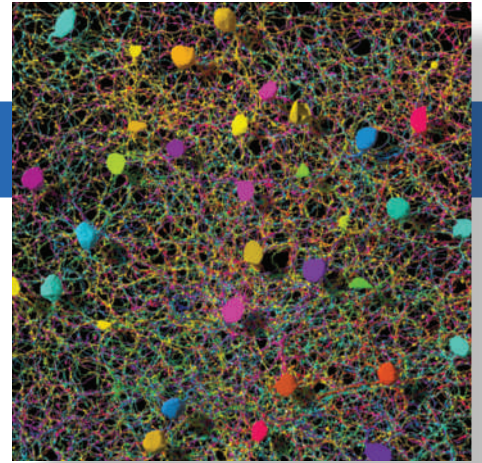
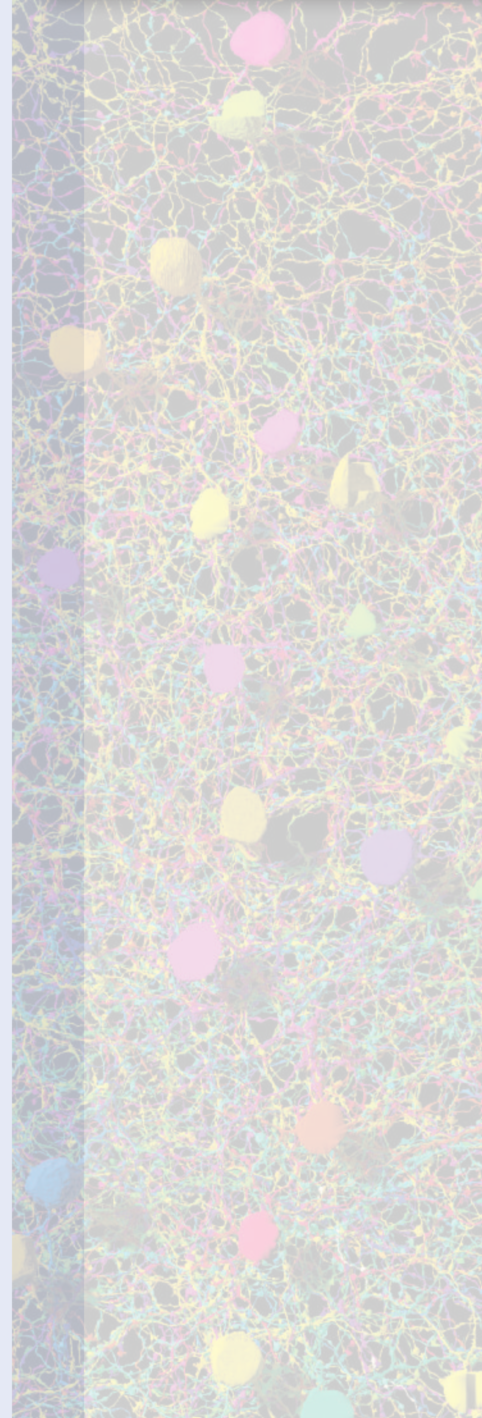


CHAPTER TWO

Neurons and Glia



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INTRODUCTION

All tissues and organs in the body consist of cells. The specialized functions of cells and how they interact determine the functions of organs. The brain is an organ—to be sure, the most sophisticated and complex organ that nature has devised. But the basic strategy for unraveling its functions is no different from that used to investigate the pancreas or the lung. We must begin by learning how brain cells work individually and then see how they are assembled to work together. In neuroscience, there is no need to separate *mind* from *brain*; once we fully understand the individual and concerted actions of brain cells, we will understand our mental abilities. The organization of this book reflects this "neurophilosophy." We start with the cells of the nervous system—their structure, function, and means of communication. In later chapters, we will explore how these cells are assembled into circuits that mediate sensation, perception, movement, speech, and emotion.

This chapter focuses on the structure of the different types of cells in the nervous system: *neurons* and *glia*. These are broad categories, within which are many types of cells that differ in structure, chemistry, and function. Nonetheless, the distinction between neurons and glia is important. Although there are approximately equal numbers of neurons and glia in the adult human brain (roughly 85 billion of each type), neurons are responsible for most of the unique functions of the brain. It is the **neurons** that sense changes in the environment, communicate these changes to other neurons, and command the body's responses to these sensations. **Glia**, or **glial cells**, contribute to brain function mainly by insulating, supporting, and nourishing neighboring neurons. If the brain were a chocolate chip cookie and the neurons were chocolate chips, the glia would be the cookie dough that fills all the other space and suspends the chips in their appropriate locations. Indeed, the term *glia* is derived from the Greek word for "glue," giving the impression that the main function of these cells is to keep the brain from running out of our ears! Although this simple view belies the importance of glial function, as we shall see later in this chapter, we are confident that neurons perform most information processing in the brain, so neurons receive most of our attention.

Neuroscience, like other fields, has a language all its own. To use this language, you must learn the vocabulary. After you have read this chapter, take a few minutes to review the key terms list and make sure you understand the meaning of each term. Your neuroscience vocabulary will grow as you work your way through the book.

THE NEURON DOCTRINE

To study the structure of brain cells, scientists have had to overcome several obstacles. The first was the small size. Most cells are in the range of 0.01–0.05 mm in diameter. The tip of an unsharpened pencil lead is about 2 mm across; neurons are 40–200 times smaller. (For a review of the metric system, see Table 2.1.) Because neurons cannot be seen by the naked eye, cellular neuroscience could not progress before the development of the compound microscope in the late seventeenth century. Even then, obstacles remained. To observe brain tissue using a microscope, it was necessary to make very thin slices, ideally not much thicker than the diameter of the cells. However, brain tissue has a consistency like a bowl of Jell-O: not firm enough to make thin slices. Thus,

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TABLE 2.1 **Units of Size in the Metric System**

Unit	Abbreviation	Meter Equivalent	Real-World Equivalent
Kilometer	km	10^3 m	About two-thirds of a mile
Meter	m	1 m	About 3 feet
Centimeter	cm	10^{-2} m	Thickness of your little finger
Millimeter	mm	10^{-3} m	Thickness of your toenail
Micrometer	μm	10^{-6} m	Near the limit of resolution for the light microscope
Nanometer	nm	10^{-9} m	Near the limit of resolution for the electron microscope

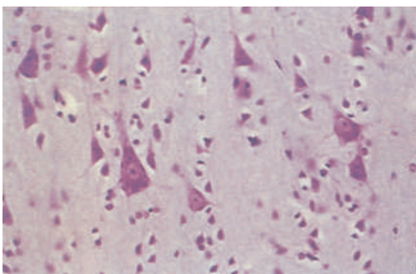
the anatomical study of brain cells had to await a method to harden the tissue without disturbing its structure and an instrument that could produce very thin slices. Early in the nineteenth century, scientists discovered how to harden, or "fix," tissues by immersing them in formaldehyde, and they developed a special device called a *microtome* to make very thin slices.

These technical advances spawned the field of **histology**, the microscopic study of the structure of tissues. But scientists studying brain structure faced yet another obstacle. Freshly prepared brain tissue has a uniform, cream-colored appearance under the microscope, with no differences in pigmentation to enable histologists to resolve individual cells. The final breakthrough in neurohistology was the introduction of stains that selectively color some, but not all, parts of the cells in brain tissue.

One stain still used today was introduced by the German neurologist Franz Nissl in the late nineteenth century. Nissl showed that a class of basic dyes would stain the nuclei of all cells as well as clumps of material surrounding the nuclei of neurons (Figure 2.1). These clumps are called *Nissl bodies*, and the stain is known as the **Nissl stain**. The Nissl stain is extremely useful for two reasons: It distinguishes between neurons and glia, and it enables histologists to study the arrangement, or **cytoarchitecture**, of neurons in different parts of the brain. (The prefix *cyto-* is from the Greek word for "cell.") The study of cytoarchitecture led to the realization that the brain consists of many specialized regions. We now know that each region performs a different function.

The Golgi Stain

The Nissl stain, however, could not tell the whole story. A Nissl-stained neuron looks like little more than a lump of protoplasm containing a



◀ FIGURE 2.1
Nissl-stained neurons. A thin slice of brain tissue has been stained with cresyl violet, a Nissl stain. The clumps of deeply stained material around the cell nuclei are Nissl bodies. (Source: Hammersen, 1980, Fig. 493.)

