CHAPTER 3
Neurocytology

This chapter focuses on the two major cell types that form the nervous system: supporting cells and conducting cells. The supporting cells consist of the glia, epedenymal cells lining cells the ventricles, the meningeal coverings of the brain, the circulating blood cells, and the endothelial lining cells of the blood vessels. The conducting cells, or neurons, form the circuitry within the brain and spinal cord and their axons can be as short as a few microns or as long as one meter. The supporting cells are constantly being replaced, but the majority of conducting cells/neurons, once formed, remain throughout our lives.

Any investigation of the structure of the nervous system is complicated by the fact that no single stain demonstrates all details of a neuron or of the glia. Instead, many techniques are used for microscopic examination of the nervous system. But before nerve tissues can be examined, they must be preserved (fixed). Neutral-buffered formalin is the most commonly used fixative in light microscopy.

Golgi Neuronal Method (Figs. 3-1 and 3-2)

The shapes of neurons and glia can best be seen by means of the Golgi neuronal method. Brain slices 3 to 5 mm thick (either fixed or unfixed, normal or abnormal, from vertebrates or invertebrates) are encrusted with heavy metals (usually dichromate or mercury) and then immersed in silver nitrate. With the Golgi method, only about 1 in every 70 cells stains completely and reveals the axon, soma, dendrites, and dendritic spines in full detail. With either a camera lucida or the modern technique of image analysis, one is able to fully reconstruct the cell and determine the morphology of normal or diseased cells.

With the Golgi neuronal method some of the most elegant cells have been identified, including the Purkinje cell of the cerebellum, the pyramidal cell of the cerebrum *(Fig 3-1),* the stellate cells of the cerebrum *(Fig 3-3)* and the mitral cell of the olfactory bulb. A disadvantage is that it does not

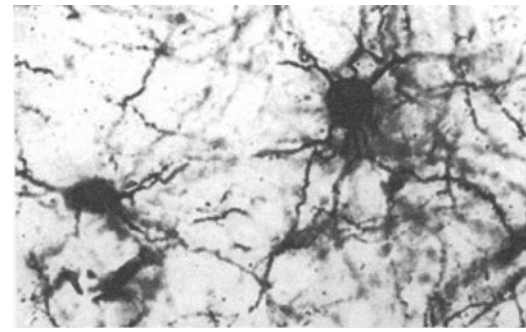

Figure 3-1. Golgi type II cells in the motor cortex of the rat. (Golgi-Cox stain, <X>450.)

reveal details of the neuron=s internal structure. However, the most pronounced organelle in the soma, the rough endoplasmic reticulum, or Nissl substance, is demonstrable with basophilic dyes.

The Neuron

The basic functional unit of the nervous system is the neuron. The neuron doctrine (postulated by Waldeyer in 1891, described the neuron as having one axon, which is efferent, and one or more dendrites, which are afferent. It was also noted that nerve cells are contiguous, not continuous, and all other elements of the nervous system are there to feed, protect, and support the neurons.

Although muscle cells can also conduct electric impulses, only neurons, when arranged in networks and provided with adequate informational input, can respond in many ways to a stimulus. Probably the neuron's most important feature is that each is unique. If one is damaged or destroyed, no other nerve cell can provide a precise or complete replacement. Fortunately, though, the nervous system was designed with considerable redundancy, so it takes a significant injury to incapacitate the individual (as in Alzheimer's disease).

Neuronal parts and their functions are shown in Figure 3-2 and Table 3-1.

Neurons in the adult nervous system are either pseudounipolar, bipolar, or multipolar.

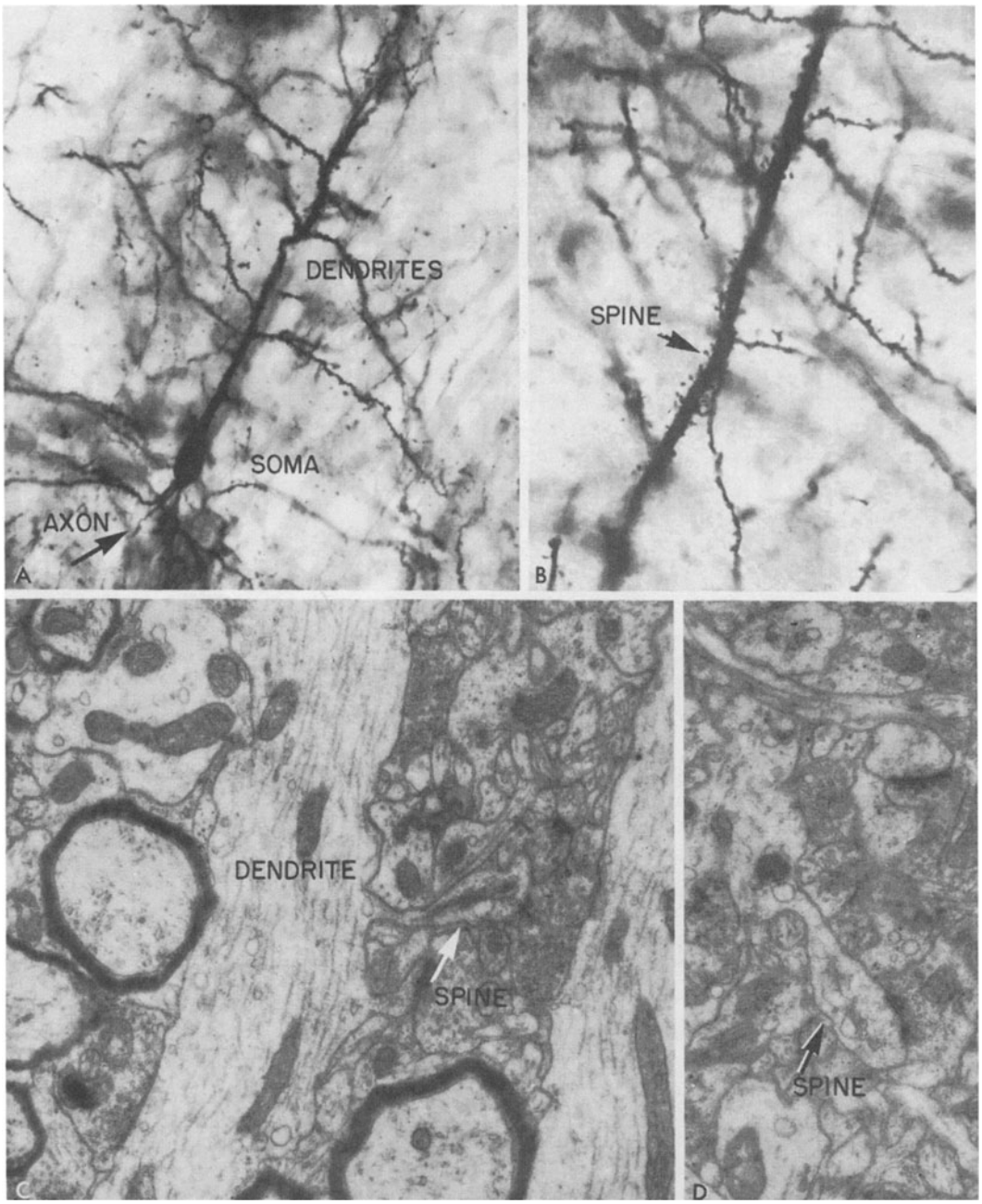

Figure 3-2. Golgi type I cells in the motor cortex of a rat. A, shows entire cell--soma, axon, and dendrite (Golgi rapid stain, <X>100). B demonstrates dendritic spines (Golgi rapid stain, <X>350). C and D are electron micrographs of dendritic spines (<X>30,000).

True unipolar cells are found in the invertebrate nervous system (Fig 1-1). In the mammalian central nervous system pseudounipolar cells (fig 1-1) are found in the sensory ganglia of the spinal cord (dorsal root ganglia) and cranial nerves and in the mesencephalic nucleus of cranial nerve V, as a single process acts as the axon and the dendrite. *Bipolar neurons(fig 1-1)*, which are sensory in function, are found in the rods and cones of the retina, in the olfactory neuroepithelial cells, in the olfactory mucosa at the upper end of the nasal passages, and in the vestibular and auditory

TABLE 3-1. PARTS OF A NEURON

Soma	The neuron=s trophic center, containing the nucleus, nucleolus, and many organelles. The majority of inhibitory synapses are found on its surface.
Dendrites	Continuation of the soma; has many neurotubules and majority of synapses on its surface; type I neurons have dendritic spines
Axon	Conducts action potentials to other neurons via the synapse. Ranges from a few millimeters to a meter in length; In CNS covered by myelin, an insulator.
Synapse	The site where an axon connects to the dendrites, soma or axon of another neuron. Consists of a presynaptic part containing neurotransmitters and postsynaptic portion with membrane receptors separated by a narrow cleft.

receptors of the inner ear. *Multipolar neurons(Fig 1-1)* are found throughout the central nervous system and in the sympathetic ganglia of the peripheral nervous system. They convey both sensory and motor impulses. Multipolar neurons vary greatly in size and in the complexity of their axonal and dendritic fields.

Dendrites

The dendritic zone receives input from many different sources. The action potential originates at the site of origin of the axon and is transmitted down the axon in an all-or-nothing fashion to the synapse, where the impulse is transmitted to the dendritic zone of the next neuron on the chain.

Dendrites have numerous processes that increase the neuron=s receptive area. The majority of synapses on a nerve cell are located on the dendrite surface. With the electron microscope the largest dendrites can be identified by the presence of parallel rows of neurotubules, which may help in the passive transport of the action potential *(Fig. 3-5, 3-12)*. The dendrites in many neurons are also studded with small membrane extensions, the dendritic spines.

Soma

The soma (perikaryon, or cell body) of the neuron varies greatly in form and size. Unipolar cells have circular cell bodies; bipolar cells have ovoid cell bodies; multipolar cells have polygonal cell bodies.

Golgi Type I and II Neurons

Neurons can also be grouped by axon length: those with long axons are called Golgi type I (or pyramidal) cells; those with short axons are called Golgi type II (or stellate) cells (Gray, 1959).

Golgi type I axons are projectional (Fig. 3-2). They form the tracts and commissures in the central nervous system, as well as the axons of the peripheral nervous system. In Figure 3-6 #1, a cerebral pyramidal cell (Golgi type I) with a long axon is compared in figure 3-6 #2, with a stellate (Golgi type II) cell with a short axon.

The *Golgi type I cell (Fig 3-1)* has an apical and basal dendrite, each of which has secondary, tertiary, and quaternary branches, with smaller branches arising from each of these branches that extend into all planes. Spines are absent from the initial segment of the apical and basal dendrite of pyramidal neurons, but they become numerous farther along the dendritic branches. The axons of pyramidal neurons run long distances within the cortex, but they may also exit from the cor-

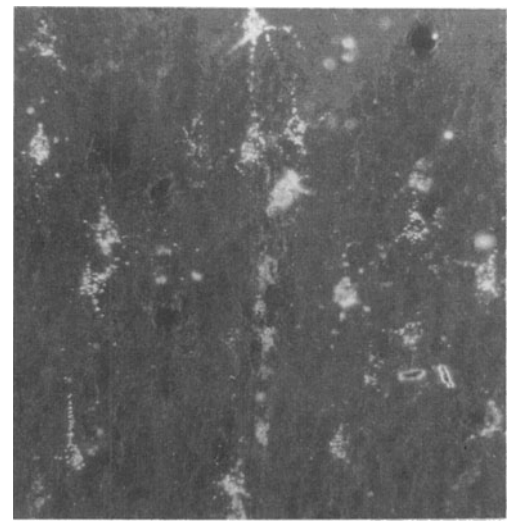

Figure 3-3. Demonstration of axoplasmic flow with the horseradish peroxidase method. A, Pyramidal cells in the sensory cortex after injection in the opposite hemisphere. B, Pyramidal cell in motor cortex labeled after injection into gyrate hemisphere. (Dark field, <X>350.)

tex and distribute to the subcortical nuclei.

The *Golgi type II cell* has a small axonal field and dendrites (fig 3-3). The axon usually extends only a short distance within the cerebral cortex (0.3 to 5 mm). Golgi type II cells have fewer dendritic spines than type I cells. Spines, which are common to many neurons, are small knob-shaped structures approximately 1 to 3 microns in diameter (Fig. 3-1). Their importance stems from the fact that they greatly expand the dendrite=s receptive synaptic surface.

Neuronal Cytoplasmic Organelles

Organelles found in the cytoplasm allow each neuron to function (Fig. 3-9) In these eukaryotic cells the organelles tend to be compartmentalized and include the nucleus, polyribosomes, rough endoplasmic reticulum, smooth endoplasmic reticulum, mitochondria, and inclusions (Fig. 3-10). Most neuronal cytoplasm is formed in the organelles of the soma and flows into the other processes. Newly synthesized macromolecules are transported to other parts of the nerve cell, either in membrane-bound vesicles or as protein particles. As long as the somas with a majority of its organelles are intact, the nerve cell can live. Thus it is the *trophic* center of the neuron. Separation of a process from the soma produces death of that process.

Nucleus. The large ovoid nucleus is found in the center of the cell body (Figs. 3-7, 3-8 and 3-9. Within the nucleus there is usually only a single spherical nucleolus, which stains strongly for RNA. The DNA, which can be demonstrated by staining the neuron by the Feulgen method or fluorescent markers, appears dispersed (heterochromatic) in mature neurons. (These cells are very active in metabolizing protein; consequently, the DNA is dispersed.)

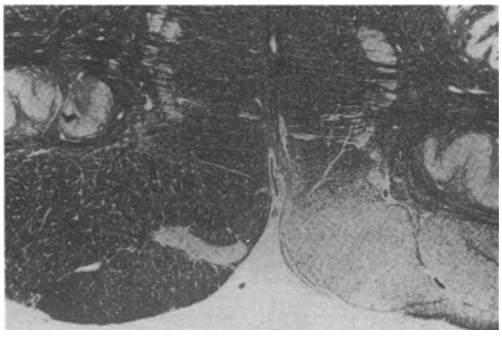

Figure 3-4. Wallerian degeneration. Medullary pyramids in a human some months after an infarct in the motor-sensory strip. Left side is normal; note the absence of myelin on right side. (Weigert myelin sheath stain, <X>80.)

In females, the nucleus also contains a perinuclear accessory body, called the *Barr body* (Fig.3-9). The Barr body is an example of the inactivation and condensation of one of the two female sex, or X, chromosomes (Barr and Bertram, 1949). The process of inactivation of one of the X chromosomes is often called *lyonization,* after the cytogenetist who discovered it, Mary Lyons.

Recently much progress has been made in the localization of genes associated with neurologic processes, e.g., Huntington's Chorea, Down=s syndrome.

Endoplasmic Reticulum. The largest membrane in the eukaryotic nerve cell is the endoplasmic reticulum (ER). It consists of a rough endoplasmic reticulum, which is the site of ribosome and protein synthesis, and the smooth endoplasmic reticulum, which is the site of the synthesis and metabolism of fatty acids and phospholipids.

Rough Endoplasmic Reticulum (Figs. 3-10, 3-11 and 3-12). The rough endoplasmic reticulum, or Nissl substance, is the chromidial substance found in the cell body. It can be demonstrated by using a light microscope and basic dyes, such as methylene blue, cresyl violet, and toluidine blue. The appearance and amount vary from cell to cell. With electron microscopy, cisterns containing parallel rows of interconnecting rough endoplasmic reticulum are revealed (Fig. 3-11). Ribosomes (clusters of ribosomal RNA) are attached to the outer surfaces of the membranes and consist of a large and a small RNA-protein subunit. Protein synthesis begins when there is a combination of initiation factors, messenger RNA (mRNA) and transfer RNA (tRNA) with the small subunit. This is then followed by the presence of an elongation factor, which then starts the peptide chain to grow.

