura mater is a third interface, though a significantly smaller one than the BBB and BCSFB.

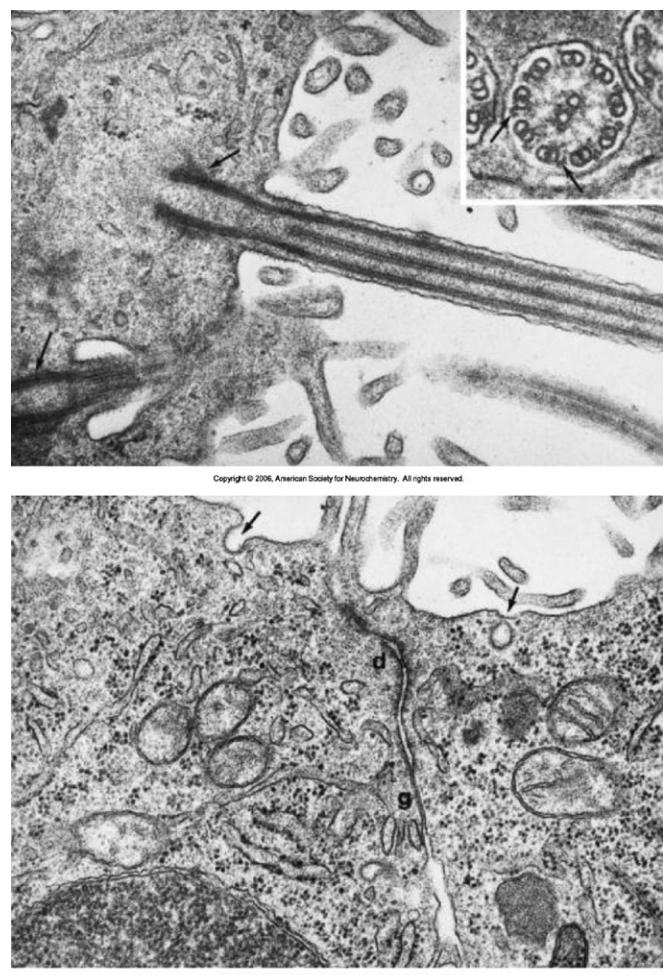


FIGURE 1.15 Ependymal cells are highly ciliated and linked by tight junctions. **Top panel:** The surface of an ependymal cell. Surface contains basal bodies (arrows) connected to the microtubules of cilia, seen here in longitudinal section. Several microvilli are also present. $\times 37,000$. **Inset:** Ependymal cilia in transverse section possess a central doublet of microtubules surrounded by nine pairs, one of each pair having a characteristic hook-like appendage (arrows). $\times 100,000$. **Bottom panel:** A typical desmosome (d) and gap junction (g) between two ependymal cells. Microvilli and coated pits (arrows) are seen along the cell surface. $\times 35,000$.

The BBB, with an estimated surface area 5000 times larger than the BCSFB, is the largest barrier and forms a primary role in the barrier function of the brain.

The BBB is defined by the restricted permeability of brain capillaries compared to capillaries of other organs, particularly with regard to hydrophilic (water-soluble) molecules. The BBB comprises the largest area of brain–blood contact, with a surface area between 10–20 m². The extent of this vascularization is such that it is estimated that each neuron is perfused by its own blood vessel. Specialized brain endothelial cells (BECs) that line brain capillaries are a key component of the BBB. Neighboring BECs are connected by tight junctions (TJ), also called zonulae occludens. TJ reduce the paracellular (between cells) movement of molecules, creating a