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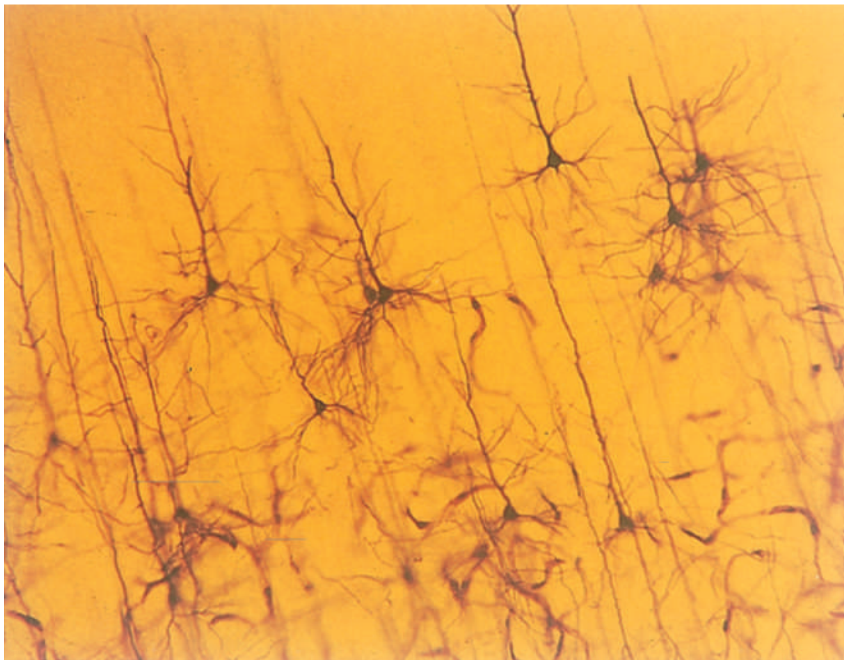
▲ FIGURE 2.2
Camillo Golgi (1843–1926).
(Source: Finger, 1994, Fig. 3.22.)

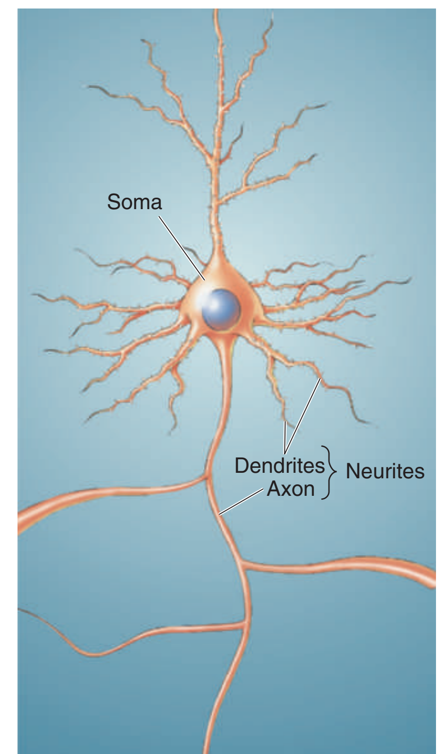
nucleus. Neurons are much more than that, but how much more was not recognized before Italian histologist Camillo Golgi devised a new method (Figure 2.2). In 1873, Golgi discovered that soaking brain tissue in a silver chromate solution, now called the **Golgi stain**, makes a small percentage of neurons become darkly colored in their entirety (Figure 2.3). This revealed that the neuronal cell body, the region of the neuron around the nucleus that is shown with the Nissl stain, is actually only a small fraction of the total structure of the neuron. Notice in Figures 2.1 and 2.3 how different histological stains can provide strikingly different views of the same tissue. Today, neurohistology remains an active field in neuroscience, along with its credo: "The gain in brain is mainly in the stain."

The Golgi stain shows that neurons have at least two distinguishable parts: a central region that contains the cell nucleus and numerous thin tubes that radiate away from the central region. The swollen region containing the cell nucleus has several names that are used interchangeably: **cell body**, **soma** (plural: somata), and **perikaryon** (plural: perikarya). The thin tubes that radiate from the soma are called **neurites** and are of two types: **axons** and **dendrites** (Figure 2.4).

The cell body usually gives rise to a single axon. The axon is of uniform diameter throughout its length, and any branches from it generally extend at right angles. Because axons can extend over great distances in the body (a meter or more), histologists of the day immediately recognized that axons must act like "wires" that carry the output of the neurons. Dendrites, on the other hand, are rarely longer than 2 mm. Many dendrites extend from the cell body and generally taper to a fine point.



▲ FIGURE 2.3
Golgi-stained neurons. (Source: Hubel, 1988, p. 126.)



▲ FIGURE 2.4
The basic parts of a neuron.

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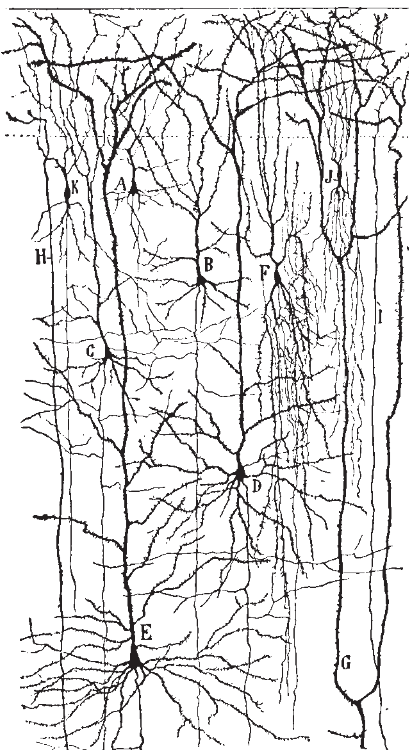
Early histologists recognized that because dendrites come in contact with many axons, they must act as the antennae of the neuron to receive incoming signals, or input.

Cajal's Contribution

Golgi invented the stain, but a Spanish contemporary used it to greatest effect. Santiago Ramón y Cajal was a skilled histologist and artist who learned about Golgi's method in 1888 (Figure 2.5). In a remarkable series of publications over the next 25 years, Cajal used the Golgi stain to work out the circuitry of many regions of the brain (Figure 2.6). Curiously, Golgi and Cajal drew completely opposite conclusions about neurons. Golgi championed the view that the neurites of different cells are fused together to form a continuous reticulum, or network, similar to the arteries and veins of the circulatory system. According to this reticular theory, the brain is an exception to the cell theory, which states that the individual cell is the elementary functional unit of all animal tissues. Cajal, on the other hand, argued forcefully that the neurites of different neurons are not continuous with each other and *communicate by contact, not continuity*. This idea that cell theory also applies to neurons came to be known as the **neuron doctrine**. Although Golgi and Cajal shared the Nobel Prize in 1906, they remained rivals to the end.

The scientific evidence over the next 50 years strongly supported the neuron doctrine, but final proof had to wait for the electron microscope in the 1950s (Box 2.1). With the increased resolving power of the electron microscope, it was finally possible to show that the neurites of different neurons are not continuous with one another (Figure 2.7). Thus, our starting point in the exploration of the brain must be the individual neuron.

▲ FIGURE 2.5
Santiago Ramón y Cajal (1852–1934). (Source: Finger, 1994, Fig. 3.26.)



◀ FIGURE 2.6
One of Cajal's many drawings of brain circuitry. The letters label the different elements Cajal identified in an area of the human cerebral cortex that controls voluntary movement. We will learn more about this part of the brain in Chapter 14. (Source: DeFelipe and Jones, 1998, Fig. 90.)

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BOX 2.1 OF SPECIAL INTEREST

Advances in Microscopy

The human eye can distinguish two points only if they are separated by more than about one-tenth of a millimeter (100 μm). Thus, we can say that 100 μm is near the limit of resolution for the unaided eye. Neurons have a diameter of about 20 μm, and neurites can be as small as a fraction of a micrometer. The light microscope, therefore, was a necessary development before neuronal structure could be studied. But this type of microscopy has a theoretical limit imposed by the properties of microscope lenses and visible light. With the standard light microscope, the limit of resolution is about 0.1 μm. Because the space between neurons is only 0.02 μm (20 nm), it's no wonder that two esteemed scientists, Golgi and Cajal, disagreed about whether neurites were continuous from one cell to the next. This question could not be answered until about 70 years ago when the electron microscope was developed and applied to biological specimens.