The Nissl substance is most concentrated in the soma and adjacent parts of the dendrite (Fig. 3-12A). It is, however, also found throughout the dendrite (Fig. 3-12B). Before the electron microscope it was always presumed that the axon hillock was devoid of Nissl substance, but it has

now been shown that there are polyribosomes in this region.

Smooth Endoplasmic Reticulum (Fig. 3-10). All nerve cells have some smooth endoplasmic reticulum, but in neurosecretory cells in the hypothalamus, the smooth endoplasmic reticulum is greatly enlarged. The smooth endoplasmic reticulum consists of GERL--Golgi apparatus, endoplasmic reticulum, and lysosomes--which work together to synthesize, modify, or even degrade secretory proteins. The Golgi apparatus is found in all cells and is visible by a light microscope with osmium and silver stains as an irregular network in a perinuclear location. In electron micrographs, the Golgi apparatus consists of stacks of flattened smooth-surface membranes called saccules.

The protein secretion from the Nissl substance is transferred to the Golgi apparatus where a carbohydrate component is added to the protein. The product is released in a secretory vesicle.

Lysosomes (Figs. 3-10, and 3-11). Lysosomes are common in the cell body, appear as dense bodies, and function as centers of degradation. They are membrane-bound, vary in size from 0.35 to 3.0 microns in diameter, and commonly contain small granules. Lysosomes contain acidic hydrolytic enzymes (4.8 pH) that are capable of breaking down proteins, DNA, RNA,

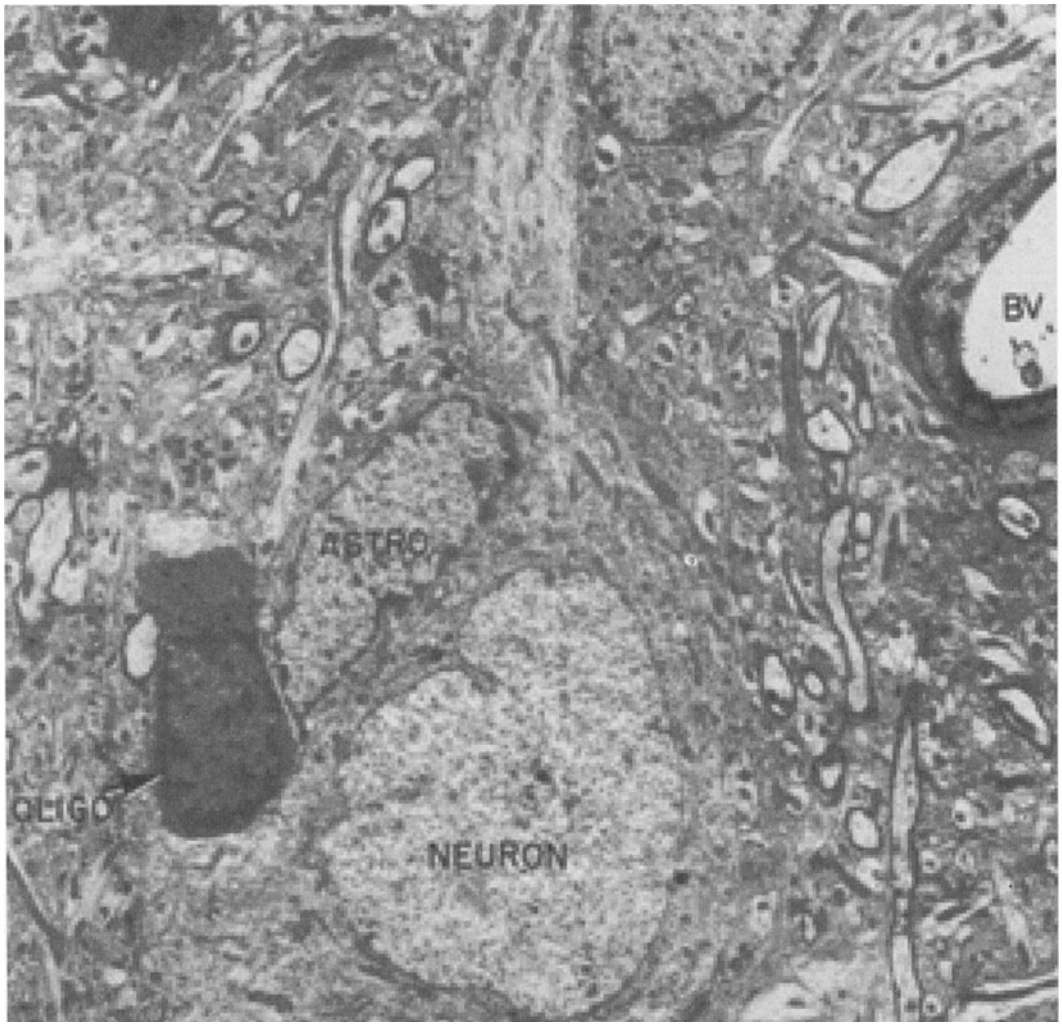

Figure 3-5. Electron micrograph (<X>6000) of the cerebral cortex showing the principal cell types in the nervous system: neuron, astrocyte (astro), oligodendrocyte (oligo), and a blood vessel (BV)

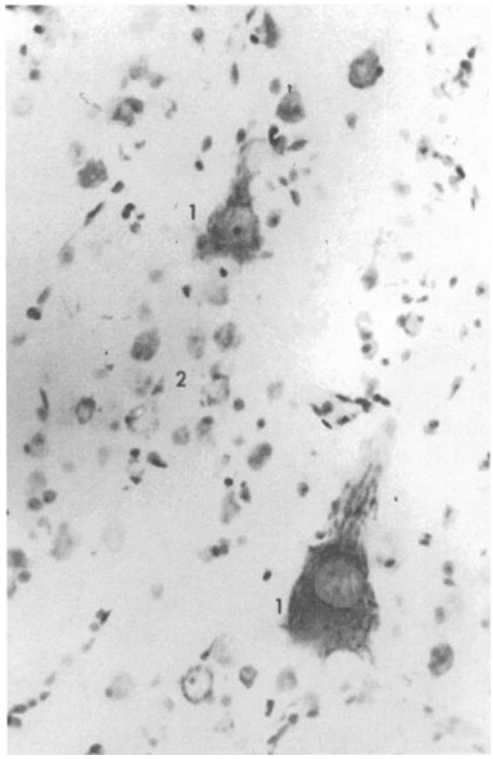

Figure 3-6 Motor cortex of the chimpanzee, demonstrating a pyramidal neuron. 1, Golgi type I cells (neurons with long axons). 2, Golgi type II cells (with short axons). (Nissl stain, <x> 1500.)

and certain carbohydrates. The lysosomes help digest macromolecular polymers into subunits (de Duve and Wattiaux, 1966). They are necessary for the degradation of older portions of membranes as newer ones are formed and also help in the elimination of deleterious toxins from the nerve cells.

Tay-Sachs disease illustrates the importance of the lysosome in the normal function of the nerve cells. When a specific lysosomal hydrolase is missing, *B-N*-hexosaminidase A, the degradation of the ganglioside G_{M3} is stopped, and Tay-Sachs results. Other instances of lysosomal enzymatic defects can also result in lysosomal storage diseases in the brain and spinal cord (Greenfield, 1993).

Peroxisomes. These small membrane-limited organelles are similar in appearance to lysosomes but have different functions. Peroxisomes contain enzymes that break down fatty acids, amino acids, and the enzyme catalase, which degrades the deleterious hydrogen peroxide formed by many reactions.

Mitochondria (Figs. 3-10, 3-11, and 3-12). Mitochondria are the principal site of adenosine triphosphate (ATP) production in the cell. These organelles, found throughout the neuron, are the third largest organelles after the nucleus and endoplasmic reticulum. They are rod-shaped and vary from 0.35 to 10 microns in length and 0.35 to 0.5 microns in diameter. Mitochondria can be demonstrated in light microscopy, but details of their structure are best seen in electron micrographs.

The wall of a mitochondrion consists of two layers--an outer and inner membrane. The outer membrane contains pores that render the membrane soluble to proteins with molecular weights of up to 10,000. The inner membrane is less permeable and has folds called cristae that project into the center of the mitochondrial matrix. The interior of the mitochondrion is filled with a fluid denser than cytoplasm. Cations and mitochondrial DNA have been demonstrated in the mitochondrial matrix. Mitochondrial DNA is derived from the mother. An intriguing study links this mitochondrial DNA to a common human female ancestor, Lucy, who lived in Africa over 300,000 years ago.

On the inner membrane are found enzymes that provide much of the energy required for the nerve cell. These respiratory enzymes (flavoproteins and cytochromes) catalyze the addition of a phosphate group to adenosine diphosphate (ADP), forming ATP. ATP is broken down in

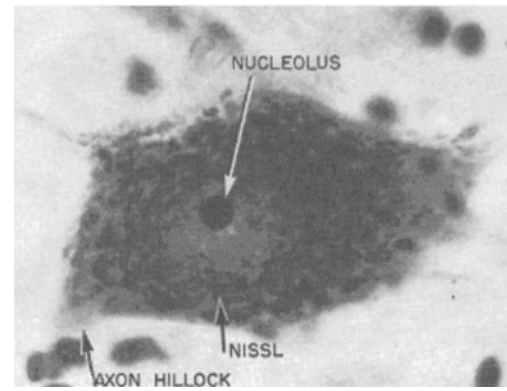

Figure 3-7. Motor neuron; ventral horn cell from a human cervical spinal cord, demonstrating the Nissl substance (rough endoplasmic reticulum), axon hillock, and nucleolus. (Nissl stain, <X>600.)

the cytoplasm to ADP, providing the energy required for cellular metabolic functions. In the cytoplasm are found enzymes that break down glucose into pyruvic and acetoacetic acid. These substances are taken into the mitochondrial matrix and participate in the Krebs citric-acid cycle, which allows the mitochondria to metabolize amino acids and fatty acids.

Centrosomes. Centrioles within the centrosome are seen in the immature dividing neuroblast as well as the adult neuron. However, since mature neurons are incapable of dividing, the function of centrosomes there is not clear.

Inclusions. Substances stored in a cell include pigments, glycogen, and lipid droplets. Pigment granules (melanin) are common in certain parts of the brain, particularly the substantia nigra, locus ceruleus, and reticular formation . In humans, lipochrome pigment (lipofuscin) is found in most cells *(Fig. 3-11A)*. The amount appears to increase with age. Lipofuscin consists of pigment combined with fatty material and probably is a metabolic by-product of lysosomal activity that is not readily disposable. It is commonly referred to as the "wear-and-tear" pigment.

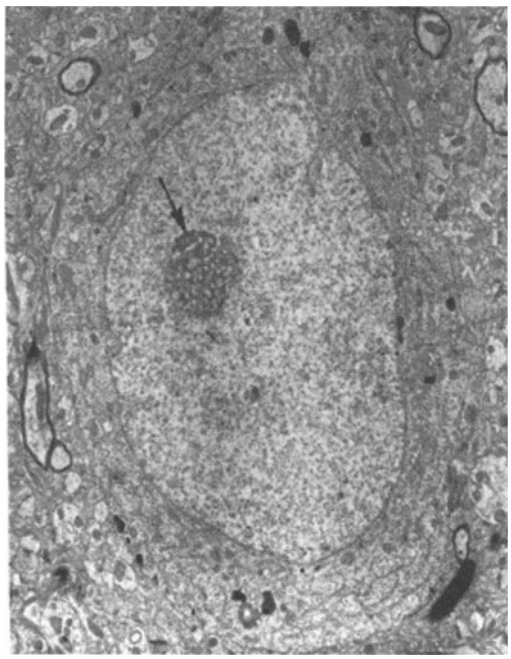

Figure 3-8. Electron micrograph of neuron in the rat sensory cortex, demonstrating the nucleus and nucleolus (arrow). (<X>10,000.)

Glycogen (Fig. 3-11B). Glycogen is a polymer made up of D-glucose monomers and is commonly seen in electron micrographs of nerve cells and glia. Glycogen appears in the form of electron-dense rosettes, which are much larger than the RNA rosettes. It is a local source of energy.

Lipid Droplets (Fig. 3-11). Lipid droplets are also seen in the soma. They represent a local store of energy as well as a source of carbon chains for membrane formation.

Neurosecretory Granules. Neurons in the supraoptic and paraventricular nuclei of the hypothalamus form neurosecretory material (Bodian, 1963 and 1966; Palay, 1957; Scharrer, 1966). The axons of these cells form the hypothalamic-hypophyseal tract, which runs through the median eminence, down the infundibular stalk to the neurohypophysis (pars nervosa), where the axons end in close proximity to the endothelial cells. The secretory granules are 130 to 150 millimicrons in diameter and are found in the tract *(Fig. 3-13)*. They increase in size as one approaches the endothelial end of the axons.

The protein in the secretory granules is made in the Nissl substance; the granules are formed in the Golgi apparatus and transported by the axons of the hypothalamic-hypophyseal tract to the infundibulum, where they are stored in the neural lobe. The sites of storage are called Herring bodies (Fig. 3-14B). Interruption of the hypothalamic-hypophyseal tract produces diabetes insipidus.

Neuronal Cytoskeleton

In silver-stained sections examined in a light microscope, a *neurofibrillary* network can be seen in the neurons (Fig. 3-14). Electron micrographs can distinguish microtubules, 3 to 3 nm in diameter, and neurofilaments, 1 nm in diameter. It appears that fixation produces clumping of the tubules and filaments into the fibrillar network seen in light micrographs.

Neurons in common with other eukaryotic cells contain a cytoskeleton that maintains it shape. This cytoskeleton consists of at least three types of fibers:

1. Microtubules 30 nm in diameter
3. Microfilaments 7 nm in diameter
3. Intermediate filaments 10 nm in diameter.

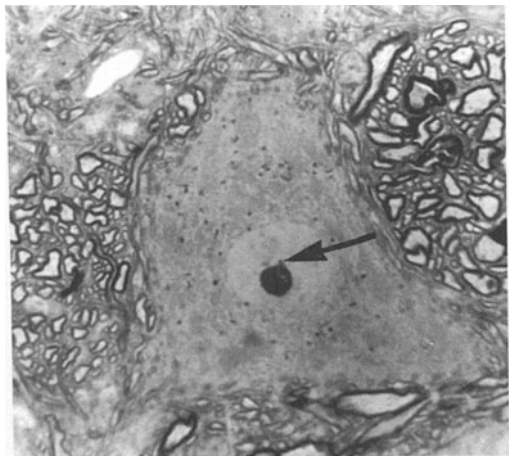

Figure 3-9. Motor neuron from the ventral horn of a female squirrel monkey. Note the nucleus, nucleolus, and accessory body of Barr (arrow). (One-micron epoxy section, <X>1400.)

If the plasma membrane and organelle membrane are removed, the cytoskeleton is seen to consist of actin microfilaments, tubulin-containing microtubules, and criss-crossing intermediate filaments.

Neurotubules (microtubules) predominate in dendrites and in the axon hillock, whereas microfilaments are sparse in dendrites and most numerous in axons (Fig. 3-15). Microtubules and intermediate filaments are found throughout the axon.

The microtubules help to transport membrane-bound vesicals, protein, and other macromolecules. This orthograde transport, or anterograde axonal transport, is the means whereby these molecules formed in the soma are transported down the axon into the axonal telodendria.

The individual microtubules in the nervous system are 10 to 35 nm in length and together form the cytoskeleton. The intermediate filaments are associated with the microtubules. The wall of the microtubule consists of a helical array of repeating tubulin subunits containing the A and B tubulin molecule. The microtubule wall consists of globular subunits 4 to 5 nm in diameter; the subunits are arranged in 13 protofilaments that encircle and run parallel to the long axis of the tubule. Each microtubule also has a defined polarity. Associated with the microtubules are protein motors, kinesins and dyneins, which when combined with cAMP may well be the mechanism of transport in the central nervous system (Brady, 1985; Vale et al., 1985 and 1987). The products transported down the microtubules probably move like an inch worm and not like a train on a track. During mitosis, microtubules disassemble and reassemble; however, a permanent cytoskeleton lattice of microtubules and intermediate filaments in the neuron is somehow maintained. It is not yet known how long each microtubule exists, but there is evidence of a constant turnover.