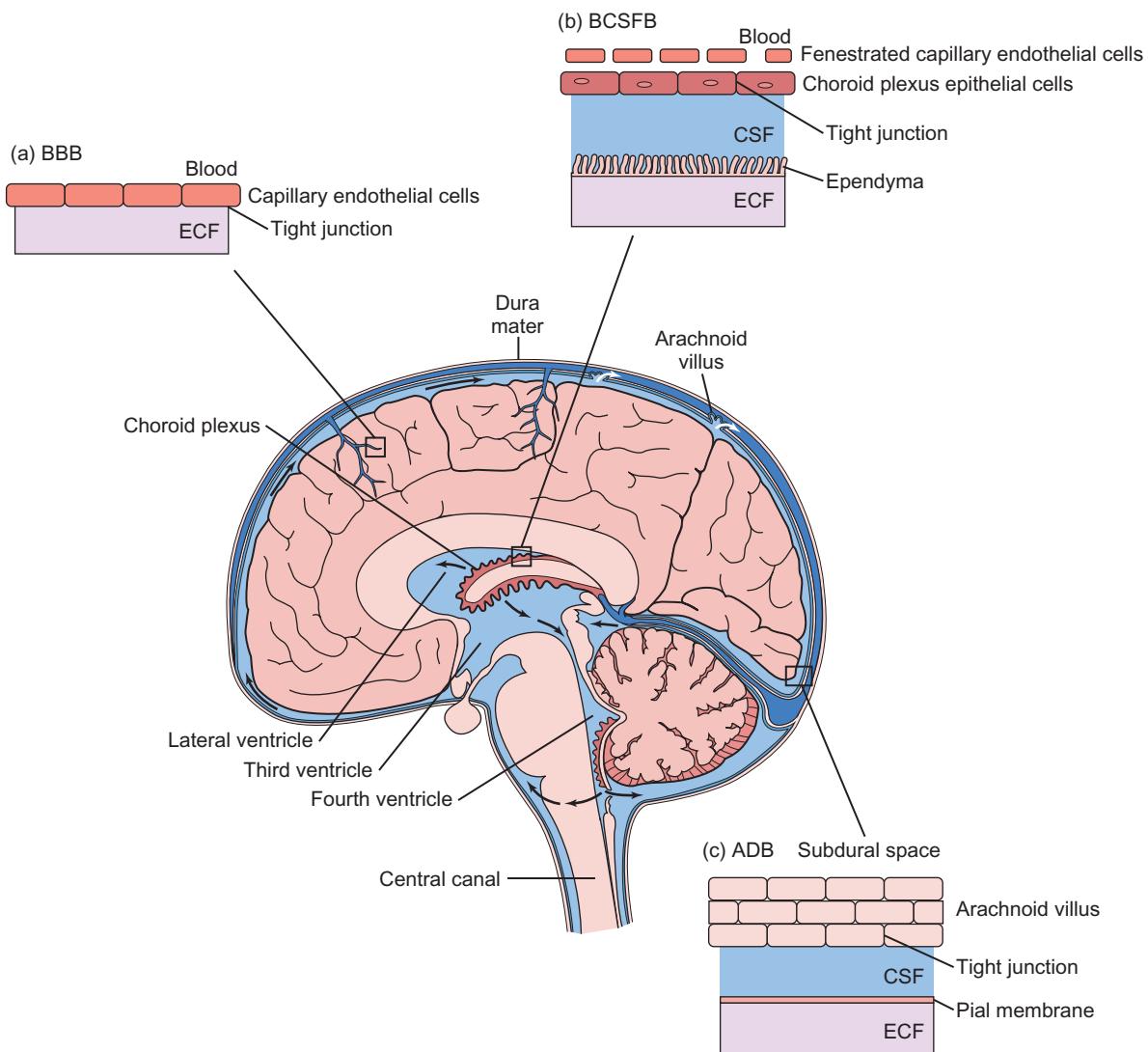


FIGURE 1.16 The brain tissue is separated from the plasma by three main interfaces: (a) blood–brain barrier (BBB), (b) blood–cerebral spinal fluid barrier (BCSFB) and (c) arachnoid cells underlying the dura mater. (a) The BBB comprises the largest area of blood–brain contact with a surface area of $10\text{--}20\text{ m}^2$. Specialized brain endothelial cells form the barrier function of the BBB restricting paracellular (via tight junction proteins) and transcellular (via transporters and enzymes) transport. (b) BECs at the choroid plexuses are fenestrated and leaky; however, tight junctions (TJs) of epithelial cells form the BCSFB. (c) Arachnoid cells under the dura mater comprise the barrier of the arachnoid–dura barrier. Adapted from Abbott et al., (2010).

physical barrier. BECs also form metabolic and transport barriers to many molecules through enzymes and transporters, while allowing access of essential nutrients to the brain (see Ch. 3).

The BSCFB occurs at the choroid plexuses. The choroid plexuses are highly vascular, branched structures that project into the ventricles of the brain. Epithelial cells at the choroid plexus produce cerebral spinal fluid (CSF) with a turnover rate of around four times every 24 hours. BECs at the choroid plexus differ from those in most of the brain in that BECs are fenestrated. However, epithelial cells surrounding the endothelial cells have TJ that constitute the BCSFB barrier.

In addition to the choroid plexus, there are a few other regions in the CNS where BECs are fenestrated. These are

collectively called the circumventricular organs (CVOs). CVOs are ventricularly located structures that carry out the two main functions of neu-ropeptide secretion (e.g., medial eminence, pineal gland) and hormone/neurotransmitter detection (e.g., subfornical organ). At these sites, specialized ependymal cells/tanyocytes are linked by TJ to act as a barrier.

The BBB and BCSFB serve a number of key functions critical for brain function

First, the BBB regulates *ionic gradients*. Neuronal action potentials depend on the intra- and extra-neuronal

TABLE 1.1 Concentration of Plasma and CSF Levels for Selected Ions and Molecules in Humans. Notice that K⁺ ions, amino acids like glutamate and selected plasma proteins are all present in the plasma at much higher concentrations than the CSF, and could cause neuronal toxicity if allowed to enter unhindered into the brain.

Solute	Units	Plasma	CSF	Ratio
Na ⁺	mM	140	141	1
K ⁺	mM	4.6	2.9	0.63
Ca ²⁺	mM	5	2.5	0.5
Mg ²⁺	mM	1.7	2.4	1.4
Cl ⁻	mM	101	124	1.23
Glucose	mM	5	3	0.6
Amino Acids				
Glutamate	μM	83	1.8-14.7	0.02-0.18
Glycine	μM	249	4.7-8.5	0.012-0.034
Taurine	μM	78	5.3-6.8	0.07-0.09
Alanine	μM	330	23.2-37.8	0.07-0.1
Proteins				
Albumin	mg/ml	42	0.192	0.005
Transferrin	mg/ml	2.6	0.014	0.005
Fibrinogen	mg/ml	325	0.00275	0.000008
Immunoglobulin	mg/ml	9.87	0.012	0.001
a2-macroglobulin	mg/ml	3	0.0046	0.0015

Adapted from Abbott et al., (2010)

concentrations of ions, specifically K⁺ and Na⁺. These ionic concentrations must be maintained within a narrow range to allow efficient neuronal firing and neurotransmitter release. Plasma K⁺ levels are 1.5 times higher than the CSF (CSF levels are a guide for brain concentrations in the absence of interstitial fluid measurements), and K⁺ ions are excluded from the brain by the BBB (Table 1-1).