The electron microscope uses an electron beam instead of light to form images, dramatically increasing the resolving power. The limit of resolution for an electron microscope is about 0.1 nm—a million times better than the unaided eye and a thousand times better than a light microscope. Our insights into the fine structure of the inside of neurons—the ultrastructure—have all come from electron microscopic examination of the brain.

Today, microscopes on the leading edge of technology use laser beams to illuminate tissue and computers to create digital images (Figure A). Neuroscientists now routinely introduce into neurons molecules that fluoresce when illuminated by laser light. The fluorescence is recorded by sensitive detectors, and the computer takes these data and reconstructs the image of the neuron. Unlike the traditional methods of light and electron microscopy, which require tissue fixation, these new techniques give neuroscientists the ability to peer into brain tissue that is still alive. Furthermore, they have allowed "super-resolution" imaging, breaking the limits imposed by traditional light microscopy to reveal structures as small as 20 nm across.

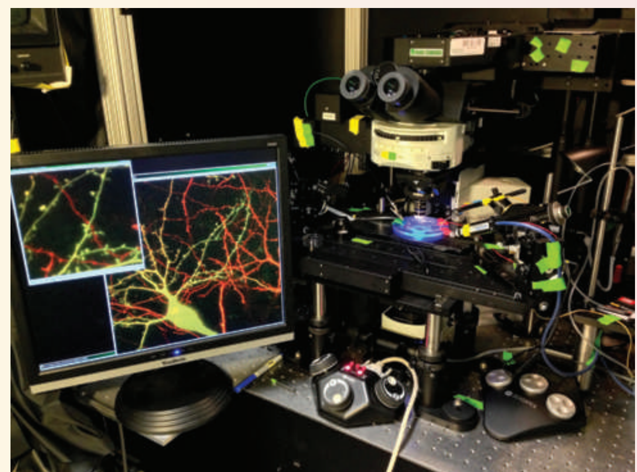
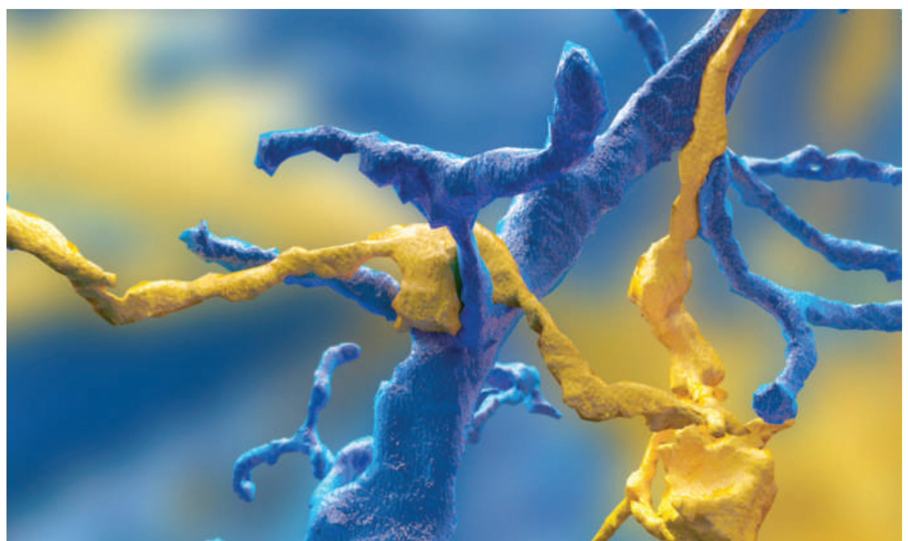


Figure A
A laser microscope and computer display of a fluorescent neuron and dendrites. (Source: Dr. Miquel Bosch, Massachusetts Institute of Technology.)



▶ FIGURE 2.7
Neurites in contact, not continuity.
These neurites were reconstructed from a series of images made using an electron microscope. The axon (colored yellow) is in contact with a dendrite (colored blue). (Source: Courtesy of Dr. Sebastian Seung, Princeton University, and Kris Krug, Pop Tech.)

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THE PROTOTYPICAL NEURON

As we have seen, the neuron (also called a *nerve cell*) consists of several parts: the soma, the dendrites, and the axon. The inside of the neuron is separated from the outside by the *neuronal membrane*, which lies like a circus tent on an intricate internal scaffolding, giving each part of the cell its special three-dimensional appearance. Let's explore the inside of the neuron and learn about the functions of the different parts (Figure 2.8).

The Soma

We begin our tour at the soma, the roughly spherical central part of the neuron. The cell body of the typical neuron is about 20 μm in diameter. The watery fluid inside the cell, called the **cytosol**, is a salty, potassium-rich solution that is separated from the outside by the neuronal membrane. Within the soma are a number of membrane-enclosed structures called **organelles**.

The cell body of the neuron contains the same organelles found in all animal cells. The most important ones are the nucleus, the rough endoplasmic reticulum, the smooth endoplasmic reticulum, the Golgi apparatus, and the mitochondria. Everything contained within the confines of the cell membrane, including the organelles but excluding the nucleus, is referred to collectively as the **cytoplasm**.

The Nucleus. Its name derived from the Latin word for "nut," the **nucleus** of the cell is spherical, centrally located, and about 5–10 μm across. It is contained within a double membrane called the *nuclear envelope*. The nuclear envelope is perforated by pores about 0.1 μm across.

Within the nucleus are **chromosomes** which contain the genetic material **DNA** (**deoxyribonucleic acid**). Your DNA was passed on to you from your parents and it contains the blueprint for your entire body. The DNA in each of your neurons is the same, and it is the same as the DNA in the cells of your liver and kidney and other organs. What distinguishes a neuron from a liver cell are the specific parts of the DNA that are used to assemble the cell. These segments of DNA are called **genes**.

Each chromosome contains an uninterrupted double-strand braid of DNA, 2 nm wide. If the DNA from the 46 human chromosomes were laid out straight, end to end, it would measure more than 2 m in length. If we were to compare this total length of DNA to the total string of letters that make up this book, the genes would be analogous to the individual words. Genes are from 0.1 to several micrometers in length.

The "reading" of the DNA is known as **gene expression**. The final product of gene expression is the synthesis of molecules called **proteins**, which exist in a wide variety of shapes and sizes, perform many different functions, and bestow upon neurons virtually all of their unique characteristics. **Protein synthesis**, the assembly of protein molecules, occurs in the cytoplasm. Because the DNA never leaves the nucleus, an intermediary must carry the genetic message to the sites of protein synthesis in the cytoplasm. This function is performed by another long molecule called **messenger ribonucleic acid**, or **mRNA**. mRNA consists of four different nucleic acids strung together in various sequences to form a chain. The detailed sequence of the nucleic acids in the chain represents the information in the gene, just as the sequence of letters gives meaning to a written word.

The process of assembling a piece of mRNA that contains the information of a gene is called **transcription**, and the resulting mRNA is called