Neruofibrillar tangles are bundles of abnormal filaments within a neuron. They are helical filaments, that are different from normal cytoskeletal proteins and they contain the tau protein a microtubule binding protein (MAP) that is a normal component in neurons. In Alzheimer's disease there are accumulations of abnormally phosphorylated and aggregated forms the microtubule binding protein tau. These large aggregates form the tangles that can be physical barriers to transport, may interfere with normal neuronal functions, and are probably toxic. Mutations in the human tau gene are found in autosomal domininant neuronal degenerative disorders isolated to chromosome. 17. These familial disorders are characterized by extensive neurofibrillar pathology and are often called "taupathies" (Hutton 2000). Functions of neuronic organelles are listed in Table 3-3.

Axon and Axon Origin

The axon contains some elongate mitochondria, many filaments oriented parallel to the long axis of the axon *(Figs. 3-15, and 3-16)*, and some tubules. In contrast, a dendrite contains a few filaments and any tubules, all arranged parallel to the long axis of the dendrite *(Fig. 3-5)*. Polyribosomes are present, but the highly organized, rough endoplasmic reticulum is absent.

Axon Hillock. The axon hillock is a slender process that usually arises from a cone-shaped region on the perikaryon (Fig. 3-16). This region includes filaments, stacks of tubules, and polyribosomes (Fig. 3-16B). The initial segment of the axon, arising from the axon hillock, is covered by dense material that functions as an insulator membrane at the hillock is covered by an electron dense material *(Fig. 3-16)*.

Myelin. In the nervous system axons may be

TABLE 3-3. FUNCTIONS OF DYNAMIC ORGANELLES IN THE NEURON

Microtubules	Provide the structural basis for transport, and axoplasmic flow. Found throughout the neuron; part of the neuronal cytoskeleton.
Microfilaments	Form much of the cytoskeleton of the entire neuron.
Nissl substance (rough endoplasmic reticulum)	Protein manufacturing unit in the nerve cell. The most commonly stained organelle with basophilic dyes; very sensitive to cell injury.
Golgi bodies	Form the lipophilic portion of all the membranes in the neuron.
Nucleus	Chromatin is dispersed as nerve cells are very active metabolically. Eukaryotic in the adult nerve cell; important in all normal cell functions. Demonstrable abnormalities in many diseases, including trisomies, Alzheimer= s, Huntington= s, and Parkinson= s.
Nucleolus	Contains the messenger RNA that is activated by chromatin.

myelinated or unmyelinated. Myelin is formed by a supporting cell, which in the central nervous system is called the oligodendrocyte and in the peripheral nervous system, the Schwann cell. The immature Schwann cells and oligodendrocyte have on their surface the myelin-associated glycoprotein that binds to the adjacent axon and may well be the trigger that leads to myelin formation. Thus, the myelin sheath is not a part of the neuron; it is only a covering for the axon. Myelin consists of segments approximately 0.5 to 3 mm in length. Between these segments are the nodes of Ranvier. The axon, however, is continuous at the nodes, and axon collaterals can leave at the nodes. The myelin membrane like all membranes contains phospholipid bi-layers (Fig. 3-17). In the central nervous system myelin includes the following proteins:

Proteolipid protein (50%)
Myelin basic protein (40%)
Myelin-associated glycoprotein (1%)
3,3-cyclic nucleotide (4%)

The oligodendrocytic process forms the

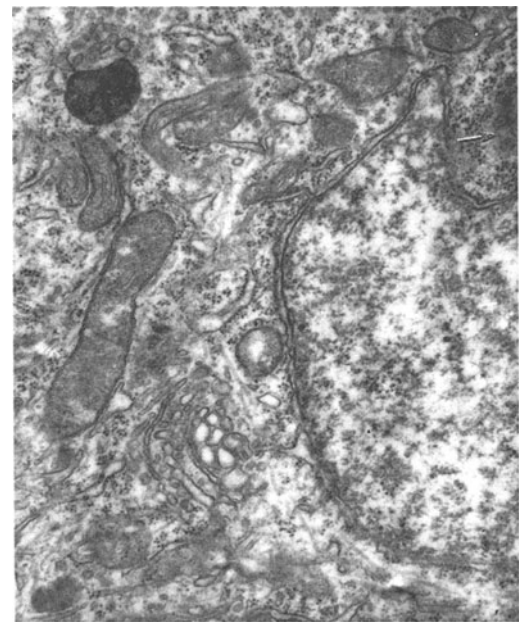

Figure 3-10. Electron micrograph of a small pyramidal neuron in the rat cerebral cortex, demonstrating the following organelles: Golgi apparatus, mitochondria, lysosome, and Nissl substance. Note the nuclear pore (arrow). (<X>60,000.)

myelin sheaths by wrapping around the axon. The space between the axonal plasma membrane and the forming myelin is reduced until most of the exoplasmic and cytoplasmic space is finally forced out. The result is a compact stack of membranes. The myelin sheath is from 3 to 100 membranes thick and acts as an insulator by preventing the transfer of ions from the axonal cytoplasm into the extracellular space.

Myelin Sheath. Myelin sheaths are in contact with the axon. In light microscopy they appear as discontinuous tubes 0.5 to 3 mm in length, interrupted at the node *(Fig. 3-29)*. The axon is devoid of myelin at the site of origin (the nodes) and at the axonal telodendria. At the site of origin the, axon is covered by an electron-dense membrane, and at the site of the synaptic telodendria the various axonal endings are isolated from one another by astrocytic processes.

In electron micrographs each myelin lamella actually consists of two-unit membranes with the entire lamella being 130 to 180 Å thick (Fig. 3-17). Myelin is thus seen to consist of a series of light and dark lines. The dark line, called the major dense line, represents the apposition of the

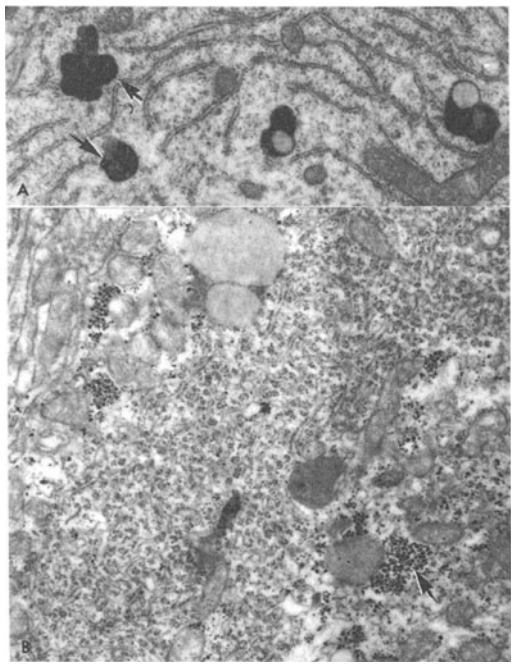

Figure 3-11. Electron micrographs showing inclusions. A, Lipofuscin. B, Glycogen, lipid, and Nissl substance. (<X>30,000.)

inner surface of the unit membranes. The less dense line, called the interperiod line, represents the approximation of the outer surfaces of adjacent myelin membranes.

Only at the node of Ranvier is the axonal plasma membrane in communication with the extracellular space. The influx of Na+ at each node causes the action potential to move rapidly down the axon by jumping from node to node (see Chapter 5-Part II).

Myelination. The process of myelination has been followed with the electron microscope. An axon starts with just a covering formed by the plasma membrane of either the Schwann cell or the oligodendrocyte. More and more layers are added until myelination is complete. One theory is that myelin is laid down by the processes of the Schwann cell twisting around the axon (Geren, 1956; Robertson, 1955); this indeed occurs in the peripheral nervous system. In the central nervous system each oligodendrocyte enwraps many axons, and they also appear to twist around the axons as they myelinate.

The sequence of myelination has been studied in great detail; it begins in the spinal cord, moves into the brain stem, and finally ends up

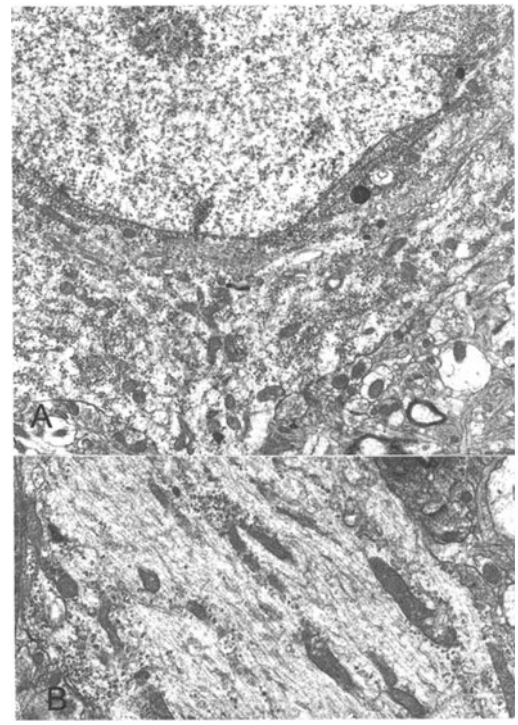

Figure 3-12. Electron micrographs of a pyramidal neuron in the rat cerebral cortex. A, Soma and nucleus. B, Dendrite. Note the large amount of Nissl substance in the soma, but the dendrites have less Nissl substance and many microtubules. (<X>35,000.)

with the diencephalon and cerebrum last. A delay in myelination can result from many factors, including genetic and nutritional ones, and is usually very harmful to the fetus.

Peripheral Nervous System

In the peripheral nervous system, there is usually only one Schwann cell for each length or internode of myelin. In the central nervous system each oligodendrocyte may form and maintain myelin sheaths on 30 to 60 axons.

The unmyelinated axons in the peripheral nervous system are found in the cytoplasm of the Schwann cell. There can be as many as 13 unmyelinated axons in one Schwann cell. The unmyelinated axons in the central nervous system are usually found in small bundles without any special covering.

Axoplasmic Flow (Fig. 3-18).

With the protein manufacturing apparatus present only in the soma, and to a lesser degree in the dendrites, a mechanism must exist to

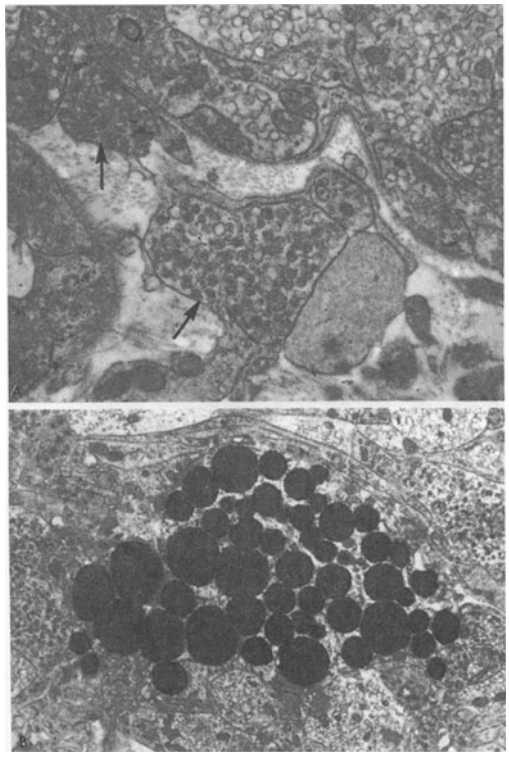

Figure 3-13. Electron micrograph of a rat neurohypophysis. A shows neurosecretory granules in the axoplasm of fibers of the hypothalamo-hypophyseal tract (<X>30,000). B demonstrate a Herring body, a storage site of neurosecretory material. (<X>8,000.)
Figure 3-16. Cytoskeleton. Neurofibrillary stain of a ventral horn cell in the cat spinal cord, showing neurofibrillary network in soma and dendrites (A) and in the axons (B). (<X>400).

transport proteins and other molecules from the soma, down the axon, and into the presynaptic side. Weiss and Hisko (1948) demonstrated by tieing off a peripheral nerve, which caused swelling proximal to the tie, that material flows from the soma, or trophic center, into the axon and ultimately to the axon terminal. The development of techniques that follow this axoplasmic flow has revolutionized the study of circuitry within the central nervous system. The ability to map this circuitry accurately has given all neuroscientists a better understanding of the integrative mechanisms in the brain. There are many compounds now available to follow circuitry in the brain and they include horseradish peroxidase, wheat germ agluttin, tetanus toxin, fluorescent molecules and radiolabeled compounds.

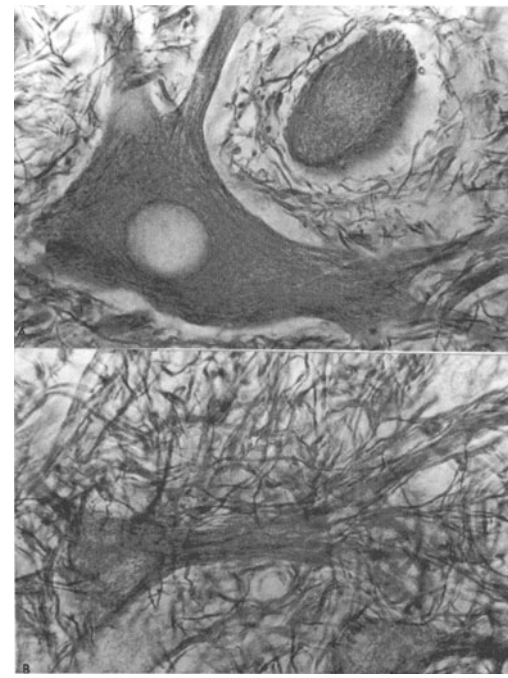

Figure 3-14. Cytoskeleton. Neurofibrillar stain of a ventral horn cell in the cervical spinal cord of the cat, showing neurofibrillar network in soma and dendrites(A): and in axons(B). X400

This mechanism of transport is not diffusion but rather retrograde axonal transport associated with the microtubule network that exists throughout the nerve cell. The rate of flow varies depends upon the product being transported and ranges from more than 300 mm/day to less than 1 mm a day. The main direction of the flow is anterograde, from the cell body into the axon and synapse. There is also a very active retrograde flow from the synaptic region back to the cell body that may be a source for recycling many of the substances found at the synaptic ending.

The particles that move the fastest consist of small vesicles of the secretory and synaptic vesicles, and the slowest group is the cytoskeletal components. Mitochondria are transported down from the cell body at an intermediate rate. The retrograde flow from the synaptic telodendria back into the soma, returns any excess of material for degradation or reprocessing. The retrograde flow permits any excess proteins or amino acids to recycle. It also permits products synthesized or released at the axonal cleft to be absorbed and then transported back to the cell

body, where they can affect the basic function of the cell--the signaling process.

Fast axonal transport is associated with the microtubules. The slower components including membrane associated proteins (MAPS) are transported inside the microtubules, but the mitochondria actually descend the axonal cytoplasm (Table 3-3).

TABLE 3-3. RATE OF AXONAL TRANSPORT OF CELLULAR STRUCTURES (DATA FROM GRAFTSTEIN AND FORMAN, 1980; MCQUARRIE, 1988; WUJEK AND LASEK, 1983.)