Second, the BBB controls protein, metabolite and toxin exchange. Blood plasma contains many elements that are relatively nontoxic in the periphery, but interfere with neuronal function and may be neurotoxic if allowed to enter the brain along their concentration gradients (Table 1-1). For example, plasma levels of the excitatory neurotransmitter glutamate are more than five times greater than levels in CSF. Similarly, some high-molecular weight proteins abundant in plasma (e.g., albumin, plasmin and thrombin) can induce neuronal apoptosis, as can certain ingested xenobiotics and by-products of digestion or physiological metabolism.

Third, the BBB allows nutrient exchange. The brain utilizes 20% of the total body oxygen consumption and a comparable

fraction of glucose. However, energy reserves in the brain are limited and a constant supply is critical. Since the BBB excludes many hydrophilic molecules, transporters allow nutrients and signaling molecules brain access e.g., glucose transport via glucose transporter-1 (glut-1) (Ch. 3).

Fourth, the BBB and choroid plexus control the composition of extracellular fluid (ECF). CSF produced by the choroid plexuses, in particular at the lateral ventricles, acts to cushion the brain, reduces brain weight by up to a third and provides drainage for the ECF. The ECF supplied by the BBB is often referred to as the interstitial fluid (ISF), although the distinction between CSF and ISF is not always clear because the ISF drains partially into the CSF. The BBB also produces a slow ISF drainage via osmotic and ionic gradients. Overall, the BBB and BCSFB not only control ECF composition but also generate a flow to allow metabolite drainage.

Evolution of the blood–brain barrier concept

The concept of the BBB was first proposed over 100 years ago by Paul Erlich as an explanation for exclusion of hydrophilic aniline dyes from the brain when administered into the peripheral vasculature. Trypan blue injected directly into the CSF stained all brain cell types, whereas intravenous application did not, adding further evidence for a barrier function protecting the brain. These and other findings sparked a search for the cellular basis for the BBB, but it was not until the advent of electron microscopy that BECs emerged as a central component of the BBB.

During the 1960s, horseradish peroxidase (HRP) had been employed as an enzymatic tracer for vascular transport studies due to high sensitivity of detection and low molecular weight (MW 43,000). HRP passes from the circulation to extravascular spaces by diffusion through gaps between ECs and via pinocytotic vesicles in cardiac and skeletal muscle. However, Reese and Karnovsky (1967) demonstrated that HRP administered via the tail veins of mice was undetectable in the brain tissue. These studies established that neighboring BECs overlap and are linked together by TJ (Fig. 1-17), thereby preventing the intercellular/paracellular passage of HRP. Based on this identification of BECs as a key component of the BBB, subsequent experiments yielded a detailed understanding of the BBB.

THE NEUROVASCULAR UNIT INCLUDES MULTIPLE COMPONENTS

The lumen of the cerebral capillaries that penetrate and course through the brain tissue are enclosed by BECs interconnected by TJ

Pericytes are small connective tissue cells containing smooth muscle myosin sparingly attached to the abluminal (brain-side) surface of BECs, with both cells surrounded by a basal lamina or basement membrane. In addition, astrocytic end feet are contiguous with the basal lamina and surround the BECs. The term neurovascular unit has recently been introduced to describe the interactions between BECs,

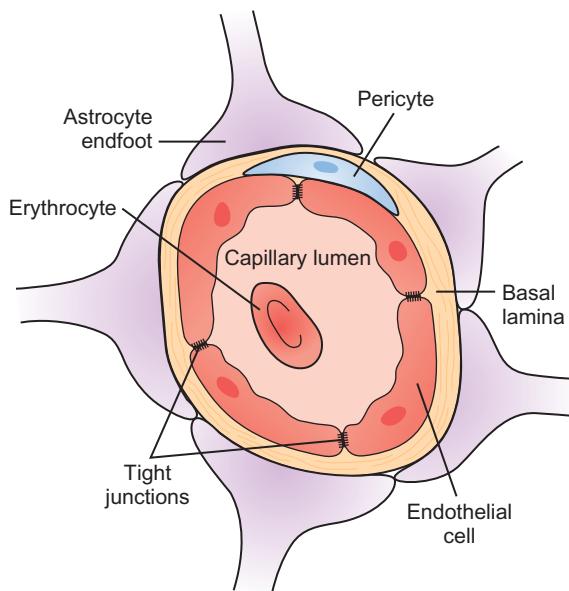


FIGURE 1.17 Neurovascular unit. Brain capillaries are lined by specialized endothelial cells, which are intermingled with pericytes and surrounded by a basal lamina. Astrocytic end feet surround the basal lamina.

astrocytes, pericytes, neurons and neighboring glia (Paolinelli et al., 2011). Astrocytes and pericytes lie closest to BECs. Both cell types have been demonstrated to play a role in the maintenance and induction of the BBB. The key features of the neurovascular unit shall now be discussed in detail.

The basement membrane (BM)/basal lamina is a vital component of the BBB

The BM surrounds BECs and pericytes and acts to hold the cells in their place (Ch. 9). The 20–200 nm thick BM is a mixture of different classes of extracellular matrix proteins including structural proteins (collagen type IV); adhesion proteins (laminin, fibronectin); and heparan sulfate proteoglycans (perlecan, agrin) among others. BECs, astrocytes and pericytes all bind to the BM via specific receptors. Integrins are the major class of receptors expressed by all cell types in the neurovascular unit, and exist in different subclasses to allow binding to different components of the BM, e.g., collagen or laminin. In addition, the non-integrin receptor dystroglycan mediates binding to the proteoglycans and laminin. All cells of the neurovascular unit secrete components of the basement membrane. Matrix metalloproteinases (MMP) can actively digest the membrane, and are found up-regulated in inflammatory disease, e.g., multiple sclerosis (Ch. 39).