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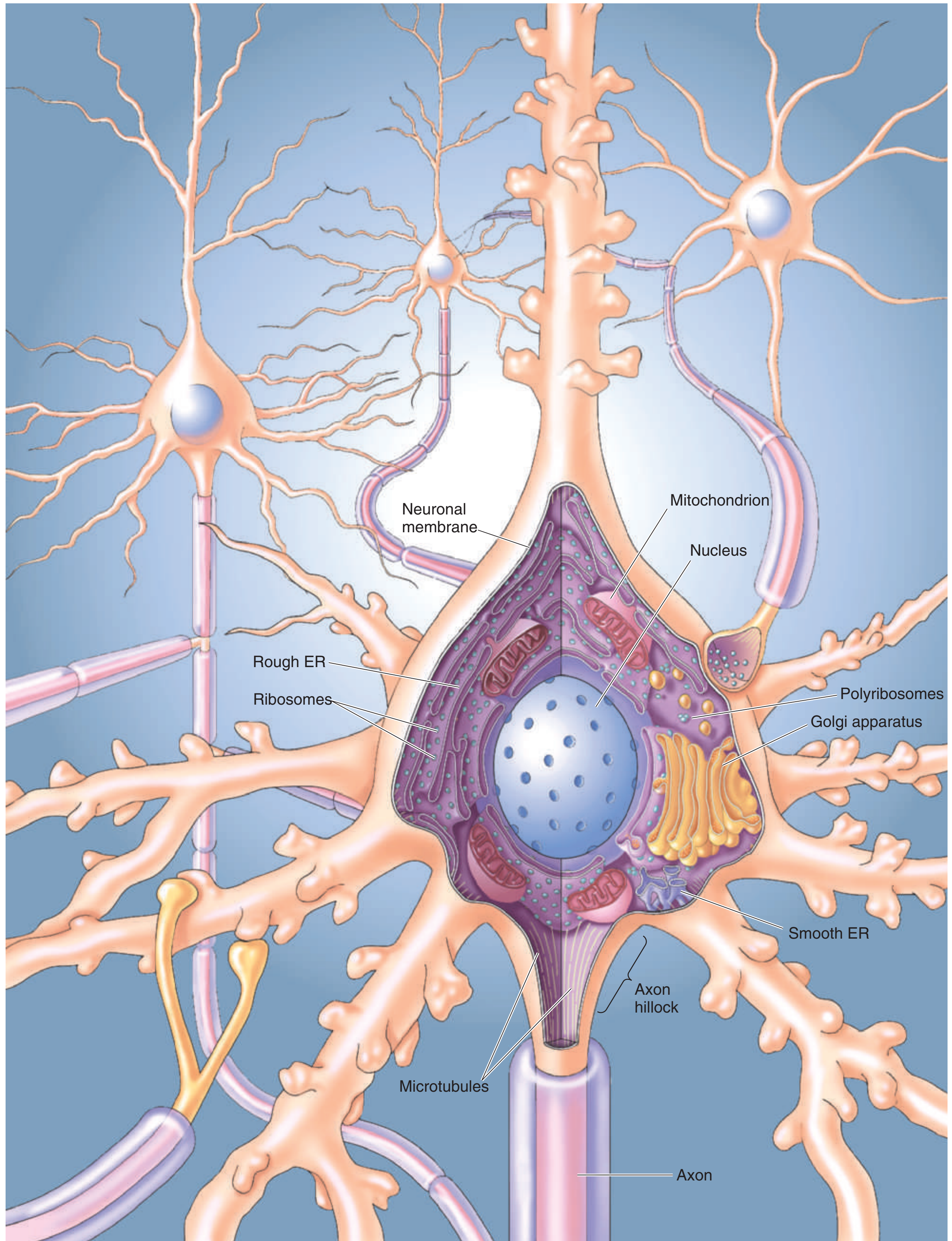

▲ FIGURE 2.8
The internal structure of a typical neuron.

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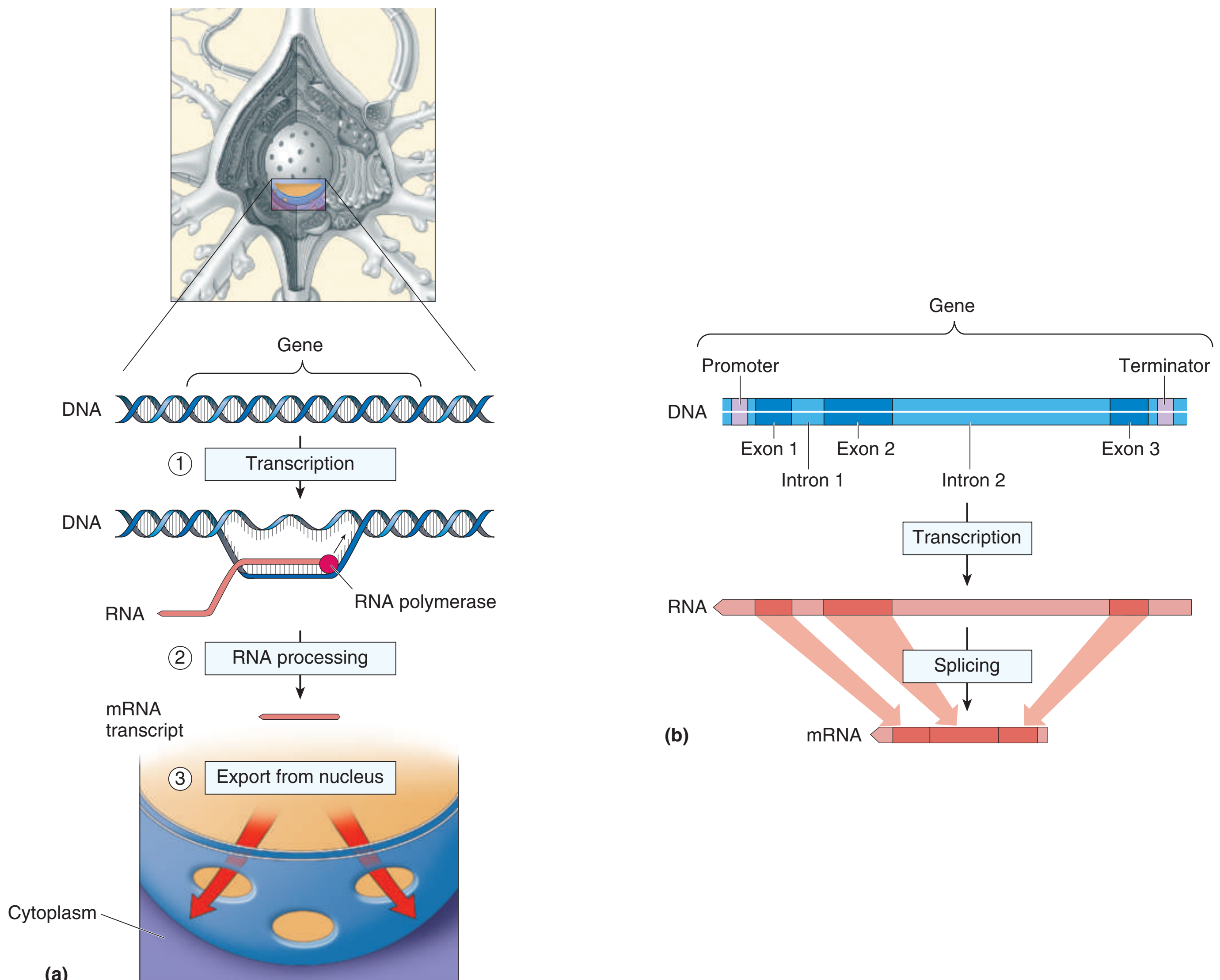

▲ FIGURE 2.9
Gene transcription. **(a)** RNA molecules are synthesized by RNA polymerase and then processed into mRNA to carry the genetic instructions for protein assembly from the nucleus to the cytoplasm. **(b)** Transcription is initiated at the promoter region of the gene and stopped at the terminator region. The initial RNA must be spliced to remove the introns that do not code for protein.

the *transcript* (Figure 2.9a). Interspersed between protein-coding genes are long stretches of DNA whose functions remain poorly understood. Some of these regions, however, are known to be important for regulating transcription. At one end of the gene is the **promoter**, the region where the RNA-synthesizing enzyme, *RNA polymerase*, binds to initiate transcription. The binding of the polymerase to the promoter is tightly regulated by other proteins called **transcription factors**. At the other end is a sequence of DNA called the *terminator*, or *stop sequence*, that the RNA polymerase recognizes as the end point for transcription.

In addition to the non-coding regions of DNA that flank the genes, there are often additional stretches of DNA within the gene itself that cannot be used to code for protein. These interspersed regions are called *introns*, and the coding sequences are called *exons*. Initial transcripts contain both introns and exons, but then, by a process called **RNA splicing**,

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the introns are removed and the remaining exons are fused together (Figure 2.9b). In some cases, specific exons are also removed with the introns, leaving an "alternatively spliced" mRNA that actually encodes a different protein. Thus, transcription of a single gene can ultimately give rise to several different mRNAs and protein products.

mRNA transcripts emerge from the nucleus via pores in the nuclear envelope and travel to the sites of protein synthesis elsewhere in the neuron. At these sites, a protein molecule is assembled much as the mRNA molecule was: by linking together many small molecules into a chain. In the case of protein, the building blocks are **amino acids**, of which there are 20 different kinds. This assembling of proteins from amino acids under the direction of the mRNA is called **translation**.