Transport	Rate (mm/day)	Cellular Structure
Fast	300<->400	Vesicles, smooth endoplasmic reticulum, and granules
Intermediate	50 15	-- Mitochondria --Filament proteins
Slow component B	3<->4	Actin, fodrin, enolase, CPK, calmodulin, and clathrin
Slow component A	0.3<->1	Neurofilament protein, tubulin, and MAPS

Peripheral Versus Central Nerve Structure

Peripheral Nerve Structures. The structure of a peripheral nerve is different from that of fiber bundles in the central nervous system (Table 3-4). Peripheral nerves consist of many axons held together in a fascicle by connective tissue of mesodermal origin *(Fig. 3-19)*. The outer layer that covers the nerve trunks and fills between the individual fascicle is called the *epineurium*. It consists of connective tissue cells, collagen, and some fat cells. Each of the fascicles is wrapped in a dense layer of connective tissue, which is called the *perineurium*. Strands of collagen, fibroblasts, and other cells that run between the individual nerve fibers are called the *endoneurium*. The term endoneurium is also applied to the delicate trabeculum surrounding each nerve fiber, which is also called the sheath of Key-Retzius. The peripheral nerve fiber or axon is engulfed in the Schwann cytoplasm (the neurilemmal sheath), which also forms the myelin sheath. Large blood vessels are found in the epineurium and perineurium and capillaries are seen in the endoneurium.

TABLE 3-4. CONTENTS OF A PERIPHERAL NERVE BUNDLE

Epineurium	The outer layer, covering the nerve trunks and filling between the individual fascicles consists of connective tissue cells, collagen, and some fat cells.
Perineurium	A connective tissue layer that surrounds the nerve fascicles
Endoneurium (sheath of Key-Retzius)	Strands of collagen and fibroblasts between individual axons. Endoneurium also refers to the delicate trabeculum surrounding each nerve fiber.
Sheath of Schwann	Engulfs each individual axon and forms myelin.

Fibers can vary in diameter from less than 0.5 microns to 33 microns (see Chapter 5 Part II). Axons can be classified by size and function into three major groups (Table 3-5).

Central Nervous Structure. Axons in the central nervous system also vary in size (5 to 33 microns) and in length (0.5 mm to 1 m), but these axons cannot be separated into functional categories based on axonal diameter. The axons in the central nervous system run in groups called tracts that are enwrapped by the processes of fibrous astrocytes. However, no specific cov-

TABLE 3-5. FUNCTIONAL COMPONENTS OF PERIPHERAL NERVES

Fiber Type	Description	Diameter (microns)	Conduction Speed (meters per second)
Type A	Myelinated somatic afferent and efferent fibers.	1<->33	5<->130
Type B	Myelinated efferent preganglionic autonomic fibers.	1<->3	3<->15
Type C	Unmyelinated afferentor efferent fibers,pain fibers, and postganglionic sympathetics fibers.	0.3<->1.3	0.5<->3

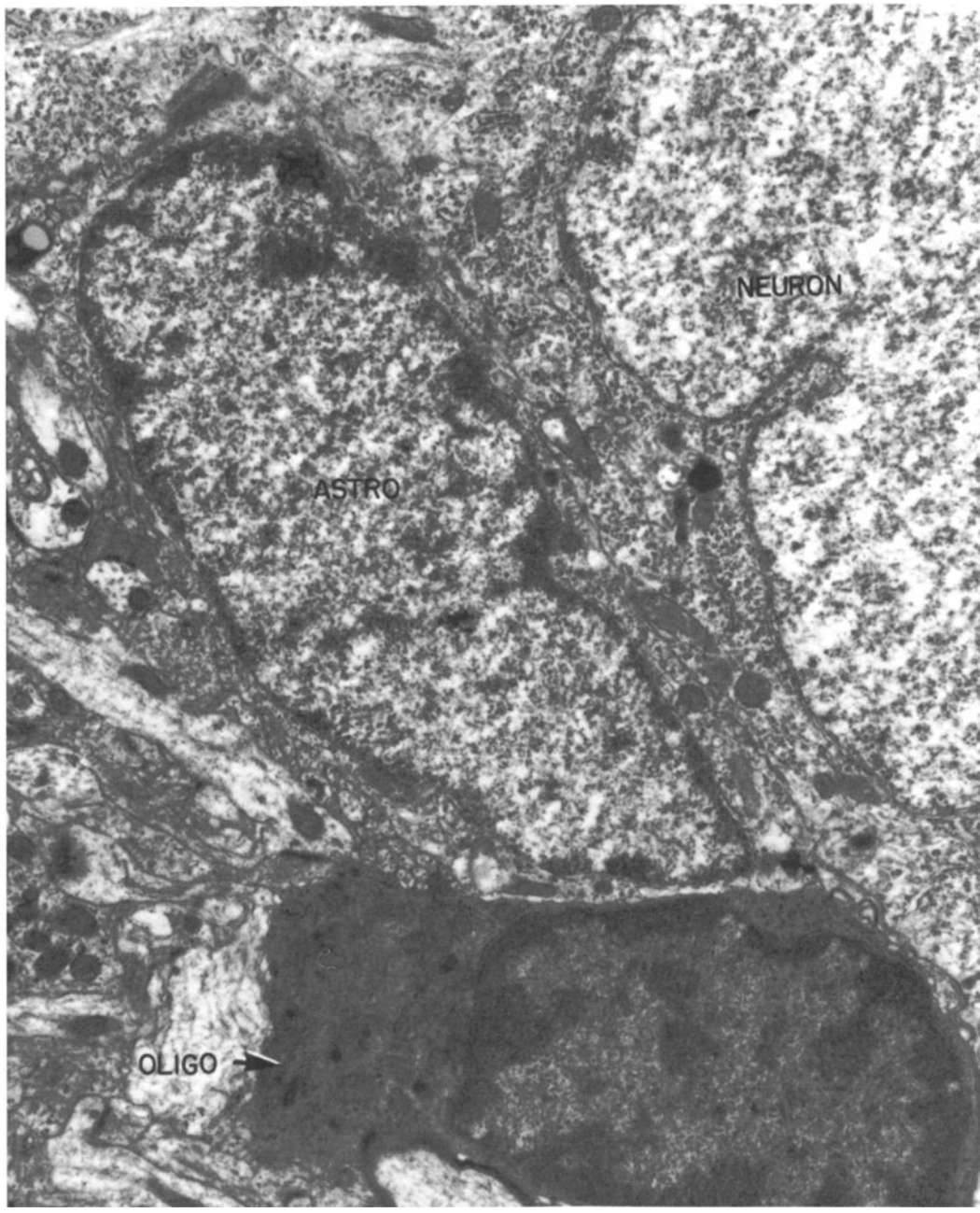

Figure 3-15. Electron micrograph of the rat cerebral cortex, demonstrating the difference between a myelinated axon and a dendrite. Note the axon has numerous microfilaments while the dendrite has numerous microtubules. (<X>30,000.)

ering corresponds to the sheath of Schwann in the peripheral nervous system. Each tract has a distinct function in the nervous system.

The axonal arborization of neurons is not as elaborate as that of the dendrite, but it can be very extensive. Although the surface area of many dendrites may total more than that of an axon, some axons run very long distances. For example, from the type I pyramidal cell in the cerebral cortex to the ventral horn cells in the sacral spinal cord, or from a ventral horn cell in the spinal cord, down a peripheral nerve to a foot muscle.

Action Potentials. The action potential is

generated at or near the axon hillock and is then propagated down the axon as an all-or-none phenomenon to the synapse (see Chapter 5 - Part II). Several axon terminals that come from many different sources are found on any neuron. Unlike the axon, the dendrite does not respond in an all-or-none fashion like the axon. Instead, each nerve impulse at a given site on the dendrite produces a change in the electrical activity. The sum total of all these electrical changes result in a variation in the membrane potential in the neuron either below or above the firing threshold.

Synapse: Synapses can be seen at the light microscopic level *(Fig 3-20)*, however to identify all the components of a synapses the electron microscope must be use. At the electron microscopic level the synapse consists of the axonal ending, which forms the presynaptic side, and the dendritic zone, which forms the postsynaptic side *(Fig. 3-21)*. Collectively, the pre- and postsynaptic sides and the intervening synaptic cleft are called the synapse.

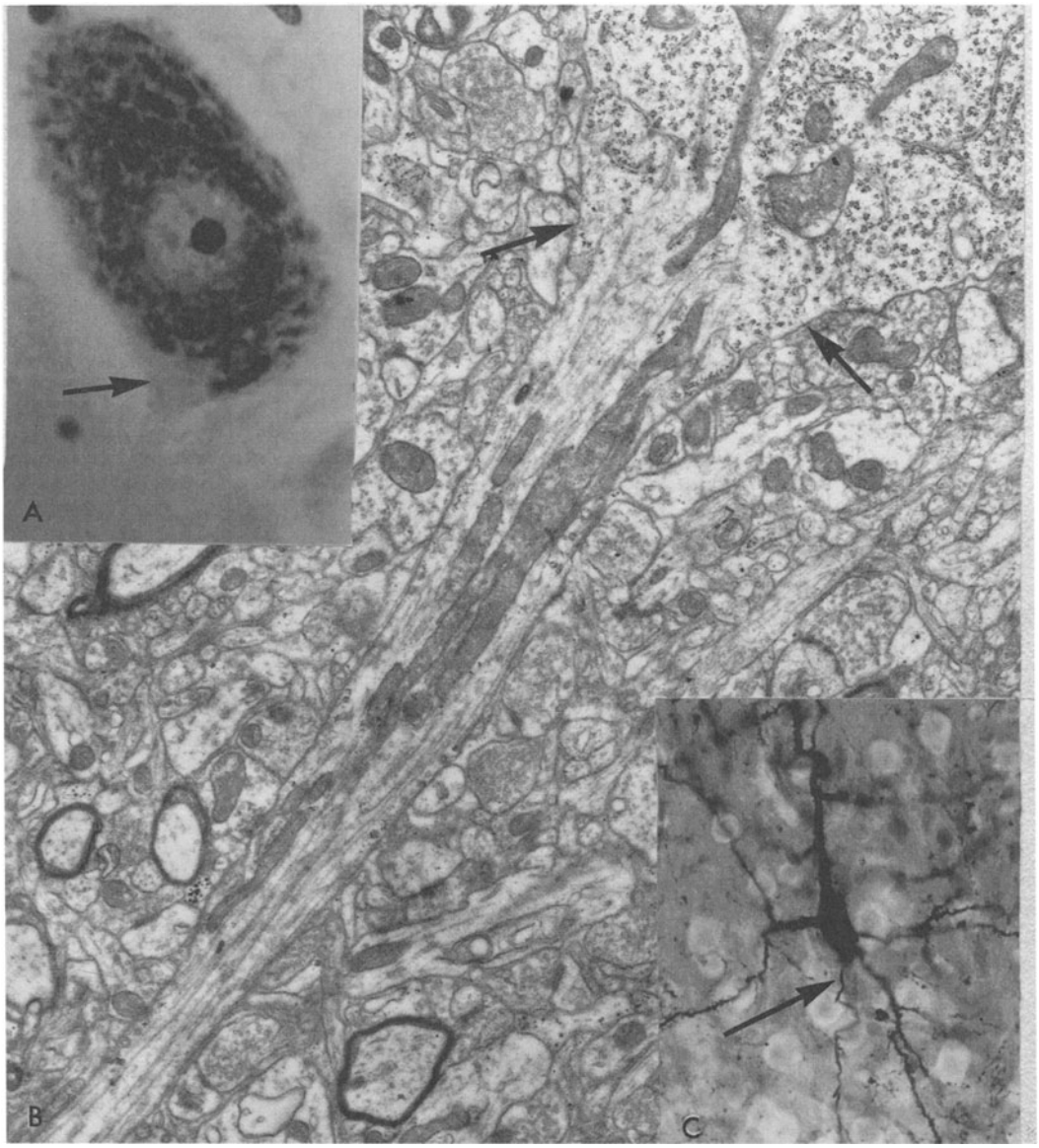

Figure 3-16. Appearance of the axon hillock: A, after Nissl staining (<X>400), B, in an electron micrograph (<X>15,000), and C, after Golgi rapid staining (<X>350).

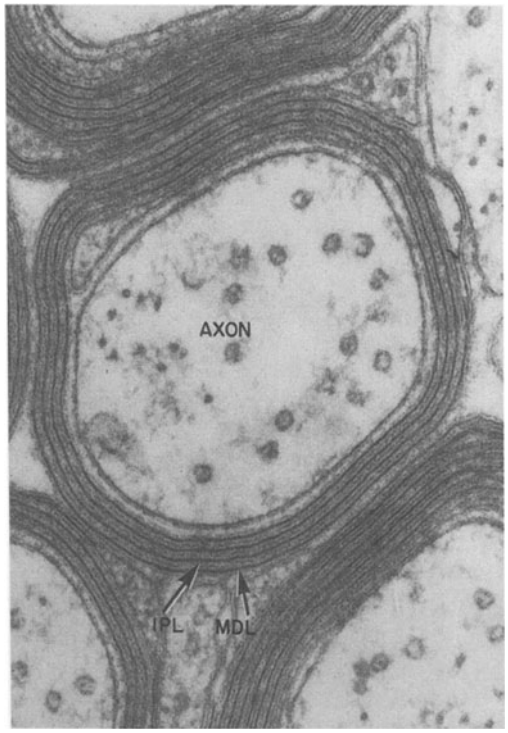

Figure 3-17. Electron micrograph of myelin sheath from the optic nerve of the mouse demonstrating repeating units of the myelin sheath, consisting of a series of light and dark lines. The dark line, called the major dense line (MDL), represents the apposition of the inner surface of the unit membranes. The less dense line, called the interperiod line (IPL), represents the approximation of the outer surfaces of adjacent myelin membranes. (<X>67,000.) (Courtesy of Alan Peters, Department of anatomy, Boston University School of Medicine)

At the synapse the electrical impulse from one cell is transmitted to another. Synapses vary in size from the large endings on motor neurons (1 to 3 microns) to smaller synapses on the granule and stellate cells of the cortex and cerebellum (less than 0.5 microns). Synapses primarily occur between the axon of one cell and the dendrite of another cell. Synapses are usually located on the dendritic spins but are also seen on the soma and rarely between axons. At the synapse the axon arborizes and forms several synaptic bulbs that are attached to the plasma membrane of the opposing neuron by intersynaptic filaments (Fig. 3-21).

Structure. Synapses can be identified by light microscopy, but the electron microscope

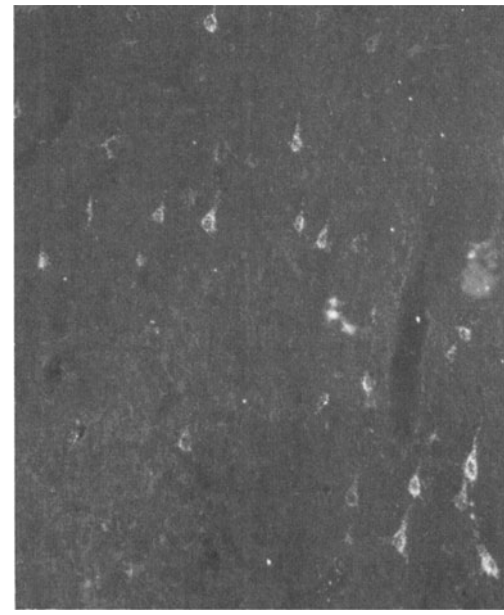

Figure 3-18. Demonstration of the cells of origin of callosal axons with the horseradish peroxidase<->diaminobenzidine reaction method in layers III, and V of the somatosensory cortex. The horseradish peroxidase was injected into the contralateral cortex 34 hours previously. The predominant cell type is pyramidal. (Dark field, <X>100.)

has revealed many new details in synaptic structure (Bodian, 1970; Colonnier, 1969; Gray, 1959; Palay, 1967). In an electron micrograph, the presynaptic or axonal side of the synapse contains mitochondria and many synaptic vesicles (Fig. 3-21). Synaptic vesicles are concentrated near the presynaptic surface with some vesicles actually seen fusing with a membrane (Fig. 3-21), illustrating that this site releases neurotransmitters. Neurofilaments are usually absent on the presynaptic side. Pre- and postsynaptic membranes are electron-dense and are separated by a 30 to 40 nm space, the synaptic cleft, which is continuous with the extracellular space of the central nervous system.