Astrocytes contribute to the maintenance of the BBB

The close proximity of astrocytic foot processes to BECs provided the basis for the view that astrocytes regulate the

BBB. Indeed, the loss of astrocytes is associated with a loss of BBB integrity *in vivo*. Co-culture of isolated BECs with astrocytes or astrocyte-conditioned media modifies BEC functions such as reduced permeability, and increased transporter protein expression (e.g., glut-1 and TJ proteins). A combination of astrocyanic secreted factors (e.g., basic fibroblast growth factor, transforming growth factor beta, angiopoietin-1, glia-derived neurotrophic factor, lipoproteins) and the physical presence of astrocytes contribute to the maintenance of the BBB. BECs can also influence astrocytic properties through a two-way communication.

Pericytes at the BBB are more prevalent than in other capillary types

At the rat BBB, there is one pericyte to every 5 ECs compared to 1 per 100 in skeletal muscle capillaries. Thus, association of pericytes is correlated with TJ expression and barrier function in endothelia. The presence of pericytes appears critical in BBB development and BM formation. Recruitment of pericytes to BEC during development is in part mediated by platelet-derived growth factor (PDGF), and accordingly PDGF knockout mice develop numerous aneurisms, possibly due to capillary wall instability. An early observation of pericytes' ultrastructure was that they contain smooth muscle actin. Pericytes have been demonstrated to contract in *in vivo* and *in vitro*, indicating a role in controlling blood flow. During hypoxia and traumatic brain injury, both conditions associated with an increased BBB permeability, pericytes migrate away from BECs, highlighting the importance of pericytes in BBB maintenance.

Brain endothelial cells restrict the transport of many substances while permitting essential molecules access to the brain

BECs at the BBB are thin cells (500 nm), but create an effective barrier. The presence of TJ between BECs results in a decreased paracellular permeability of the BBB to many molecules, especially ions. Reduced ion permeability induces a high BBB electrical resistance of 1000–2000 ohms·cm² compared to peripheral vessels (2–20 ohms·cm²). BECs also differ from peripheral EC by high mitochondrial content (\approx 10% of total cytoplasmic volume compared to \approx 4% in peripheral ECs), consistent with high levels of energy utilization. ATP-dependent transporters act to transport blood-borne harmful agents back into the blood (e.g., P-glycoprotein, see below) and to maintain ionic and nutrient homeostasis. BECs may have lower levels of endocytosis.

BECs are linked together by a complex of proteins, which span the inter-endothelial space and form homo (to the same protein) or hetero (to different proteins) interactions with adjacent cells, sealing the interendothelial cleft. Accessory proteins link these transmembrane proteins to the actin cytoskeleton, providing structural support. Adheren junctions and TJ are the main types of interendothelial protein complexes present on BECs at the BBB. TJ proteins are regulated both in normal physiology and in pathological states and are thus highly dynamic. In addition to reducing paracellular permeability, TJ help maintain the polarization of BECs by

preventing exchange of transporters between the abluminal (brain-side) membrane and luminal (blood-side) membrane.

A detailed description of TJ components and structure is beyond the scope of this chapter (Abbott et al., 2010). Adheren junctions are found throughout the vasculature and are essential for TJ formation, EC adhesion and paracellular permeability, but TJ differentiates BECs from other ECs. TJ proteins are composed of transmembrane proteins, which span the membrane and bind to those on adjacent cells. Cytoplasmic accessory proteins bind to the transmembrane proteins and mediate their connection to a pericellular ring of actin filaments. Junctional adhesion molecules (JAMs), occludin and claudin family members, form the transmembrane components of TJ, while zonulae occludens 1, 2 and 3 (ZO-1,2,3), cingulin, AF-6 and 7H6 act as cytoplasmic accessory proteins. Signaling molecules associated with the TJ complex affect the TJ permeability.

TJ structures are highly dynamic and can be modulated continually by locally secreted molecules or over longer periods of time (Nag, Kapadia et al., 2011). BEC permeability is regulated via TJ translocation, decreased TJ protein expression and/or phosphorylation of the TJ complex. There are multiple extracellular mediators that can induce a leaky BBB, including cytokines (e.g., TNF-, IL-1); oxidative stress (nitric oxide); inflammatory mediators (e.g., prostaglandins and histamine); and disease-associated cells (HIV virus, bacteria) or proteins (amyloid beta) (see also Chs. 34–36). TJ-altering mediators often act via intracellular signaling pathways including protein kinase family (A, G and C); mitogen-activated protein kinase family (MAPK, including JNK, ERK and p38MAPK); the small G protein family (especially rho and rac); and wnt and eNOS pathways. Intracellular Ca^{2+} ions are also key