The scientific study of this process, which begins with the DNA of the nucleus and ends with the synthesis of protein molecules in the cell, is known as *molecular biology*. The "central dogma" of molecular biology is summarized as follows:

Neuronal Genes, Genetic Variation, and Genetic Engineering. Neurons differ from other cells in the body because of the specific genes they express as proteins. A new understanding of these genes is now possible because the human **genome**—the entire length of DNA that comprises the genetic information in our chromosomes—has been sequenced. We now know the 25,000 "words" that comprise our genome, and we know where these genes can be found on each chromosome. Furthermore, we are learning which genes are expressed uniquely in neurons (Box 2.2). This knowledge has paved the way to understanding the genetic basis of many diseases of the nervous system. In some diseases, long stretches of DNA that contain several genes are missing; in others, genes are duplicated, leading to overexpression of specific proteins. These sorts of mishaps, called *gene copy number variations*, often occur at the moment of conception when paternal and maternal DNA mix to create the genome of the offspring. Some instances of serious psychiatric disorders, including autism and schizophrenia, were recently shown to be caused by gene copy number variations in the affected children. (Psychiatric disorders are discussed in Chapter 22.)

Other nervous system disorders are caused by *mutations*—"typographical errors"—in a gene or in the flanking regions of DNA that regulate the gene's expression. In some cases, a single protein may be grossly abnormal or missing entirely, disrupting neuronal function. An example is fragile X syndrome, a disorder that manifests as intellectual disability and autism and is caused by disruption of a single gene (discussed further in Chapter 23). Many of our genes carry small mutations, called *single nucleotide polymorphisms*, which are analogous to a minor misspelling caused by a change in a single letter. These are usually benign, like the difference between "color" and "colour"—different spelling, same meaning. However, sometimes the mutations can affect protein function (consider the difference between "bear" and "bare"—same letters, different meaning). Such single nucleotide polymorphisms, alone or together with others, can affect neuronal function.

Genes make the brain, and understanding how they contribute to neuronal function in both healthy and diseased organisms is a major goal of neuroscience. An important breakthrough was the development of tools for **genetic engineering**—ways to change organisms by design with gene mutations or insertions. This technology has been used most in mice because they are rapidly reproducing mammals with a central nervous

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BOX 2.2 **BRAIN FOOD**

Expressing One's Mind in the Post-Genomic Era

Sequencing the human genome was a truly monumental achievement, completed in 2003. The Human Genome Project identified all of the approximately 25,000 genes in human DNA. We now live in what has been called the "post-genomic era," in which information about the genes expressed in our tissues can be used to diagnose and treat diseases. Neuroscientists are using this information to tackle long-standing questions about the biological basis of neurological and psychiatric disorders as well as to probe deeper into the origins of individuality. The logic goes as follows. The brain is a product of the genes expressed in it. Differences in gene expression between a normal brain and a diseased brain, or a brain of unusual ability, can be used to identify the molecular basis of the observed symptoms or traits.

The level of gene expression is usually defined as the number of mRNA transcripts synthesized by different cells and tissues to direct the synthesis of specific proteins. Thus, the analysis of gene expression requires comparing the relative abundance of various mRNAs in the brains of two groups of humans or animals. One way to perform such a comparison is to use DNA *microarrays*, which are created by robotic machines that arrange thousands of small spots of synthetic DNA on a microscope slide. Each spot contains a unique DNA sequence that will recognize and stick to a different specific mRNA sequence. To compare the gene expression in two brains, one begins by collecting a sample of mRNAs from each brain. The mRNA of one brain is labeled with a chemical tag that fluoresces green, and the mRNA of the other brain is labeled with a tag that fluoresces red. These samples are then applied to the microarray. Highly expressed genes will produce brightly fluorescent spots, and differences in the relative gene expression between the brains will be revealed by differences in the color of the fluorescence (Figure A).

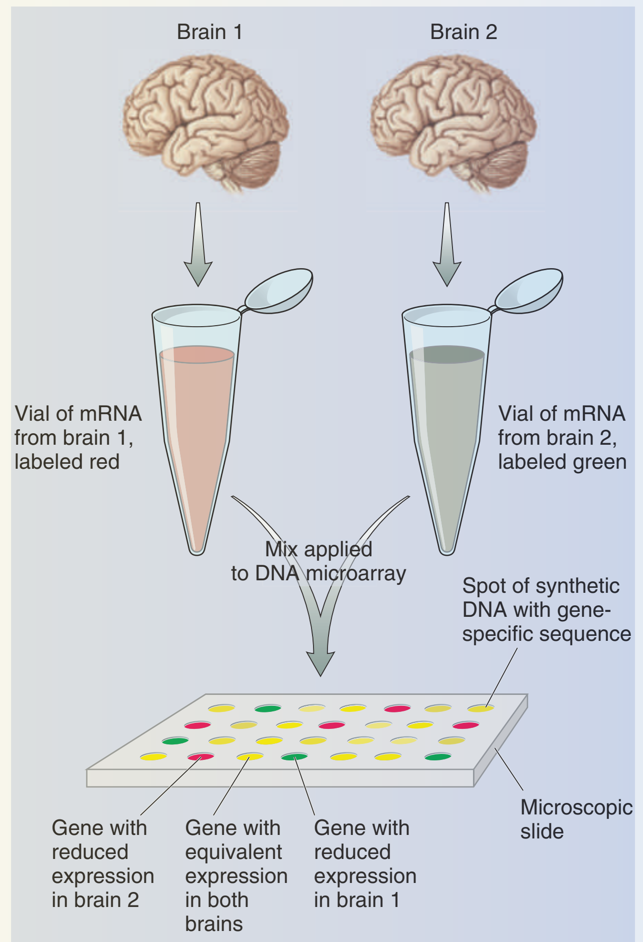

Figure A
Profiling differences in gene expression.

system similar to our own. Today, it is common in neuroscience to hear about **knockout mice**, in which one gene has been deleted (or "knocked out"). Such mice can be used to study the progression of a disease, like fragile X, with the goal of correcting it. Another approach has been to generate **transgenic mice**, in which genes have been introduced and overexpressed; these new genes are called *transgenes*. **Knock-in mice** have also been created in which the native gene is replaced with a modified transgene.

We will see many examples in this book of how genetically engineered animals have been used in neuroscience. The discoveries that allowed genetic modification of mice have revolutionized biology. The researchers who did this work were recognized with the 2007 Nobel Prize in Physiology or Medicine: Martin Evans of Cardiff University, Oliver Smithies of the University of North Carolina at Chapel Hill, and Mario Capecchi of the University of Utah (Box 2.3).

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BOX 2.3 PATH OF DISCOVERY

Gene Targeting in Mice

by Mario Capecchi

How did I first get the idea to pursue gene targeting in mice? From a simple observation. Mike Wigler, now at Cold Spring Harbor Laboratory, and Richard Axel, at Columbia University, had published a paper in 1979 showing that exposing mammalian cells to a mixture of DNA and calcium phosphate would cause some cells to take up the DNA in functional form and express the encoded genes. This was exciting because they had clearly demonstrated that exogenous, functional DNA could be introduced into mammalian cells. But I wondered why their efficiency was so low. Was it a problem of delivery, insertion of exogenous DNA into the chromosome, or expression of the genes once inserted into the host chromosome? What would happen if purified DNA was directly injected into the nucleus of mammalian cells in culture?

To find out, I converted a colleague's electrophysiology station into a miniature hypodermic needle to directly inject DNA into the nucleus of a living cell using mechanical micromanipulators and light microscopy (Figure A). The procedure worked with amazing efficiency (Capecchi, 1980). With this method, the frequency of successful integration was now one in three cells rather than one in a million cells as formerly. This high efficiency directly led to the development of transgenic mice through the injection and random integration of exogenous DNA into chromosomes of fertilized mouse eggs, or zygotes. To achieve the high efficiency of expression of the exogenous DNA in the recipient cell, I had to attach small fragments of viral DNA, which we now understand to contain enhancers that are critical in eukaryotic gene expression.