Synaptic Types. Two types of synapses, electrical and chemical, differ in location and appearance. Most of the synapses in the mammalian central nervous system are chemical.

Electrical synapses are connected by membrane bridges, gap junction connections, which permit the electric impulse to pass directly from one cell to the other. Electric synapses have almost no delay and little chance of misfiring.

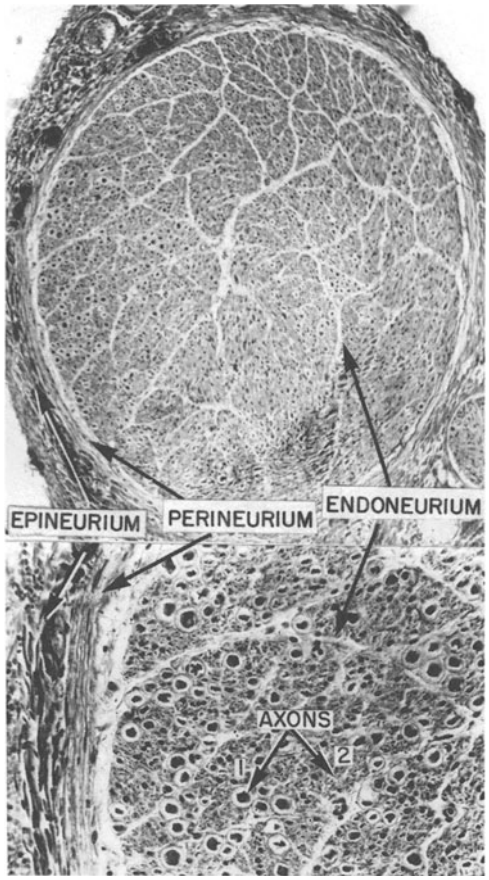

Figure 3-19. Peripheral nerve of a cat. A shows wrapping of the nerve trunk, the epineurium; each nerve fascicle is surrounded by the perineurium while each nerve fiber is embedded in endoneurium (Bodian stain, <X>100). In B, 1 demonstrates a large myelinated axon; 3 points to a small myelinated and unmyelinated axon (Bodian stain, <X>350).

These synapses are seen in many fish.

Chemical synapses have a presynaptic side, containing vesicles and a gap, and the postsynaptic side with membrane receptors. The neurotransmitter released by the action potential is exocytosed and diffuses across the synaptic cleft and binds to the specific receptor on the postsynaptic membrane.

Synapses are either excitatory or inhibitory. Synapses that depolarize the membrane potential (make it more positive) are *excitatory.* Synapses that hyperpolarize the membrane potential (make it more negative) are *inhibitory.*

Excitatory synapses in the central nervous system are asymmetrical, having a prominent

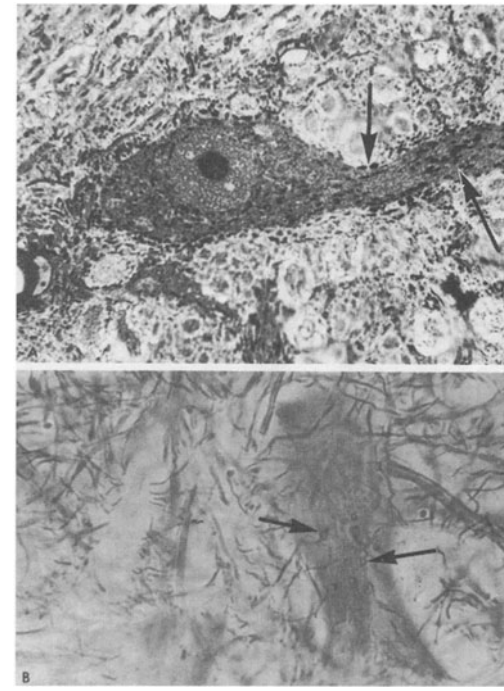

Figure 3-20. Silver stain of a one-micron plastic embedded section. A demonstrates synaptic boutons on neurons in the reticular formation. B demonstrates boutons on ventral horn cells. (<X>400.)

postsynaptic bush with presynaptic vesicles *(Fig 3-21).* This type of synapse is most commonly seen on dendrites. Glutamate has been identified in excitatory synapses. At the excitatory synapse there is a change in permeability that leads to depolarization of the postsynaptic membrane and which can lead to the generation of an action potential. Glutamate has been identified in excitatory synapses.

Inhibitory synapses in the central nervous system are symmetrical with thickened membranes on the pre- and postsynaptic side and vesicles only on the presynaptic side. GABA has been identified in the inhibitory synapses. At an inhibitory synapse the neurotransmitter binds to the receptor membrane, which changes the permeability and tends to block the formation of the action potential. Synapses on the soma are symmetrical and they are considered inhibitory.

Throughout much of the central nervous system, spines are found on the dendrites. These dendritic spines are bulbous, with a long neck connecting to the dendrite. Many axon terminals are located on the spines. In the cerebral

cortex, a spine apparatus is found within the spine, which seems to function like a capacitor, charging and then discharging when its current load is exceeded (see Fig. 3-2, 3-15).

Synaptic Vesicles. The synaptic vesicles differ in size and shape and may be agranular, spherical, flattened, or round with a dense core. The method of fixation for electron micrographs affects the shape of a vesicle. Bodian (1970) has shown that osmium fixation produces only spheroidal vesicles. Aldehyde followed by osmium produces spheroidal and flattened vesicles. The shape of flattened vesicles may also be modified by washing the tissue in buffer or placing the tissue directly from the aldehyde into the osmium. The spheroidal vesicles retain their

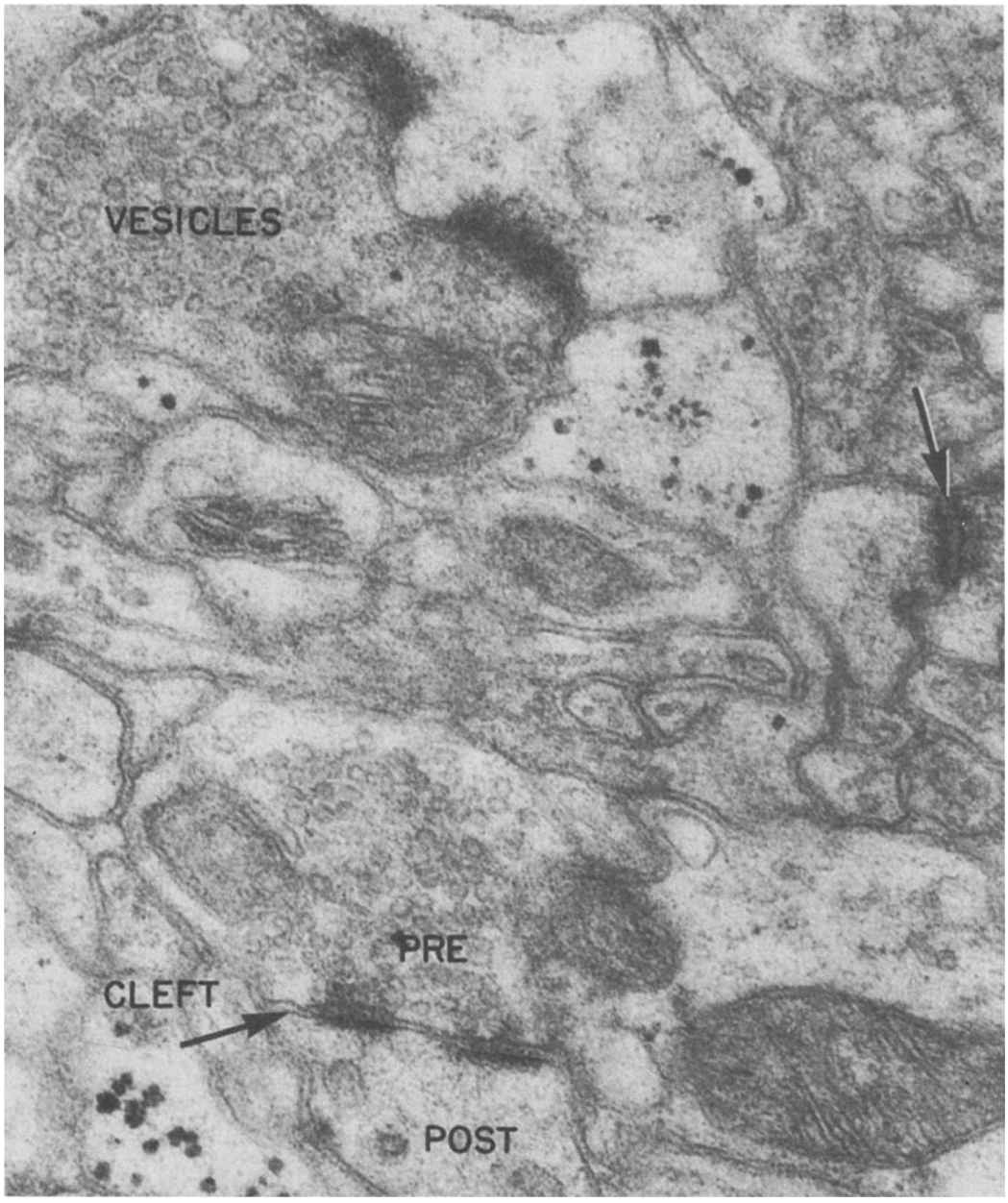

Figure 3-21. Synapse in the sensory cortex of the rat demonstrating agranular synaptic vesicles (300 to 400 Å) in the presynaptic axonal side. Note the electron-dense synaptic membranes and the intersynaptic filaments in the synaptic cleft. Electron micrograph (<X>65,000.)

shape regardless of any manipulation.

There are four basic categories of synaptic vesicles (Palay, 1967), as described in Table 3-6.

Treating the Type 1 vesicles with high osmolality aldehyde fixatives produces the commonly seen spherical vesicles and also a group of flattened, ovoid, or disc-shaped vesicles. Flattened vesicles are known to be inhibitory synapses, and the spherical vesicles are assumed to be excitatory synapses.

Synaptic Transmission. Current evidence in the mammalian central nervous system suggests that synaptic transmission is primarily a chemically and not an electrically mediated phenomenon, based on the presence of:

1. A 30 to 40 nm cleft
3. Synaptic vesicles
3. Appreciable synaptic delay due to absorbance of the chemical onto the postsynaptic receptor site.

In contrast electrical synapses have cytoplasmic bridges that interconnect the pre- and postsynaptic membranes resulting in a minimal synaptic delay as transmission is ionic rather than by release of chemical from a vesicle.

Neurotransmitters

Many compounds have been identified as neurotransmitters. These substances are found in synaptic vesicles on the presynaptic side. Introduction of the compound into the synaptic cleft produces the same change in the resting membrane potential as stimulation of the presynaptic axon; the compound is rapidly degraded, and the membrane potential returns to the resting state.

The neurotransmitters are either amino acids, derived from amino acids, or small neuropeptides. The classic neurotransmitters in the central nervous system include acetylcholine, epinephrine, norepinephrine, serotonin, glycine, glutamate, dopamine, and GABA. At certain synaptic sites the following compounds may also function as modulators (usually a slower transmitter) form of neurotransmission: adenosine, histamine, octopamine, B-alanine, ATP, and taurine. Many of the neuropeptides, such as substance P, vasoactive peptide, peptide Y, and somatostatin are also active in neurotransmission or neuromodulation. Catecholamines and 5-hydroxytryptamine are transmitters linked to synaptic transmission in the central nervous system. Noradrenaline is a transmitter at the preganglionic synapses.

Many steroids and hormones have also been linked to synaptic transmission. It is still uncertain whether these compounds play a direct role in nervous transmission or if they are just related by their importance to the ongoing functions of the entire nervous system (Table 3-7).

At excitatory synapses the following com-

TABLE 3-6. CATEGORIES OF SYNAPTIC VESICLES

Type	Diameter	Locations
1. Spheroidal or flattened with a clear center; most common type (Figs. 3-33 and 3-34).	30 to 40 nm	At neuromuscular junction and throughout central nervous system.
2. Spheroidal with 38 nm electron-dense granule in the center	40 to 80 nm	Found in autonomic endings in the intestines, vas deferens, and pineal body; contains catecholamines.
3. Spheroidal with a 50 nm electron-dense granule in the center	80 to 90 nm	Found at preganglionic sympathetic synapses, at neuromuscular junctions in smooth muscle, and in part of the hypothalamus, basal nuclei, brain stem, and cerebellum; catecholamines present in vesicles.
4. Spheroidal with a large droplet that nearly fills the vesicle (Fig. 3-15).	130 to 300 nm	Characteristic of nerve endings in the hypothalamus and neurohypophysis; also found in the soma, axons, and presynaptic endings of nerve cells of the hypothalamic-hypophyseal tract; vesicles contain vasopressin and oxytocin.

TABLE 3-7. LOCATION AND FUNCTION OF NEUROTRANSMITTERS

Agent	Location	Function
L-Glutamine	Excitatory neurons	Excitation
GABA	Inhibitory neurons	Inhibition (fast/slow)
Acetylcholine	Motor neurons, basal forebrain, and midbrain and pontine tegmentum	Excitation and modulation
Monoamines -- Norepinephrine -- Serotonin -- Histamine	Brain stem and hypothalamus --Locus ceruleus --Raphe nuclei --Hypothalamus	Modulation
Neuropeptides	Limbic, hypothalamus, autonomics, and pain pathways	Modulation

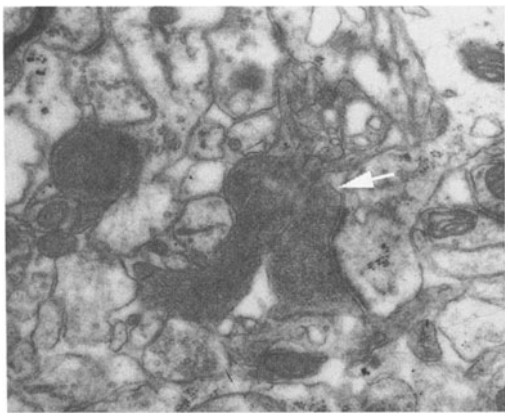

Figure 3-22. Degenerating synaptic ending in the sensory cortex of the adult rat, showing dense vesicles and filaments replacing the normal appearance seen in Figure 3-21. Electron micrograph <X> 115,000

pounds have been found: acetylcholine, norepinephrine, dopamine, serotonin, glutamate, and aspartate. Inhibitory neurotransmitters include GABA, histamine, neurotensin, and angiotensin.

Acetylcholine, the best documented transmitter in the peripheral nervous system, has been isolated in synaptic vesicles. Acetylcholine esterase has been found throughout the central and peripheral nervous systems and at postganglionic sympathetic endings.

Effectors and Receptors

Each peripheral nerve, whether sensory, motor, or secretory, terminates by arborizing in a peripheral structure (Fig.1-3).

Effectors

The motor nerves of the somatic nervous system end in skeletal muscles and form the *motor end plates* (see Chapter 6). Nerve endings in smooth and cardiac muscle and in glands resemble the synaptic endings in the central nervous system. *Visceral motor endings* are found on muscles in arterioles (vasomotor), muscles in hair follicles (pilomotor), and sweat glands (sudomotor).

Sensory Receptors. A stereogram of the skin is shown in Figure 1-3. Table 3-8 lists the mechanicoreceptors in the body.

Sensory Endings (Fig 1-3). Sensory endings, found throughout the body, subserve pain, touch, temperature, vibration, pressure, heat, and cold in the skin, muscles, and viscera as well as the specialized somatic and visceral sensations of taste, smell, vision, audition, and balance. Visceral sensory receptors are similar to somatic sensory receptors associated with the somatic nervous system, except that they are located in the viscera and their accessory organs.