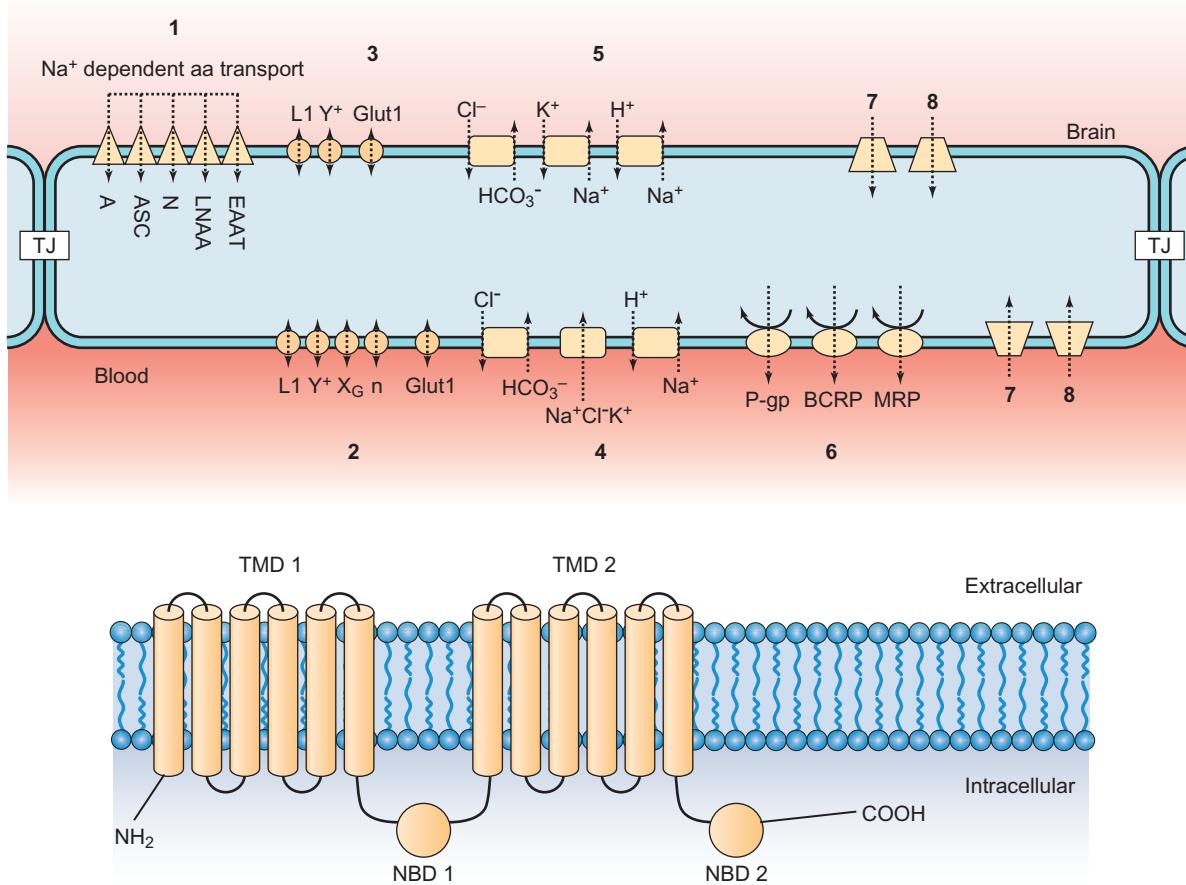


FIGURE 1.18 Transport mechanisms at the blood–brain barrier. **(a)** BECs contain a number of transport mechanisms to allow homeostatic control of nutrients, ions and signaling molecules. **(1)** Na^+ dependent symporters (A, ASC, LNAA, EAAT) eliminate amino acids from the brain, thus preventing excess accumulation. **(2)** Facilitated diffusion allows essential amino acids ($\text{L}1, \text{Y}^+$) and glucose (Glut-1) into the brain and elimination of excitatory amino acids (N, X_G) into the blood across the luminal and **(3)** abluminal membrane. **(4–5)** Ion transporters at the BBB regulate extracellular K^+ and Na^+ ions and intracellular pH. **(6)** ABC transporters (P-gp, BCRP, MRP) protect the brain from toxins circulating in the blood. **(7)** Receptor-mediated transport allows essential proteins and signaling molecules into the brain (e.g., insulin receptor, transferrin receptor). Receptors can also mediate export of materials from brain. **(8)** Adsorptive mediated transcytosis (AMT). At physiological pH the glycocalyx of the luminal BEC membrane has an overall net negative charge, which allows cationic molecules access to the brain via non-specific transcytosis. AMT also goes in the brain-to-blood direction. **(b)** Structure of P-gp. P-gp is a transmembrane efflux transporter consisting of two transmembrane (TMD) domains and two nucleotide-binding (NBD) domains. P-gp uses the energy derived from ATP to actively prevent the blood-to-brain transport of many substances.

regulators of TJ protein distribution (see specific discussions in relevant portions of Chs. 21–27).

There are multiple transporters and transport processes for bidirectional transport at the BBB

BBB transport mechanisms are often divided into different categories: passive diffusion, efflux transporters, solute carriers, receptor-mediated transcytosis, and immune cell migration (Fig. 1-18). Further subdivisions are based on their

specific transport properties, such as Na^+ -dependent facilitated diffusion or macromolecule transport (Ch. 3). Table 1-2 shows examples of some important receptors of the BBB.