But what fascinated me most was our observation that when many copies of a gene were injected into a cell nucleus, all of these molecules ended up in an ordered head-to-tail arrangement, called a *concatemer* (Figure B). This was astonishing and could not have occurred as a random event. We went on to unequivocally prove that homologous recombination, the process by which chromosomes share genetic information during cell division, was responsible for the incorporation of the foreign DNA (Folger et al., 1982). These experiments demonstrated that all mammalian somatic cells contain a very efficient machinery for swapping segments of DNA that have similar sequences of nucleotides. Injection of a thousand copies of a gene sequence into the nucleus of a cell resulted in chromosomal insertion of a concatemer containing a thousand copies of that sequence, all oriented in the same direction. This simple observation directly led me to

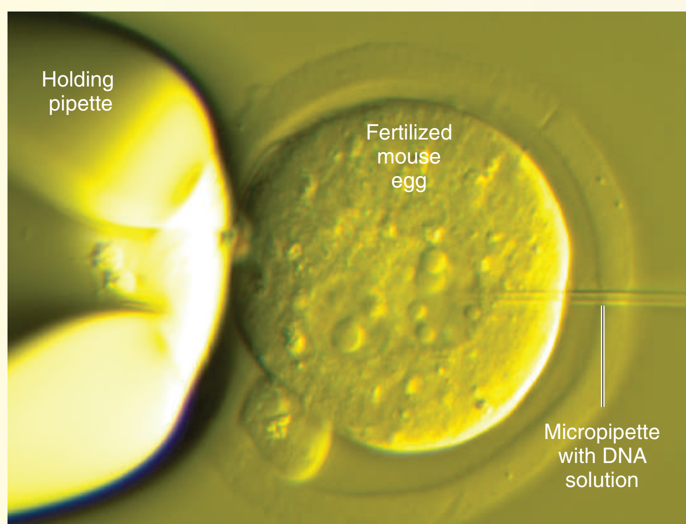

Figure A
Fertilized mouse egg receiving an injection of foreign DNA. (Image courtesy of Dr. Peimin Qi, Division of Comparative Medicine, Massachusetts Institute of Technology.)

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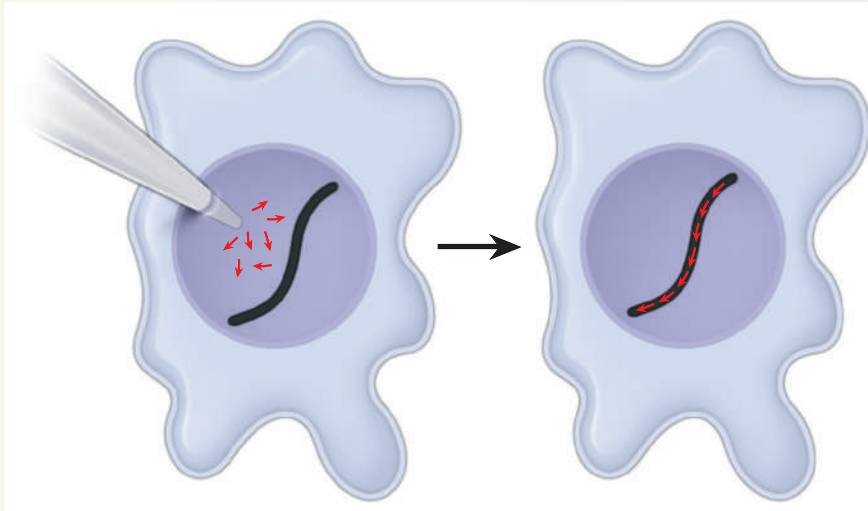

Figure B

(EC). Then I heard a rumor that Martin Evans in Cambridge, England was isolating more promising cells, which he called *EK cells*, that resembled EC cells but were derived from a normal mouse embryo rather than from tumors. I called him and asked if the rumor was correct, and he said it was. My next question was whether I could come to his laboratory to learn how to work with those cells, and his answer again was yes. Christmas time, 1985, was beautiful in Cambridge. My wife, who worked with me, and I had a wonderful couple of weeks learning how to maintain these marvelous cells and use them to generate mice capable of germ line transmission.

envision mutating any chosen gene, in any chosen manner, in living mice by gene targeting.

Excited by this possibility, in 1980, I submitted a grant to the U.S. National Institutes of Health (NIH) proposing to directly alter gene DNA sequences in mammalian cultured cells by homologous recombination. They rejected the proposal, and their arguments were not unreasonable. They argued that the probability of the exogenously added DNA sequence ever finding the DNA sequence similar enough to enable homologous recombination in living mammalian cells (containing 3×10^9 nucleotide base pairs) was vanishingly small. Fortunately, my grant application contained two other proposals that the NIH reviewers liked, and they funded those projects. I used those funds to support the gene targeting project. Four years later, we had results supporting our ability to do gene targeting in cultured mammalian cells. I then resubmitted a new NIH grant application to the same review panel, now proposing to extend gene targeting to generating mutant mice. Their evaluation sheet in response began this way: "We are glad you didn't follow our advice."

It took 10 years to develop gene targeting in mice (Thomas & Capecchi, 1987). Prior to this success, we had to understand the homologous recombination machinery in eukaryotic cells. Also, because the frequency of gene targeting was low, if we were to be successful in transferring our technology to mice, we needed mouse embryonic stem cells capable of contributing to the formation of the germ line—the sperm and eggs—in mature animals. I was getting depressed from our lack of success using cells derived from embryonal carcinoma

Investigators often have a preconceived idea about the particular role of their gene of interest in mouse biology, and they are usually very surprised by results when the gene is knocked out. Gene targeting has taken us in many new directions, including most recently pursuing the role of microglia, cells that migrate into the brain after being generated in the bone marrow along with immune and blood cells. Mutating these cells in mice results in a pathology remarkably similar to the human condition called trichotillomania, a type of obsessive-compulsive disorder characterized by strong urges to pull out one's hair. Amazingly, transplanting normal bone marrow into mutant mice permanently cures them of this pathological behavior (Chen et al., 2010). Now, we are deeply immersed in trying to understand the mechanism of how microglia control neural circuit output and, more importantly, exploring the intimate relationship between the immune system (in this case microglia) and neuropsychiatric disorders such as depression, autism, schizophrenia, and Alzheimer's disease.

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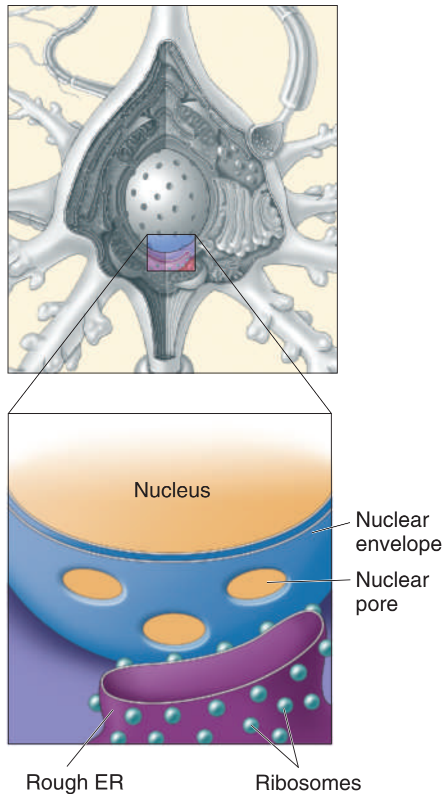

▲ FIGURE 2.10
Rough endoplasmic reticulum, or rough ER.

Rough Endoplasmic Reticulum. Neurons make use of the information in genes by synthesizing proteins. Protein synthesis occurs at dense globular structures in the cytoplasm called **ribosomes**. mRNA transcripts bind to the ribosomes, and the ribosomes translate the instructions contained in the mRNA to assemble a protein molecule. In other words, ribosomes use the blueprint provided by the mRNA to manufacture proteins from raw material in the form of amino acids.

In neurons, many ribosomes are attached to stacks of membrane called **rough endoplasmic reticulum**, or **rough ER** (Figure 2.10). Rough ER abounds in neurons, far more than in glia or most other non-neuronal cells. In fact, we have already been introduced to rough ER by another name: Nissl bodies. This is the organelle stained with the dyes that Nissl introduced over 100 years ago.

Rough ER is a major site of protein synthesis in neurons, but not all ribosomes are attached to rough ER. Many are freely floating and are called *free ribosomes*. Several free ribosomes may appear to be attached by a thread; these are called **polyribosomes**. The thread is a single strand of mRNA, and the associated ribosomes are working on it to make multiple copies of the same protein.

What is the difference between proteins synthesized on the rough ER and those synthesized on the free ribosomes? The answer appears to depend on the intended fate of the protein molecule. If it is destined to reside within the cytosol of the neuron, then the protein's mRNA transcript shuns the ribosomes of the rough ER and gravitates toward the free ribosomes (Figure 2.11a). However, if the protein is destined to be inserted into the membrane of the cell or an organelle, then it is synthesized on the rough ER. As the protein is being assembled, it is threaded back and forth through the membrane of the rough ER, where it is trapped (Figure 2.11b). It is not surprising that neurons have so much rough ER because, as we shall see in later chapters, special membrane proteins are what give these cells their remarkable information-processing abilities.