Free Nerve Endings (Fig. 1-3). Free nerve

TABLE 3-8. MECHANICORECEPTORS

Modality	Receptors
Light touch and vibration	Encapsulated endings--Meissner=s and Pacinian corpuscles
Proprioception	Muscle spindles and Golgi tendon organs in joints
Pain and temperature	Free nerve endings

endings are formed by sensory fibers and arborize in various tissues, including the stratified epithelium, muscles, tendons, connective tissues, mucosa, and serous membranes in joints. They are considered to be pain receptors because they are found in tissues where pain is the primary sensation, such as tooth pulp, dentin, and the cornea. Crude touch may also be subserved by these receptors.

Free nerve endings are also found in terminal networks around the disc-shaped tactile cells of Merkel and around the hair follicles in the dermal sheath and outer root sheath. These structures appear to subserve touch.

Encapsulated Sensory Endings (Fig. 1-3). In these endings, the nerve is surrounded by a specialized connective tissue capsule of varying thickness. Encapsulated endings include Meissner=s and pacinian corpuscles, muscle and tendon spindles, the cylindrical end bulb of Krause, and the end bulb of Golgi-Mason.

Meissner's Corpuscles(Fig 1-3). Meissner=s (tactile) corpuscles are presumed to subserve touch. They are elliptical and may have from one to five myelinated nerve fibers arborizing in their lamellated capsule. These end organs are found in dermal papillae, being most numerous in the fingertips, soles, palms, lips, glans penis, and clitoris.

Pacinian Corpuscles (fig 1-3). These corpuscles resemble a sliced onion. Many concentric layers built upon the centrally placed axon. They are found throughout subcutaneous tissue and are especially numerous in the hand, foot, mammary glands, clitoris, and penis. Pacinian corpuscles are pressure-sensitive receptors. Herbst=s corpuscles are similar to pacinian corpuscles but smaller.

End Bulbs of Krausse and Golgi-Mason(fig 1 -3). These endings are found throughout the body and contain a single, extensively ramified axon within the matrix. Many variants of this structure have been identified. This organ is presumed to record changes in heat and cold.

Muscle and Tendon Spindles (fig 7-20). The muscle spindles and annulospiral endings (see Chapter 5), as well as the tendon spindles, transmit information concerning muscular activity and tendon stretching to the central nervous system.

Remember that all of the sensory endings form the primary neurons in the sensory system. Their cell bodies are located in the spinal dorsal root ganglia or cranial nerve ganglia, and their axons enter the central nervous system. The motor or effector axons represent the lower motor neuron or final neuron in the motor system.

Supporting Cells of the Central Nervous System

The central nervous system has billions of neurons, but the number of supporting cells exceeds them by a factor of five or six. Supporting cells form a structural matrix and play a vital role in transporting gases, water, electrolytes, and metabolites from blood vessels to the neural parenchyma and in removing waste products from the neuron. In contrast to the neuron, the supporting cells in the adult central nervous system normally undergo mitotic division. The supporting cells are divided into macroglia and microglia.

The *macroglia,* which include astrocytes, oligodendrocytes, and ependyma, are the supporting cells or neuroglia (nerve glue) of the central nervous system (fig 3-23). *Schwann cells, satellite cells,* and *fibroblasts* are supporting cells of the peripheral nervous system. Mesodermal *microglia* cells include the perivascular cells and any white blood cells found within the parenchyma of the central nervous system. Functions of the different supporting cells in the nervous system are summarized in Table 3-9.

Astrocytes (Figs. 3-23, 3-24, and 3-25)

Astrocytes are of two types: fibrous (most common in white matter) or protoplasmic (most

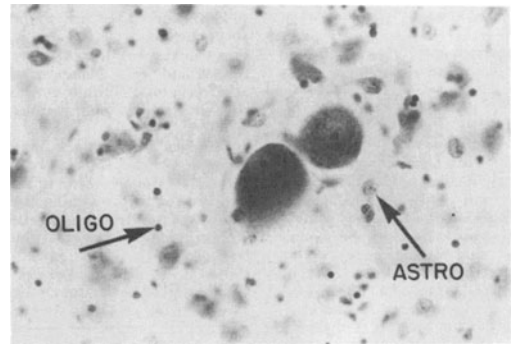

Figure 3-23. Appearance of neuron, astrocyte (astro), and oligodendrocyte (oligo). Nissl stain.(<X>300.)

TABLE 3-9. FUNCTIONS OF SUPPORTING CELLS

Cell Type	Functions
Astrocytes --Fibrous type (white matter) --Protoplasmic type (gray matter)	Major supporting cells in the brain, forming microenvironment for neurons; act as phagocyte; isolate synapses, enwrap blood vessels, and form membranes on brain=s inner and outer surface.
Oligodendrocytes	Form and maintain myelin
Ependymal cells	Ciliated lining cells of the ventricular system
Endothelial cells	Lining cells of blood vessels in the brain that form blood-brain barrier.
Microglia (pericytes)	Supporting cells and multipotential cells found in the basement membrane of blood vessels and within brain parenchyma
Mononuclear cells	White cells from the circulation that readily enter the brain (lymphocytes, monocytes, and macrophages) and function as sentinels for the immune system

common in gray matter). All astrocytes are larger and less dense than the oligodendrocytes. The astrocytes form a complete membrane on the external surface of the brain called the external glial limiting membrane, which surrounds all blood vessels, fuses with the ependymal processes, and isolates neuronal processes.

In light micrographs astrocytes appear as pale cells with little or no detail in the cytoplasm. The nuclei are smaller than those of a neuron but larger and less dense than those of an oligodendrocyte *(Fig. 3-24)*. Electron micrographs demonstrate that fibrous astrocytes have many filaments, which in places appear to fill the cytoplasm. There are few microtubules, and the processes appear pale. The nuclei of these cells have some condensed chromatin adjacent to the nuclear membrane. Glycogen is also common in astrocytic processes. Protoplasmic astrocytes have nuclei that are a little darker than those of a neuron. They resemble fibrous astrocytes except that they have just a few filaments.

Astrocytes not only form the skeleton of the central nervous system but also tend to segregate

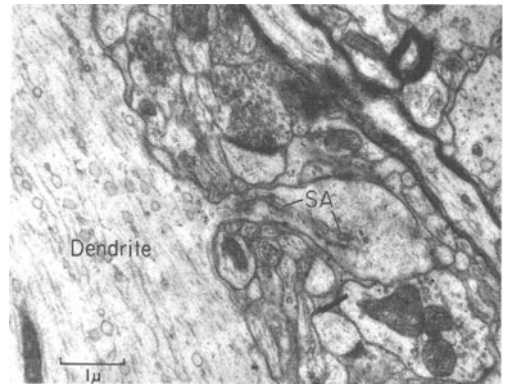

Figure 3-24. Dendrite with dendritic spine. The spines greatly increase the surface area of many neurons. Note the many microtubules in the dendrite and the neck of eh dendritic spin containing a spine apparatus. The3re are several synapses on the spine. Electorn micrograph <X> 80,000.

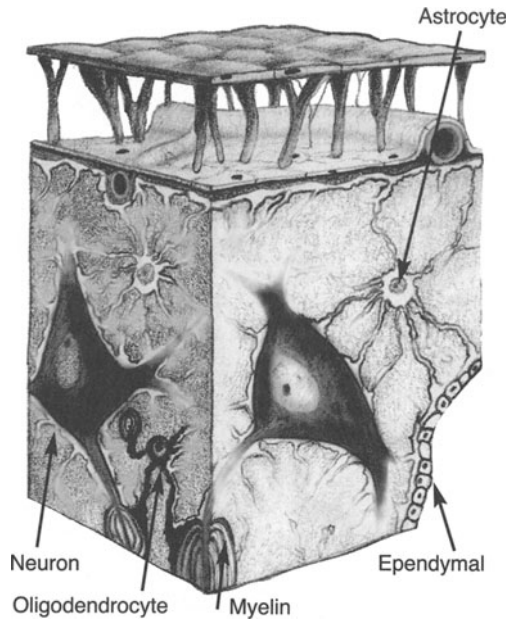

Figure 3-25. Electron micrograph demonstrating appearance of the neuron, astrocyte (astro), and oligodendrocyte (oligo). (<X> 18,000.)

synapses and help form the blood-brain barrier by enwrapping brain capillaries (outer and inner limiting membranes). If the brain is damaged by infarction, for example, astrocytes proliferate and form scars.

Astrocytes and adjacent neurons form the microenvironment of the nervous system. There are approximately 100 to 1,000 astrocytes per

neuron, depending on the size of the neuron. In areas with high glutamate concentration, such as the cerebral cortex, or areas with high dopamine content, such as the basal nuclei, the glia take on the chemical characteristics of the adjacent neuron. Glia are involved in neuronal functions because they absorb transmitters and modulators in their environment and often release them back into the synapse.

When central nervous system diseases affect only astrocytes, the reaction is called primary astrogliosis. More commonly, the disease process affects nerve cells primarily, which is called secondary astrogliosis. When an injury occurs to the nerve cell in the central nervous system without concomitant injury to blood vesicles and glia, the nerve cells are phagocytized and the astrocytes proliferate and replace the neurons, forming a glial scar (replacement gliosis). In the case of a more severe injury to the nervous system, such as an infarct that damages glia, nerve cells, and blood vessels, the astrocytes proliferate along the wall of the injury, and the dead neurons and glia are phagocytized, leaving only a cavity lined with meninges. In all other organs there are enough fibroblasts to proliferate and form a scar, but in the central nervous system there are only a few fibroblasts, so cavitation is a common sequela to extensive destruction.

Oligodendrocytes

In light micrographs the oligodendrocyte has a small darkly stained nucleus surrounded by a thin ring of cytoplasm (Fig. 3-23). In electron micrographs oligodendrocytes are dense cells with many microtubules and few neurofilaments (Figs. *3-25 and 3-26*). Dense clumps of rough endoplasmic reticulum and clusters of polyribosomes are seen in the cytoplasm, which is denser but scantier than that in neurons. The nucleus tends to be located toward one pole of the cell; the nuclear chromatin tends to be heavily clumped. In electron micrographs oligodendrocytes can be distinguished from astrocytes because they have a darker cytoplasm and nucleus, few if any filaments, and more heavily condensed chromatin (Fig. 3-26).

The role of the oligodendrocyte is to form and maintain myelin (although they may also be responsible for breaking down myelin in multiple sclerosis). The formation of myelin is under genetic control. Oligodendrocytes are usually seen in close proximity to astrocytes and neurons, and all three cell types are important in forming and maintaining myelin.

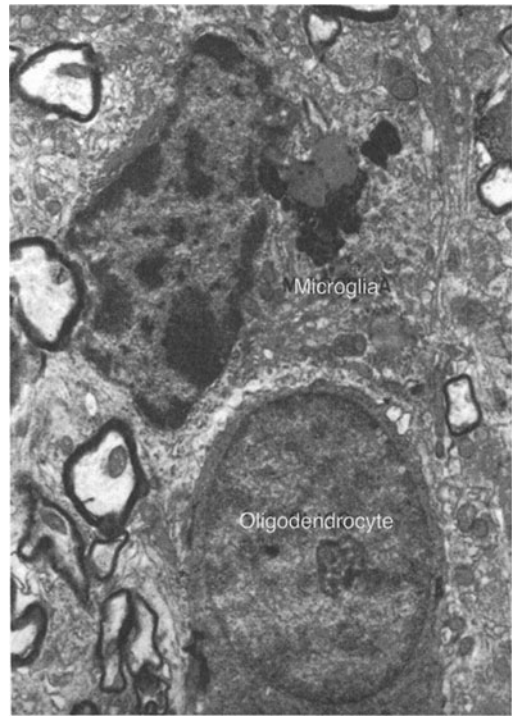

Figure 3-26. Electron micrograph of a human cerebral cortex demonstrating differences in the density of the DNA in the nuclei of oligodendrocytes (oligo) and microglia. (<X>30,000.)

Endothelial Cells

Endothelial cells form the lining of the capillaries in the central nervous system(fig 3-25). They are of mesodermal origin and bound together by tight junctions. Their tight junctions and apinocytosis provide the basis of the blood-brain barrier (see below).

Mononuclear Cells

Mononuclear cells--lymphocytes, monocytes, and histiocytes--are found in the central nervous system, where they seem to act as phagocytes, breaking down myelin and neurons. Myelin destruction always triggers intense macrophage reaction within 48 hours, followed by infiltration of monocytes first and then lymphocytes. Note that astrocytes have also been shown to engulf degenerating myelin sheaths, axonal processes, and degenerating synapses.

The central nervous system was once considered an Aimmunologically privileged site@ because:

1. No specific lymph drainage from the central nervous system alerts the immune system of infection.

2. Neurons and glia do not express the major histocompatibility complex.

3. The major cell for stimulating the immune response (leukocyte dendritic cells) is not normally present in the disease-free nervous system.

However, recent studies have shown that there is a regular immune surveillance of the central nervous system, which is sufficient to control many viral infections (Sedgwick and Dorries, 1991). It is now known that immune cells regularly enter the brain through the capillaries and that macrophages infected with human immunodeficiency virus (HIV), for example, can infect the brain directly, the so-called Trojan-horse phenomenon (Haase, 1986; Price et al., 1988).

Microglia

Neurons, astrocytes, and oligodendrocytes are ectodermal in origin, but microglial cells are mesodermal in origin (Fig. 3-26). The ovoid microglia cells are the smallest of the supporting cells and are divided into two categories: (1) those that form the perivascular cells and (3) the resting microglial cells in the brain parenchyma. Microglial cells originate from monocytes that enter the brain (Table 3-10).

Pericytes are found in relation to capillaries but external to the endothelial cells and enwrapped in the basal lamina. In electron micrographs they are not as electron-dense as oligodendrocytes and lack the neurofilaments of the astrocyte and the tubules of the oligodendrocyte. The cytoplasm is denser than that of astrocytes and contains fat droplets and laminar dense bodies. The granular endoplasmic reticulum consists of long stringy cisterns. Microglia are considered multipotential cells because with the proper stimulus they can become macrophages (Vaughn and Peters, 1968). The pericyte contains actin, and this cell may well be important in controlling the channels entering the endothelial cells (Herman and Jacobson, 1988).

During early development, *monocytes* enter the brain and, after formation of the blood-brain barrier, become trapped (Davis et al., 1994; Ling and Wong, 1993). The monocytes pass through an intermediate phase of development, the *amoeboid microglia*, which evolve into a down-regulated resting form, the *ramified microglia.* These *resting microglia* are found throughout the central nervous system and may well be the sentinels that alert the immune system to disease in the brain. With the appeearance of any central nervous system disease (e.g., multiple sclerosis, stroke, trauma, or tumors), the resting microglia are upregulated and become *activated microglial cell.* The factor or gene that upregulates or down-regulates these cells is currently unknown. Once the disease process has been resolved, the activated microglia can revert to resting microglial cells.

The activated microglia cell is a partially activated macrophage containing the CR3 complex and class I major histocompatibility complex (MHC). The active microglia then become a

TABLE 3-10. TYPES OF MICROGLIA CELLS

Cell Type	Function
Monocytes	Enters brain during early development and is the stem cell of microglia.
Pericytes (perivascular cell)	Found inside the brain in the basement membrane of the blood vessel; can act as a macrophage.
Amoeboid microglia	Transitional form leads to resting microglia.
Resting microglia (ramified)	Down-regulated from amoeboid microglia; probably the sentinels in the brain that raise the alarm for invasive diseases.
Activated microglia	Upregulated resting cell changes into partially activated macrophage with MHC class I.
Reactive microglia	Fully activated macrophage with MHC class II and phagocytic properties.
Giant multinucleated cells	Forms from fusion of reactive cells; associated with viral brain infections and Creutzfeld-Jakob disease; hallmark of AIDS dementia.

reactive microglial cell, which is a fully active macrophage containing class II MHC and phagocytic activity. These cells are very active during all major disease states in the brain. Activated microglial cells can also evolve into giant multinucleated cells by the fusion of reactive cells. They are seen in viral infections and are considered the hallmark of AIDS dementia. Also called gitter cells, *giant multinucleated cells* are often found in patients with Creutzfeldt-Jakob disease (Fig. 3-31), a disease caused by proteinaceous infectious particles, or prions.