Lipid solubility is a key factor in determining the permeability of a substance through the BBB by passive diffusion

Substances with a high oil/water-partitioning coefficient diffuse through plasma membranes and into the brain. Diffusion across the plasma membrane is the primary route of

TABLE 1.2 Examples of Transporters at the BBB and Their Ligands. Luminal = L, Abluminal = Ab, Intracellular IC. (Deli, 2009; Nag et al., 2011; Pardridge, 2008)

Transporter	Symbol	Substrate examples	Location
ION TRANSPORTERS			
Na^+/K^+	–	Na^+, K^+	Ab
Na^+/H^+	–	Na^+, H^+	L, Ab
$\text{Cl}^-/\text{HCO}_3^-$	–	$\text{Cl}^-, \text{HCO}_3^-$	L, Ab
$\text{Na}^+-\text{Cl}^--\text{K}^+$	–	$\text{Na}^+-\text{Cl}^--\text{K}^+$	Ab
SOLUTE TRANSPORTERS			
Glucose transporter -1	Glut-1	Glucose	L, Ab, IC
Essential neutral amino acid system	L1	Leucine, valine, isoleucine, phenylalanine, tryptophan	L, Ab
Cationic amino acid system	Y^+	Lysine, arginine and ornithine	L, Ab
Acidic amino acid system	Xg	Glutamate, arginine	L
Glutamine	n	Glutamine	L
<i>Na⁺-dependent amino acid transporters:</i>	A	Alanine	Ab
	ASC	Alanine, cysteine, serine	Ab
	N	Glutamine, asparagine histidine	Ab
	EAAT	Aspartate, glutamate	Ab
	L	Asparagine, isoleucine	Ab
RECEPTOR MEDIATED TRANSCYTOSIS			
Insulin	IR	Insulin	L
Transferrin	TfR	Iron-transferrin	L
Low density receptor related protein 1	LRP1	Lipoproteins, $\text{A}\beta$	L, Ab
Immunoglobulin G	$\text{fc}\gamma\text{-R}$	IgG	L
Leptin	–	Leptin	L
Receptor for advanced glycosylation end-products	RAGE	Glycosylated proteins, S-100	L
ABC TRANSPORTERS			
P-glycoprotein	P-gp	Anticancer drugs (vinblastine) Anthypertensives (Celiprolol) L Antidepressants (Amitriptyline) HIV protease inhibitors (Amprenavir, indinavir) Fluorescent dyes (rhodamine 123)	L
Breast cancer resistance protein	BCRP	Anticancer drugs (anthracyclines) Flavonoids, Sulphated estrogens	L

entry for dissolved gases to and from the brain, i.e., O₂ into and CO₂ out. In addition, BECs at the BBB carry an overall negative surface charge on their luminal membrane due to heparan sulfate proteoglycans. Thus cationic molecules have a greater brain access than anionic ones. Such considerations are important in design of therapeutic agents with target sites of action in brain.

The BBB expresses solute carriers to allow access to the brain of molecules essential for metabolism

These include glucose, amino acids and some ions (Table 1-1). Glucose is an essential energy source for neurons in the CNS. Glucose transport at the BBB is mediated by the facilitative diffusion through glucose transporter-1 (glut-1, 55 kDa form). Glut-1 is expressed symmetrically on both luminal and abluminal membranes, with an intracellular pool that may be mobilized in times of increased neuronal demand, e.g., for seizures.

The transport of amino acids at the BBB is complex and mediated by both facilitative diffusion and Na⁺-dependent transport (Table 1-2). There are four facilitative BBB amino acid carriers: an essential neutral amino acid system (L1), a cationic amino acid system (Y⁺), an acidic amino acid system (X_G) and a glutamine system (n). System L1, expressed on luminal and abluminal sides, functions to transport essential amino acids (e.g., leucine, valine, isoleucine, phenylalanine, tryptophan). Y⁺, located on abluminal and luminal membranes, is a cationic transporter facilitating lysine, arginine and ornithine transport into brain. Collectively, L1 and Y⁺ allow brain access to all essential amino acids. Systems n and X_G are expressed only on the luminal membranes and have a high affinity for glutamine and glutamate, respectively.

CSF amino acid concentrations are lower than plasma levels (Table 1-1), much lower than expected if facilitative transport were the only mechanism of amino acid transport. Five Na⁺-dependent transporters on the abluminal side eliminate non-essential toxic amino acids and maintain homeostatic control over all amino acids. The Na⁺-dependent transporters are A (non-essential preferring), ASC (alanine, serine, cysteine preferring), N (glutamine, asparagine, histidine), excitatory amino acid transporter family (EAAT, aspartate and glutamate preferring) and L (neutral amino acid system).

Glutamate regulation is an example of the polarized BBB controlling homeostatic levels of a potentially excitotoxic neurotransmitter. The Na⁺-dependent symporter family, EAAT, transports ISF glutamate into BECs using the Na⁺ gradient. Glutamate then diffuses into the blood via system X_G. Glutamate transport into the brain from the plasma is virtually impossible due to the lack of a facilitative glutamate transporter and the high expression of EAAT on the abluminal side. Glutamine is regulated in a similar manner, whereby Na⁺ transporters A and N pump glutamine into BECs. Glutamine is then either transported into the blood via system N or converted to glutamate via glutaminase (Ch. 17).

Free movement of ions across the BBB is limited by TJ, but BECs contain a number of ion transporters to maintain the Na⁺ gradient needed for Na⁺-dependent transporters, to

allow fluid movement, and to control ionic gradients. Na⁺-K⁺ ATPase is located on the abluminal BBB membrane, while a Na⁺/H⁺ exchanger is located on both abluminal and luminal membranes. Further luminal ion transporters include a Na⁺/Cl⁻ co-transporter, a Na⁺/K⁺/Cl⁻ co-transporter and a Na⁺Cl⁻/HCO₃⁻ dependent influx transporter.

Receptor-mediated transcytosis (RMT) is the primary route of transport for some essential peptides and signaling molecules

Examples include insulin, leptin and transferrin (Table 1-2). Transcytosis at the BBB requires ligand-receptor binding, followed by coupled endocytosis and exocytosis. During transcytosis, the lysosomal degradation pathway is bypassed. An important example of RMT is transferrin-bound iron. Iron is an essential trace element and virtually all iron in plasma is bound to hydrophilic transferrin. Iron-bound transferrin is taken up in endocytic compartments via the transferrin receptor. Under the relatively acidic environment of the endosome (Ch. 7), iron-transferrin is assumed to dissociate and become available for uptake after exocytosis.