Smooth Endoplasmic Reticulum and the Golgi Apparatus. The remainder of the cytosol of the soma is crowded with stacks of membranous organelles that look a lot like rough ER without the ribosomes, so much so that one type is called **smooth endoplasmic reticulum**, or **smooth ER**. Smooth ER is heterogeneous and performs different functions in different locations. Some smooth ER is continuous with rough ER and is believed to be a site where the proteins that jut out from the membrane are carefully folded, giving them their three-dimensional structure. Other types of smooth ER play no direct role in the processing of protein molecules but instead regulate the internal concentrations of substances such as calcium. (This organelle is particularly prominent in muscle cells, where it is called *sarcoplasmic reticulum*, as we will see in Chapter 13.)

The stack of membrane-enclosed disks in the soma that lies farthest from the nucleus is the **Golgi apparatus**, first described in 1898 by Camillo Golgi (Figure 2.12). This is a site of extensive "post-translational" chemical processing of proteins. One important function of the Golgi apparatus is believed to be the sorting of certain proteins that are destined for delivery to different parts of the neuron, such as the axon and the dendrites.

The Mitochondrion. Another very abundant organelle in the soma is the **mitochondrion** (plural: mitochondria). In neurons, these sausage-shaped structures are about 1 μm long. Within the enclosure of their outer membrane are multiple folds of inner membrane called *cristae* (singular: crista). Between the cristae is an inner space called *matrix* (Figure 2.13a).

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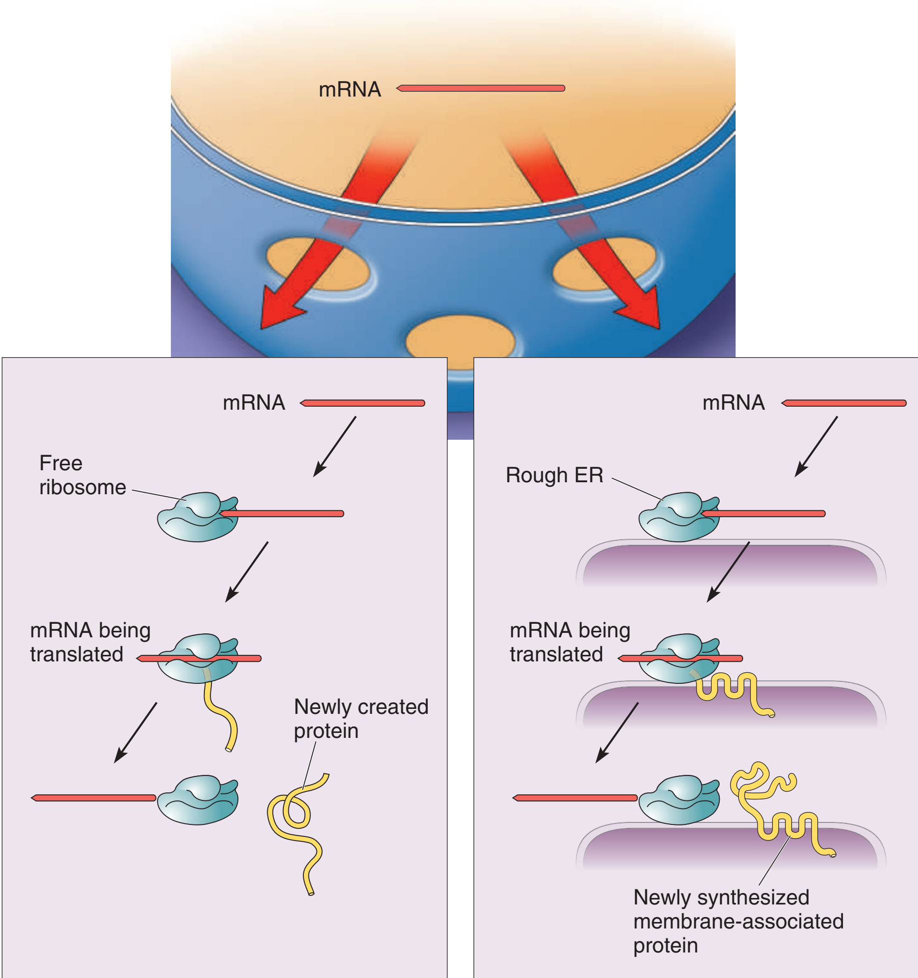
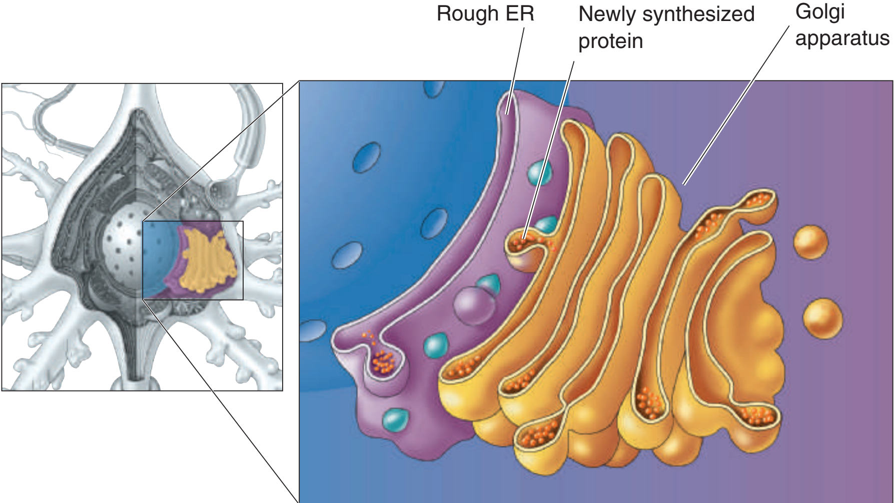


(a) Protein synthesis on a free ribosome

(b) Protein synthesis on rough ER

◀ FIGURE 2.11
Protein synthesis on a free ribosome and on rough ER. mRNA binds to a ribosome, initiating protein synthesis. **(a)** Proteins synthesized on free ribosomes are destined for the cytosol. **(b)** Proteins synthesized on the rough ER are destined to be enclosed by or inserted into the membrane. Membrane-associated proteins are inserted into the membrane as they are assembled.



◀ FIGURE 2.12
The Golgi apparatus. This complex organelle sorts newly synthesized proteins for delivery to appropriate locations in the neuron.

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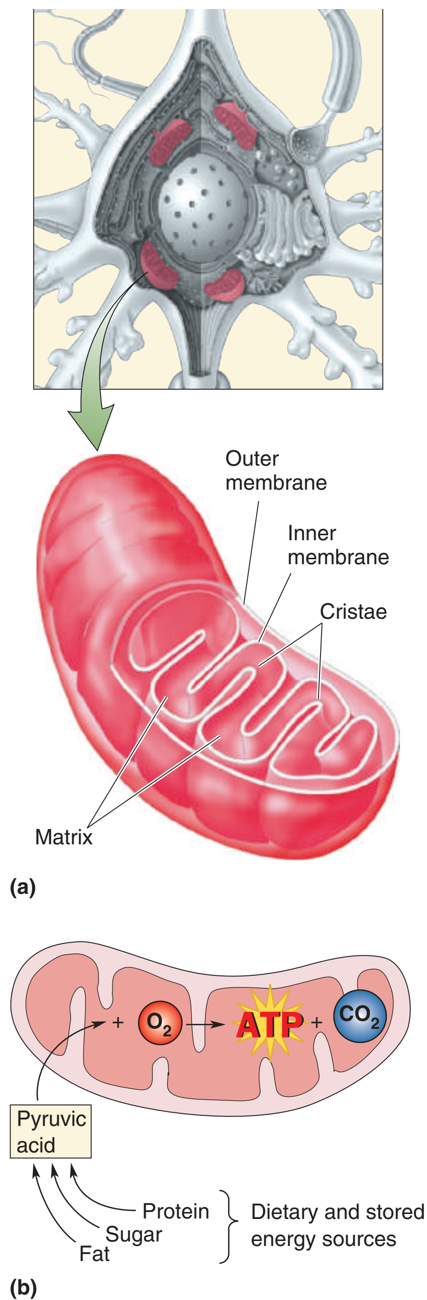

▲ FIGURE 2.13
The role of mitochondria. **(a)** Components of a mitochondrion. **(b)** Cellular respiration. ATP is the energy currency that fuels biochemical reactions in neurons.