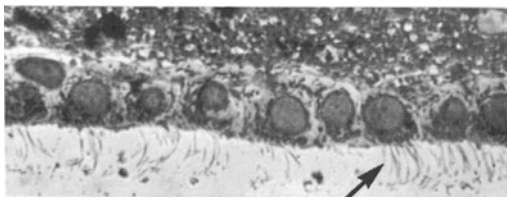

Figure 3-27. Ependymal lining cells in the third ventricle of a rat. Note prominent cilia (arrows) in this one-micron thick plastic-stained section. (<X>1,400.)

Ependymal Cells (Fig. 3-27)

Ependymal cells line all parts of the ventricular system. They are cuboidal, ciliated, and contain filaments and other organelles. The processes of these cells extend in the central nervous system and fuse with astrocytic processes to form the *inner limiting glial membrane.* Highly modified ependymal cells are found attached to the blood vessels in the roof of the body of the lateral ventricles, the inferior horn of the lateral ventricles, and the third and fourth ventricles. There they form the choroid plexus, which secretes much of the cerebrospinal fluid (Fig. 3-35). Ependymal cells originate from the germinal cells lining the embryonic ventricle, but they soon stop differentiating and stay at the lumen on the developing ventricles.

Satellite Cells (Fig. 3-28)

Satellite cells, which are found only in the peripheral nervous system among sensory and sympathetic ganglia, originate from neural crest cells. Many satellite cells envelop a ganglion cell. Functionally, they are similar to the astrocytes, although they look more like oligodendrocytes.

Schwann Cells

Schwann cells are ectodermal in origin (neural crest) in the peripheral nervous system and function like oligodendrocytes, forming the myelin and neurilemmal sheath. In addition, the unmyelinated axons are embedded in their cytoplasm. Schwann cell cytoplasm stops before the nodes of Ranvier (fig 3-33), leaving spaces between the node and Schwann cells. In an injured nerve Schwann cells can form tubes that penetrate the scar and permit regeneration of the peripheral axons. Nerve growth factor is important to proliferation of the Schwann cells.

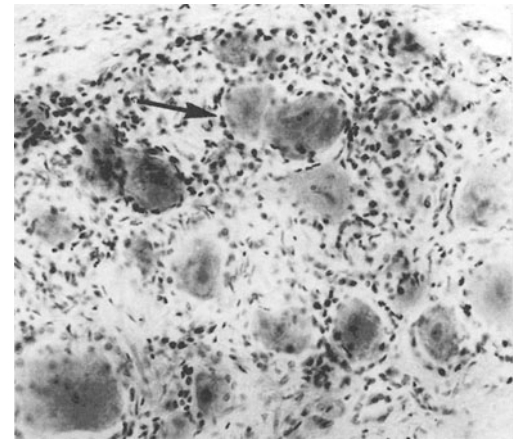

Figure 3-28. Sensory ganglion of the rhesus monkey, demonstrating pseudounipolar cells surrounded by their satellite cells (arrow). (<X>300.)

Neural Crest Cells

These cells originate embryologically as neuroectodermal cells on either side of the dorsal crest of the developing neural tube but soon drop dorsolaterally to the evolving spinal cord area. Neural crest cells migrate out to form the following: dorsal root ganglion cells, satellite cells, autonomic ganglion cells, Schwann cells of the peripheral nervous system, chromaffin cells of the adrenal medulla, and pigment cells of the integument.

Response of Nervous System to Injury

Degeneration

Neuronal death or atrophy may result from trauma, circulatory insufficiency (strokes), tumors, infections, metabolic insufficiency, developmental defects, and degenerative and heredodegenerative diseases. These neuropathologic processes produce a range of responses in the neurons and glia (Ramon y Cajal, 1938; Young, 1943). In this section, the neuronal response to

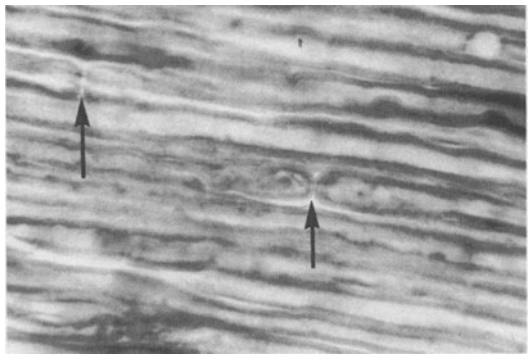

Figure 3-29. Longitudinal section of a peripheral nerve fixed in osmium, demonstrating the nodes of Ranvier (arrows). (<X>1,000.)

injury will be examined in the cell body and axon.

Retrograde Changes in the Cell Body (Fig. 3-30). Section of the axon obr direct injury to the dendrites or cell body produces the following series of responses in the soma:

1. The nucleus, cell body, and nucleoli swell. The nucleus is displaced from the center of the cell body and may even lie adjacent to the plasma membrane of the neuron.

2. A slow dissolution of Nissl substance starts centrally and proceeds peripherally, until only the most peripherally placed Nissl substance is left intact (which is probably essential to the protein metabolism of the rest of the cell). This dissolution of the Nissl substance (ribosomal RNA), called chromatolysis, allows the protein-manufacturing processes to be mobilized to help the neuron survive the injury. The mRNA then begins the manufacturing of membrane that is transported down the intact tubules into the growing axonal ending (growth cone).

3. All other organelles in the cell body and dendrites also respond to the injury. The mitochondria swell, and the smooth endoplasmic

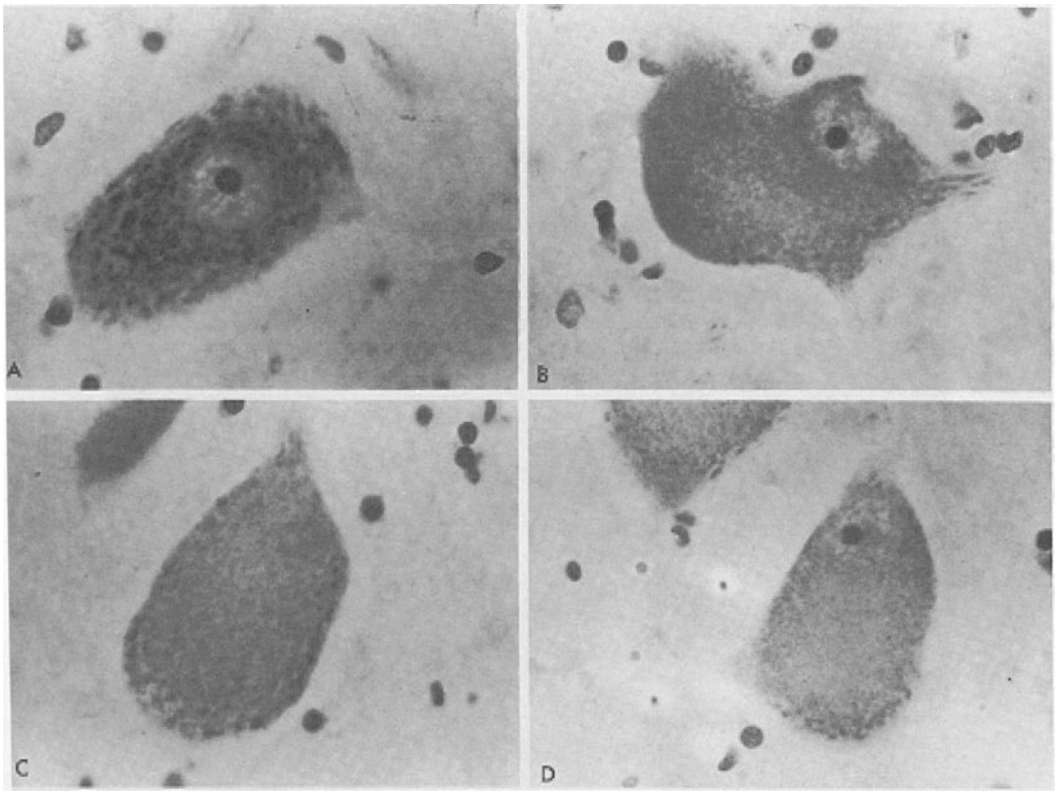

Figure 3-30. Ventral horn cells in the human lumbar spinal cord. A, Normal. B to D, Wallerian retrograde chromatolytic changes in ventral horn cells following injury to the peripheral nerve. B, Chromatolytic neuron with eccentric nucleus and some dissolution of the Nissl substance. C, Chromatolytic neurons, showing a peripheral ring of Nissl substance (peripheral chromatolysis). D, Chromatolytic neuron, showing eccentric nucleus and only a peripheral ring of Nissl substance. (Nissl stain, <X>400.)

reticulum proliferates to help in the formation of new plasma membrane and new myelin.

These responses represent the increased energy requirements of the nerve cell and the need to form plasma membrane during the regenerative process. If the cell survives the injury, all organelles return to normal: the nucleus returns to the center of the cell body, and the soma returns to its pretraumatic size. If the injury is too extensive, the neuron atrophies or dies. The responses of neuronal soma to injury (chromatolysis) can be summarized in three steps:

1. Swelling of nucleus, nucleolus, and cytoplasm with nucleus becoming eccentric as a direct response to injury of the axon or dendrite. Nissl substance appears to dissolve.

2 Proliferation of metabolic processes in the nucleus including mRNA occurs. Endoplasmic reticulum and mitochondria starts manufacturing membranes and increasing the energy available in the cell.

3. With successful recovery, the cell returns to normal size. If seriously injured, the cell becomes atrophic or may be phagocytized.

Atrophic Change. In atrophic change, the nerve cell is too severely damaged to repair itself. Consequently, the cell body shrinks and becomes smaller. This response is similar to the response of a nerve cell to insufficient blood supply, which produces an ischemic neuron. If necrosis occurs, the neuron cannot survive. The Nissl substance begins to disperse, and after 7 days the nucleus becomes dark and the cytoplasm eosinophilic. Within a few days, these cells are phagocytized.

Wallerian Degeneration. When an axon is sectioned, the distal part that is separated from the trophic center (cell body) degenerates, a process called *wallerian,* or *anterograde, degeneration.* At the same time, the cell body undergoes a process called *axonal,* or *retrograde, degeneration.* If the cell body remains intact, the proximal portion begins to regenerate. The distal stump is usually viable for a few days, but its degeneration begins within 13 hours of injury. The axon starts to degenerate before the myelin sheath. In 4 to 7 days, the axon appears beaded and is beginning to be phagocytized by macrophages, which enter from the circulatory system (Fig. 3-4). Fragments of degenerating axons and myelin are broken down in digestion chambers (Figs. 3-31 and 3-32), and it may take several months before all of the fragments are ingested. In the proximal portion degenerative changes are noted back to the first unaffected node. As the myelin degenerates, it is broken up into smaller pieces that can be ingested more easily *(Figs. 3-32 and 3-33).*

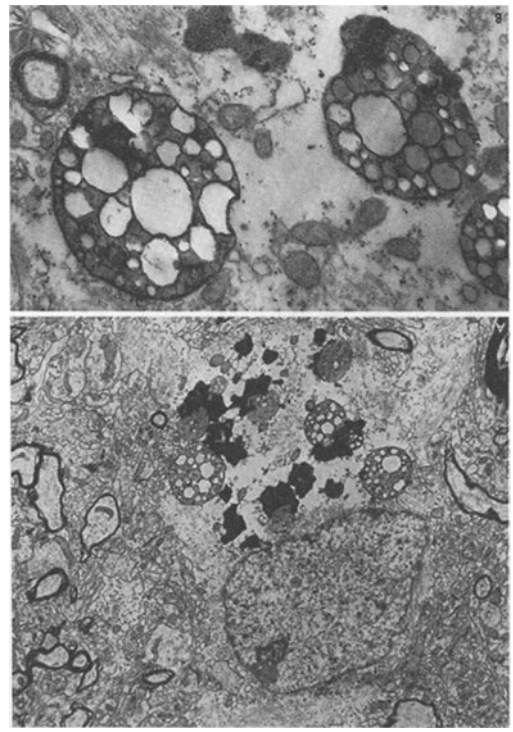

Figure 3-31. A and B, Electron micrograph of a reactive astrocyte in the cerebral cortex of a person with Jakob-Creutzfeldt disease. Note the prominent digestion vacuoles shown in higher power in B. (A, <X>8,000; B, <X> 35,000.)

REGENERATION

Peripheral Nerve Regeneration

Within a few days after section, the proximal part (attached to a functional neuronal soma) of the nerve starts regrowing. Nerve growth factor is produced after injury to the axon, and it promotes the axonal sprouting. If the wound is clean, e.g., a stab sound, sewing the nerve ends together can dramatically increase the rate of recovery in the affected limb. The regenerating nerves may cross the scar within several weeks (Fig. 3-34). The crossing is helped by the Schwann cells and fibroblasts, which proliferate from the proximal end of the nerve. The Schwann cells form new basement membrane

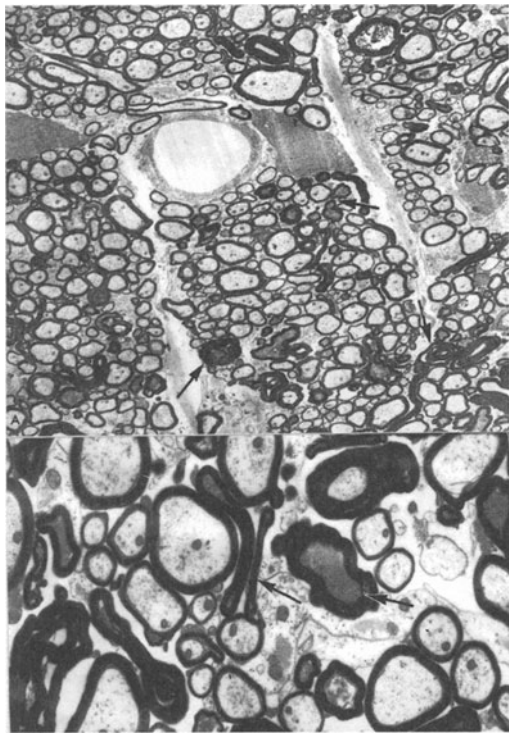

Figure 3-32. Electron micrograph of degenerating axons in several fascicles of the medullary pyramid of a rat 15 days after a cortical lesion. A, Arrows point to degenerating axons (<X>8,000). B, Detail of degenerating axons (<X>30,000). Note the collapsed axons and dense axoplasm and that many axons were unaffected by the lesion.

and provide tubes through which the regenerating axons can grow.

In certain peripheral nervous system diseases only segmental degeneration occurs. One example is diphtheria: the myelin sheath degenerates but the axon remains intact. Phagocytes break down the myelin, and Schwann cells rapidly reform myelin.

The rate of movement of the slow component of axoplasmic flow probably accounts for the rate of axonal regrowth, which is limited to about 1 mm a day. Slow components of the axoplasmic flow (-Scb) carry actin, fodrin, calmodulin, clathrina, and glycolytic enzymes that form the network of microtubules, intermediate filaments, and the axolemma, which limit the rate of daily axonal regeneration, although functional recovery may be a little faster (McQuarrie and Grafstein, 1983; Wujek and Lasek, 1983; McQuarrie, 1988; Kandel and Schwartz 2000).