ATP-binding cassette transporters (ABC) on luminal membranes of the BBB restrict brain entry of many molecules

Although lipid solubility is a good indicator of brain penetration, for some compounds, e.g., phenobarbital, brain penetration is much less than expected. ABC transporters use ATP to actively eject a range of substances, including both xenobiotics and endogenous toxic molecules (Ch. 3). At least 48 ABC transporter genes have been identified and classified into seven subfamilies. ABCA to ABCG, ABCB1/MDR1 (P-glycoprotein, P-gp), ABCG2 (breast cancer resistance protein, BCRP), and ABCC (multidrug resistance protein family, MRP) are the most significant at the BBB. A lack of specific inhibitors has hindered study of MRP, but much more is known about P-gp.

P-gp (170 kDa) was first identified in multidrug resistant cell lines, and in the epithelial cells of many tissues, including the gastrointestinal tract, P-gp expression is on the luminal membrane of the BBB. Two types of P-gp exist, termed type I and type II. Type I is located on epithelia of many organs, including gastro-intestinal tract and kidney, and on ECs in the testes and brain. Type II is expressed by canicular hepatocytes and acts as a bile salt transporter and a flippase for certain membrane lipids. P-gp contains two nucleotide-binding domains and 12 transmembrane segments. P-gp is arranged in two halves (as a barrel-like configuration), with a 75-amino-acid linker region. Each half contains a transmembrane domain (TMD) made of six transmembrane segments and an intracellular nucleotide-binding domain (NBD). Ligand binding to the transmembrane domain is thought to lead to a conformational change in the NBDs, increasing their affinity for ATP. ATP binding induces a conformational change in the TMD to induce ligand translocation. ATP hydrolysis causes dissolution of the closed NBD dimer, releasing ADP and allowing P-gp to return to a high-affinity ligand binding

state. P-gp has a diverse substrate list including endogenous molecules (e.g., steroid hormones, cytokines) and pharmaceutical drugs, including anticancer drugs (vinblastine, verapamil) and HIV-protease inhibitors. P-gp substrates are in general hydrophobic, but identifying whether a new drug will be a P-gp substrate based on structure is extremely difficult. The specificity of P-gp prevents many therapeutic drugs from reaching their target site, thus preventing their effective use.

During development, immune-competent microglia develop and reside in the brain tissue

After development, under physiological conditions, the brain is considered a relatively immune privileged site. Although leukocytes and immune cells can enter the brain, immune surveillance is much lower than in other organs. For example, leukocyte traffic into the brain has been estimated at 100 fold less than into the spleen or lungs. Decreased CNS immune surveillance is in part mediated by the barrier nature of the cerebral vasculature but, more importantly, also is mediated by the restricted expression of cerebral cell adhesion molecules required for leukocyte capture from the blood. During inflammatory conditions, there is a marked up-regulation of these markers, which can result in neuronal damage in disease states such as multiple sclerosis.

Immune cells cross into the brain at the level of the postcapillary venule, which is arranged slightly differently than other capillaries. BECs at these sites have fewer TJ compared to capillaries and are more subject to modulation. At the postcapillary venule, BECs are intermingled with pericytes and surrounded by a BM. At this site, there is a BM layer of variable size and a perivascular space, which is enclosed by a parenchymal BM and astrocytic end feet. Leukocyte diapedesis occurs in a two-step process across the postcapillary venule (Greenwood et al., 2011). Initially, leukocytes are captured by P-selectin and vascular cell adhesion molecules (VCAM) (expressed on BECs). Chemokines produced locally up-regulate integrins in leukocytes, resulting in a firm interaction between BEC and leukocytes. This tight binding allows leukocyte diapedesis both through the BECs (transcellular transport) and between cells (paracellular transport). Transcellular transport is hypothesized to occur under more physiological conditions to prevent TJ disruption, but opening of TJ under inflammatory conditions increases paracellular transport. After gaining access to the perivascular space, the leukocyte crosses the parenchymal basement membrane mediated by matrix metalloproteinase digestion.

There is increasing evidence of BBB dysfunction, either as a cause or consequence, in the pathogenesis of many diseases affecting the CNS

BBB dysfunction is found in many pathological states including Alzheimer's disease (AD), multiple sclerosis (MS)

and stroke, among others. A wide variety of conditions have the potential to compromise the BM or TJ, including brain tumors, inflammation, MS, infections, trauma and stroke. Many of these conditions are discussed in detail in other chapters. Compromises in the BBB and associated inflammation exacerbate some conditions such as AD and are a primary mechanism in certain pathologies such as MS.

BBB breakdown that occurs in acute brain injury, e.g., infarcts and trauma, can lead to severe brain edema (increase in brain fluid volume), increased intracranial pressure, and, potentially, death (Ch. 35). In the initial stages after brain injury, there is an increase in the number of caveolae/vesicles that transport plasma proteins across the BBB, beginning a process of BBB breakdown. The expression of TJ proteins also decreases and the actin cytoskeleton becomes disrupted, as does the BM. Often a repair phase occurs with angiogenesis resulting in a second stage of BBB breakdown. As an example, cerebral ischemia is an event whereby parts of the brain do not receive sufficient blood supply to maintain neuronal function. Ischemic events, e.g., stroke, can lead to increased microvascular permeability and edema both clinically *in vivo* and *in vitro*. Reduced occludin expression and increased brain permeability are found under hypoxic conditions in experimental models.