As the regenerating axon grows, the axonal end sprouts many little processes. If one axonal sprout penetrates the scar, the other sprouts degenerate, and the axon follows the path established by the penetrating sprout. If an axon reaches one of the tubes formed by the Schwann cells, it grows quickly and after crossing the scar descends the distal stump at a rate of approximately 1 mm/day (Jacobson and Guth, 1965; Guth and Jacobson, 1966).

When the motor end plate is reached, a delay occurs while the axon reinnervates the muscle and reestablishes function. At this stage the average rate of functional regeneration is 1 to 3 mm a day.

Only a small percentage of the nerves reach the effectors or receptors. The basal laminae helps direct the regenerating nerve to the motor end plate. If a sensory fiber innervates a motor end plate, it remains nonfunctional and probably degenerates, and the cell body atrophies. A sensory fiber that reaches a sensory receptor may become functional, even if the receptor is the wrong one. For example, after nerve regeneration some patients complain that rubbing or pressing the skin produces pain. In these cases it would appear that fibers sensitive to pain have reached a tactile or pressure-sensitive receptor. A

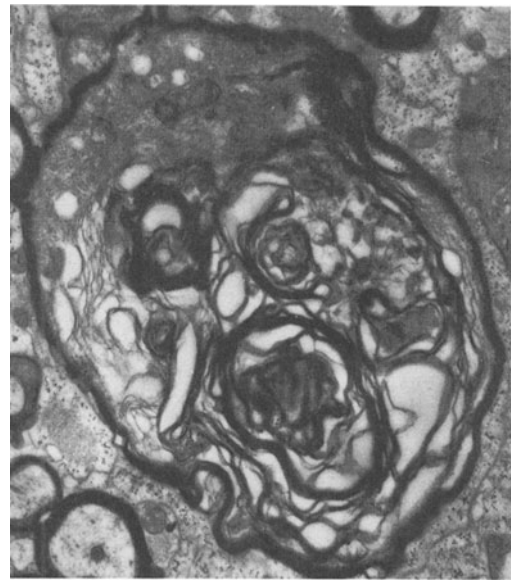

Figure 3-33. Electron micrograph of a degenerating myelin sheath in the medullary pyramid of a rat 30 days after a cortical lesion. Note the unraveling and vacuolization of the myelin. (<X>30,000.)

motor fiber may also reinnervate the wrong motor end plate, as when a flexor axon innervates an extensor. In such a case, the patient has to relearn how to use the muscle.

Muscle that is denervated assists the regenerating axons by expressing molecules that influence the regenerating axons. Some of the molecules are concentrated in the synaptic basal lamina of the muscle. Other molecules are upregulated following denervation and help in attracting and reestablishing the synapse in the muscle. These upregulated molecules include: growth factors (IGF-3 and FGF-5), acetylcholinesterase (AChE), agrin, laminin, s-laminin, fibronectin, collagen a3, and the adhesion molecules N-CAM and N-cadherin (Hall and Patterson, 1993; Horner and Gage 3000).

Successful nerve regeneration also depends on an adequate blood supply. For example, in a large gun shot wound, nerves attempt to regenerate but may not succeed. A summary of the sequence of regeneration in the peripheral nervous system can be broken down into seven steps:

1 The peripheral nerve is sectioned by an injury.

2 The axon dies back to the first unaffected node of Ranvier, with the myelin and distal axon beginning to degenerate within 34 hours.

3 At the site of injury, the axon and myelin degenerates to form a scar. Phagocytosis begins within 48 hours.

4 Axons separated from the cell body degenerate. With an adequate blood supply the portion of the axon still connected to the intact cell body begins regenerating by sprouting.

5 Within 73 hours, Schwann cells begin to proliferate and form basement membranes and hollow tubes. Nerve growth factor is also formed and released, which further encourages sprouting.

6 From each of the severed axons, sprouts attempt to penetrate the scar. After one sprout successfully grows through the scar the other sprouts die. Nerves take a month or more to grow through the scar.

7 Once an axon penetrates the scar, it grows at 1 mm/day; about a third of the severed axons actually reinnervate muscle and skin.

Central Nerve Regeneration

After an injury, axons in the central nervous system regenerate, but there seems to be no equivalent to the Schwann cell because oligodendrocytes and astrocytes do not form tubes to penetrate the scar. Instead, they form a scar that is nearly impenetrable. Even if the axons penetrate the scar, they have no means of reaching the neuron to which they were originally connected.Horner and Gage (2000)have reviewed the question of how to regenerate the damaged central nervous system in the brain that is inherelty very plastic. The y have noted that it is not the failure of neruoaln regeneration , but it is rather a feature of the dmaged environment; and it is now possible to reintroduce the factors preent in the developing nervous system tha produced this wonderful organ. The gene responsible for needed growth factors is probably missing or inactivated in adult tissue. Recently a brain-derived neurotrophic factor has been identified, which may eventually help in finding a way to guide the axon (Goodman, 1994).

Animal studies have shown that neurons have considerable *plasticity.* That is, if some axons in a region die off, bordering unaffected axons will sprout and form new synapses over many months, filling in where the synapses were and resulting in major functional reorganization. This reorganization may eventually produce some recovery of function.

Stem Cells. In the Adult Brain neuronal stem cells have been identified in the adult brain and spinal cord. These cells under the right conditions may well be activated and help to reverse the effects of lesions in the CNS (Kornack & Rackic 1999). After the implantation of immature neurons (neuroblasts) in regions affected by certain diseases (e.g., the corpus striatum of patients with Parkinson's disease), there has been some recovery (Sladek and Gash, 1984; Gage et al., 1991). In Parkinsonian patinet's the age of the individual receiving the transported cells seems to effect the outcome with younger patients(less than 50 years of age) more likely to show some improvement. Regenerating axons in the central nervous system may also grow and form a nonfunctional neuronal ball, or neuroma. Similar long-lasting neuromas may form in the

peripheral nervous system and may be a source of pain. In time, the dead neurons and axons are phagocytized by the glia and macrophages, but the astrocytic scar remains.

Factors that promote neuronal survival and axon outgrowth (e.g. brain derived neurotrophic factor-BDNF) have been identified and the focus is now on getting these cells to produce axons to grow into the injured areas and then to grow through into the uninjured area.

Nerve Growth Factors. The first nerve growth factor was isolated by Levi-Montalcini and Angeletti in 1968, but only recently have biotechnology techniques been able to produce these factors in large quantities. Attempts have been made to help regeneration in the central nervous system, for instance, by placing Teflon tubes on Schwann cells through the scarred portion of the spinal cord in the hope that the nerves would follow these channels. However, even though nerves do grow down these channels, no functional recovery occurs. Further information is needed to understand better the chemical nature of scar tissue that retards axonal regeneration.

With the identification of neurotrophic factor (netrins 1 and 3) that produces axonal growth (Serifini et al., 1994) and with the studies of programmed cell death beginning to identify genes that may be responsible for premature neuron death (Oppenheim, 1991), we may be entering an era of brain research that offers great promise to help patients with neurodegenerative diseases, Huntington=s, Parkinson=s and Alzheimer=s.

Glial Response to Injury

Neuronal death triggers an influx of phagocytic cells from the blood stream and the microglia proliferate and break down the dying neurons.

Necrosis. Within a few days of an ischemic attack with infarction, neutrophils are seen at the site of injury. Shortly thereafter, microglial cells and histocytes are seen in the region of the dying cells. Since the blood-brain barrier is usually compromised, monocytes may now migrate into the parenchyma of the central nervous system in greater numbers and assist in phagocytosis.

The time it takes for the complete removal of injured cells depends on the size of the lesion.

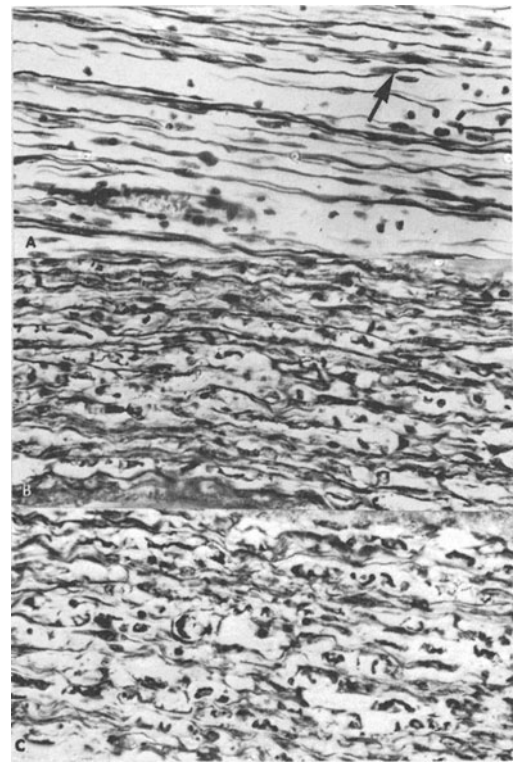

Figure 3-34. Sciatic nerve of a rat, demonstrating the appearance of regenerating nerves 3 weeks after a crushing injury. A, At the site of the crushing lesion, the nerves look normal with regenerating axons grown past the site of the injury; arrow indicates Schwann cell nucleus. B, About 30 mm distal to the site of injury, note some regenerating axons and still many degenerating axon fragments. C, About 35 mm distal to the crush site the transition from regenerating axons to only degenerating axons is shown. (Bodian silver stain, <X>500.)

Large infarcts may take several years before phagocytosis is complete. If the lesion is huge, such as a large infarct in the precentral gyrus, a cavity lined by astrocytic scar will form. In small lesions the neurons are phagocytized glia proliferate, a process called replacement gliosis. In organs with numerous fibroblasts, necrotic areas are soon filled with proliferating fibroblasts, but in the central nervous system there are few fibroblasts for this, and the astrocytes do not proliferate in sufficient numbers.

Blood-Brain Barrier

In the *peripheral nervous system* the endothelial cells are fenestrated and very active in pinocytosis: these two factors increase the ability of compounds to readily enter the nerves. Also,

molecules move through peripheral endothelial cells by fluid phase, or receptor-mediated, endocytosis. *Fluid phase endocytosis* is relatively nonspecific; the endothelial cells engulf molecules and then internalize them by vesicular endocytosis. In *receptor-mediated endocytosis,* a ligand first binds to a membrane receptor on one side of the cell. After binding to the ligand the complex is internalized into a vesicle and transported across the cell, the ligand is usually released.

In the *central nervous system* endothelial cells that line the capillaries and the choroid plexus are joined together by tight junctions, zonula occludens(fig 3-25). The capillaries are not perforated, and the endothelial cells show very little pinocytosis or receptor-mediated endocytosis (Brightman, 1988). This endothelial lining is called the blood-brain barrier because it is very selective to certain large molecules and dyes and limits the entry of other substances, including amino acids, water, glucose, and electrolytes into the brain parenchyma.

Plasma in blood vessels is separated from the central nervous system tissue by the endothelial lining of the blood vessel and the basement membrane. Pericytes (perivascular) cells are also found within the basement membrane of the capillary wall. The extracellular space of the central nervous system lies external to the basement membrane. All vascular branches within the central nervous system are surrounded by a thin covering formed by astrocytic processes *(Fig. 3-25).* However, the astrocytic processes do not fuse with the endothelial lining of the blood vessel or with the processes of other cells, so they have minimal effect on limiting the entry of solutes into the brain parenchyma. Thus the extracellular space can be entered once the materials pass through the endothelium.

The intravenous perfusion of various dye compounds (trypan blue, Evans blue, proflavin HCI, and horseradish peroxidase) demonstrates passage through the blood-brain barrier. These dyes demonstrate that the blood-brain barrier is leaky in certain midline regions of the third and fourth ventricle, the circumventricular organs, and in the choroid plexus and locus ceruleus (Brightman, 1989; Dempsey and Wislocki, 1955; Wislocki and Leduc, 1953). The circumventricular organs include: pituitary, median eminence, organum vasculosum, subfornical organ, subcommissural organ, pineal gland, and the area postrema of the fourth ventricle. These open connections between the brain and the ventricular system permit neuropeptides from the hypothalamus, midbrain, and pituitary to enter the cerebrospinal fluid and to be widely distributed in the brain and spinal cord, thus forming an alternate pathway in the neuroendocrine system.

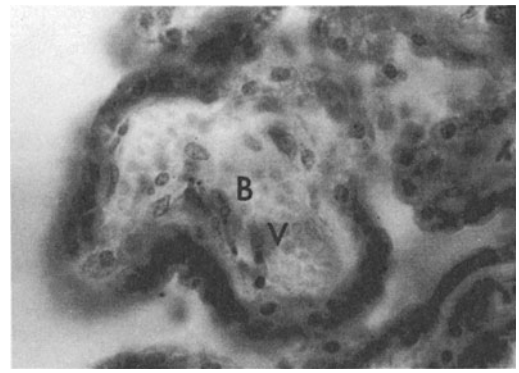

Figure 3-35. Site of cerebrospinal fluid formation: the choroid plexus in the fourth ventricle. Note the blood vessel (BV) in the center and the cuboidal epithelial cells (arrow) on the outside of the vessel. (<X>300.)

Large molecules (such as ferritin and horseradish perioxidase) injected invascularly do not pass through the endothelium; instead, they fill the extracellular spaces between the glia and neurons and do not reenter the blood vessels (Reese and Karnovsky, 1968). Studies have shown that the blood-brain barrier is impermeable to certain large molecules including proteins, but substances such as small lipid-soluable compounds, including alcohol and anesthetics, gasses, water, glucose, electrolytes (NA+, K+, and CL-), and amino acids, can pass from the plasma into the intracellular space (inside neurons and glia) or into the extracellular space between neurons and glia.

Acute lesions of the central nervous system, including those caused by infections, usually increase the permeability of the barrier and alter the concentrations of water, electrolytes, and protein. In viral diseases the infected leukocytes (macrophages) more easily penetrate into the brain by passing between the normally tight

junctions in the endothelial cells, which is one way that HIV enters the brain directly from the blood. Tumors within the central nervous system produce growth factors that cause blood vessels to sprout. These new blood vessels have immature tight junctions that are also quite leaky. The leakiness of the capillaries within tumors has been exploited with some success to deliver chemotherapeutic agents specifically to the tumor (Neuwelt and Dahlborg, 1989). There have also been attempts to interfere with the formation of the blood vessel growth factors as a way to starve tumors.

Stress has been shown to open the blood-brain barrier by activating the hypothalamic-hypophyseal-adreanal axia and releasing CRH (Esposito et al. 2001). Acute lesions of the central nervous system including those caused by infections usually increase the permeability of the barrier and alter the concentrations of water, electrolytes, and protein. In some viral diseases, for example, infected leukocytes (macrophages) more easily penetrate directly into the brain by passing between the normally tight junctions in the endothelial cells. This is one way HIV enters the brain from the blood. Also, central nervous system tumors produce growth factors that cause blood vessels to sprout. These new capillaries have immature tight junctions that are also quite leaky and have been studied with some success as a way to deliver chemotherapeutic agents specifically to the tumor (Neuwelt and Dahlborg, 1989).

Extracellular Space

Between the cells in the central nervous system is the extracellular space, measuring between 30 and 40 nm and filled with cerebrospinal fluid(CSF) and other solutes. The CSF is formed primarily by the choriod plexus in the lateral ventricle, IIIrd ventricle, and IV ventricle (Fig 3-35). The amount of extracellular space in the brain is still a matter of controversy. Some solutes can readily pass from the blood plasma through the endothelial lining into the extracellular space, and the solutes present in this space (whether deleterious or not) affect the functions of the central nervous system. A portion of the cerebrospinal fluid appears to be formed by the diffusion of extracellular fluid. Cerebrospinal fluid may also be reabsorbed after temporary storage in the extracellular space. Fat-soluble compounds that readily pass through the blood-brain barrier can enter the extracellular space and may be useful in resolving infections in the central nervous system or in improving the function of certain brain cells.