The presence of an intact BBB affects the success of potentially beneficial therapies for many CNS disorders

For example, P-gp and BCRP expressed by BECs hinder access to the brain of many drugs used to treat epilepsy (where increased P-gp expression is also found), brain tumors and depression. Similarly, many drugs effective in peripheral infectious diseases are not effective in CNS infections, due to restricted brain penetration for both low-molecular weight compounds and larger peptide and antibody pharmaceuticals. To address this issue, there is considerable interest in developing methods to overcome the BBB for drug delivery, including temporary barrier disruption and the use of receptor-mediated transport. These may take the form of receptor-mediated drug transport through modifications of the drug (Pardridge, 2008) or of opening the BBB transiently to allow drug brain access (Deli, 2009). This latter method is already in limited therapeutic use for anticancer drugs in the treatment of brain tumors. Mannitol injections cause the plasma to become hyperosmotic compared to the inside of BECs, and water leaves the brain by osmosis. The loss of water causes BECs to shrink and the TJ structures are pulled away from each other, creating a transient opening of the barrier for drug entry. However, the efficacy of these approaches in the clinics remains to be proven.

Acknowledgements

The authors would like to acknowledge their substantial debt to the chapters written for previous editions by Cedric Raine. This chapter incorporates much of the information and figures generated by Dr. Raine over the years and we are indebted to him for these contributions.

BARDET-BIEDL SYNDROME AND THE NEURONAL PRIMARY CILIUM

Scott T. Brady

One structure often overlooked in considerations of the cell biology of the brain is the primary cilium, but the importance of cilia in neuronal development and function is becoming apparent (Han & Alvarez-Buylla, 2010). In part, this awareness stems from recognition that specific defects in ciliary structure or function can have profound effects on the nervous system as well as other tissues (Cardenas-Rodriguez & Badano, 2009). Most biologists are familiar with the motile cilia, such as those on ependymal cells that line the ventricular surface (Del Bigio, 2010), but most mammalian cells also have a single primary cilium that is nonmotile. This hair-like extension is typically 200–200 nm in diameter and may be as much as 10 µm in length (Han & Alvarez-Buylla, 2010), but is rarely seen in standard histological preparations (Peters et al., 1991). The primary cilium extends from a modified centriole in neurons and neuronal progenitors as well as glia, though the prevalence of these structures in the central nervous system has only come to be appreciated in the last few decades (Louvi & Grove, 2011).

Primary cilia have multiple functions in the central nervous system (Praetorius & Spring, 2005). For example, the photoreceptors of the retina are all modified primary cilia (see Ch. 51). Indeed, primary cilia in the nervous system are best described as sensory organelles that play a key role in mechanosensory and chemosensory functions. The location of specific receptors for signals critical in neurodevelopment on primary cilia is particularly striking (see Ch. 28). Three distinct pathways critical for normal development of the nervous system have been shown to require a primary cilium: Sonic Hedgehog (Shh), platelet-derived growth factor (PDGF) and Wnt signaling (Louvi & Grove, 2011). In mammalian cells, critical components of the Shh pathway are located in the primary cilium and shuttle between cytoplasm and cilium. Shh is critical for ventralization of the neural tube, formation of spinal motor neurons and differentiation of oligodendrocytes, as well as for playing a wide range of other roles in neural development by regulating transcription of specific genes. Similarly, PDGF receptors are localized to the primary cilium and signal through cytoplasmic kinases like Akt and MAP kinases (see Ch. 25). PDGF signaling may be important for cell polarity and regulation of cell migration. Finally, the primary cilia appear to suppress the canonical Wnt pathway mediated by β-catenin and GSK3β. They may activate a noncanonical pathway that orients sheets of neuroepithelial cells and they may influence neuronal migration.

Given these diverse functions, it is not surprising that ciliopathies are pleiotropic and typically affect a wide range of cell types and tissues. Many of the phenotypes reflect alterations in brain structure or function. One example of human disease associated with defects in primary cilia is Bardet-Biedl Syndrome (BBS), a genetically heterogeneous autosomal recessive disease that results

from mutations in 1 of 12 genes (Sheffield, 2010). The syndrome was first recognized as a discrete pathology in 1920, but the role of cilia in this disease was not recognized for more than 60 years. These gene products form a complex associated with the *basal body* (kinetosome, organelle formed from a centriole and a short cylindrical array of microtubules found at the base of the cilium). BBS gene complexes are required for the generation and maintenance of cilia, both primary and motile. BBS patients exhibit a constellation of symptoms that include both neuronal and non-neuronal pathology. Pathologies involving the nervous system include degeneration of photoreceptors, anosmia, mental retardation or developmental delay, posterior encephalocele (a neural tube defect caused by the tube's failure to completely close), and obesity. Nonneuronal effects may include hypogonadism, kidney defects, polydactyly, diabetes, and *situs inversus* (randomization of normal organ locations, i.e., heart on right side instead of left side of chest). Although some of these pathologies, such as *situs inversus* and hypogonadism, are likely due to loss of motile cilia, others are clearly due to a loss of nonmotile, primary cilia. For example, failure to maintain sensory primary cilia is associated with retinal degeneration and renal failure. In turn, loss of signaling through primary cilia is likely to contribute to mental retardation, obesity, and anosmia, among other issues. Until the role of the primary cilium was recognized, the pleiotropic nature of BBS had baffled physicians looking for a common thread through all of these pathologies. Thus, a better understanding of the cell biology of the nervous system has illuminated a baffling and complex set of genetic disorders.

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