Mitochondria are the site of *cellular respiration* (Figure 2.13b). When a mitochondrion "inhales," it pulls inside pyruvic acid (derived from sugars and digested proteins and fats) and oxygen, both of which are floating in the cytosol. Within the inner compartment of the mitochondrion, pyruvic acid enters into a complex series of biochemical reactions called the *Krebs cycle*, named after the German-British scientist Hans Krebs, who first proposed it in 1937. The biochemical products of the Krebs cycle provide energy that, in another series of reactions within the cristae (called the *electron-transport chain*), results in the addition of phosphate to adenosine diphosphate (ADP), yielding **adenosine triphosphate (ATP)**, the cell's energy source. When the mitochondrion "exhales," 17 ATP molecules are released for every molecule of pyruvic acid that had been taken in.

ATP is the energy currency of the cell. The chemical energy stored in ATP fuels most of the biochemical reactions of the neuron. For example, as we shall see in Chapter 3, special proteins in the neuronal membrane use the energy released by the breakdown of ATP into ADP to pump certain substances across the membrane to establish concentration differences between the inside and the outside of the neuron.

The Neuronal Membrane

The **neuronal membrane** serves as a barrier to enclose the cytoplasm inside the neuron and to exclude certain substances that float in the fluid that bathes the neuron. The membrane is about 5 nm thick and is studded with proteins. As mentioned earlier, some of the membrane-associated proteins pump substances from the inside to the outside. Others form pores that regulate which substances can gain access to the inside of the neuron. An important characteristic of neurons is that the protein composition of the membrane varies depending on whether it is in the soma, the dendrites, or the axon.

The function of neurons cannot be understood without understanding the structure and function of the membrane and its associated proteins. In fact, this topic is so important that we'll spend much of the next four chapters looking at how the membrane endows neurons with the remarkable ability to transfer electrical signals throughout the brain and body.

The Cytoskeleton

Earlier, we compared the neuronal membrane to a circus tent draped over an internal scaffolding. This scaffolding is called the **cytoskeleton**, and it gives the neuron its characteristic shape. The "bones" of the cytoskeleton are the microtubules, microfilaments, and neurofilaments (Figure 2.14). Unlike the tent scaffolding, however, the cytoskeleton is not static. Elements of the cytoskeleton are dynamically regulated and are in continual motion. Your neurons are probably squirming around in your head even as you read this sentence.

Microtubules. Measuring 20 nm in diameter, **microtubules** are relatively large and run longitudinally down neurites. A microtubule appears as a straight, thick-walled hollow pipe. The wall of the pipe is composed of smaller strands that are braided like rope around the hollow core. Each of the smaller strands consists of the protein *tubulin*. A single tubulin molecule is small and globular; the strand consists of tubulins stuck together like pearls on a string. The process of joining small proteins to form a long strand is called *polymerization*; the resulting strand is called a *polymer*. Polymerization and depolymerization of microtubules and, therefore, of neuronal shape can be regulated by various signals within the neuron.

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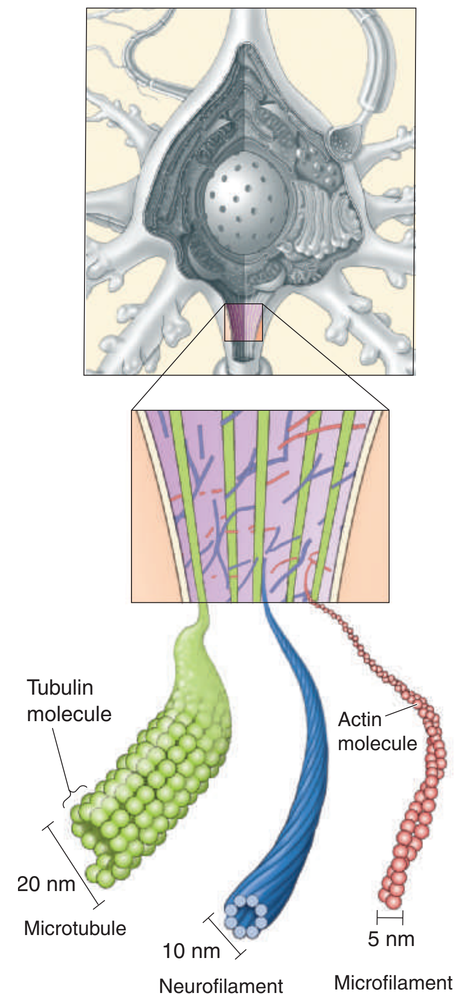
One class of proteins that participate in the regulation of microtubule assembly and function are *microtubule-associated proteins*, or *MAPs*. Among other functions (many of which are unknown), MAPs anchor the microtubules to one another and to other parts of the neuron. Pathological changes in an axonal MAP, called *tau*, have been implicated in the dementia that accompanies Alzheimer's disease (Box 2.4).

Microfilaments. Measuring only 5 nm in diameter, **microfilaments** are about the same thickness as the cell membrane. Found throughout the neuron, they are particularly numerous in the neurites. Microfilaments are braids of two thin strands that are polymers of the protein *actin*. Actin is one of the most abundant proteins in cells of all types, including neurons, and is believed to play a role in changing cell shape. Indeed, as we shall see in Chapter 13, actin filaments are critically involved in the mechanism of muscle contraction.

Like microtubules, actin microfilaments are constantly undergoing assembly and disassembly, and this process is regulated by signals in the neuron. In addition to running longitudinally down the core of the neurites like microtubules, microfilaments are also closely associated with the membrane. They are anchored to the membrane by attachments with a meshwork of fibrous proteins that line the inside of the membrane like a spider web.

Neurofilaments. With a diameter of 10 nm, **neurofilaments** are intermediate in size between microtubules and microfilaments. They exist in all cells of the body as *intermediate filaments*; only in neurons are they called *neurofilaments*. The difference in name reflects differences in structure among different tissues. For example, a different intermediate filament, keratin, composes hair when bundled together.

Of the types of fibrous structure we have discussed, neurofilaments most closely resemble the bones and ligaments of the skeleton. A neurofilament consists of multiple subunits (building blocks) that are wound together into a ropelike structure. Each strand of the rope consists of individual long protein molecules, making neurofilaments mechanically very strong.



▲ FIGURE 2.14
Components of the cytoskeleton. The arrangement of microtubules, neurofilaments, and microfilaments gives the neuron its characteristic shape.

The Axon

So far, we've explored the soma, organelles, membrane, and cytoskeleton. These structures are not unique to neurons but are found in all the cells in our body. Now we'll look at the axon, a structure found only in neurons and highly specialized for the transfer of information over distances in the nervous system.

The axon begins with a region called the **axon hillock**, which tapers away from the soma to form the initial segment of the axon proper (Figure 2.15). Two noteworthy features distinguish the axon from the soma:

1. No rough ER extends into the axon, and there are few, if any, free ribosomes in mature axons.
2. The protein composition of the axon membrane is fundamentally different from that of the soma membrane.

These structural differences translate into functional distinctions. Because there are no ribosomes, there is no protein synthesis in the axon. This means that all proteins in the axon must originate in the soma. And the different proteins in the axonal membrane enable it to serve as a wire that sends information over great distances.

Axons may extend from less than a millimeter to over a meter long. Axons often branch, and these branches, called **axon collaterals**, can

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BOX 2.4 OF SPECIAL INTEREST

Alzheimer's Disease and the Neuronal Cytoskeleton

Neurites are the most remarkable structural feature of a neuron. Their elaborate branching patterns, critical for information processing, reflect the organization of the underlying cytoskeleton. It is therefore no surprise that a devastating loss of brain function can result when the cytoskeleton of neurons is disrupted. An example is *Alzheimer's disease*, which is characterized by the disruption of the cytoskeleton of neurons in the cerebral cortex, a region of the brain crucial for cognitive function. This disorder and its underlying brain pathology were first described in 1907 by the German physician A. Alzheimer in a paper titled "A Characteristic Disease of the Cerebral Cortex." Below are excerpts from the English translation.

> One of the first disease symptoms of a 51-year-old woman was a strong feeling of jealousy toward her husband. Very soon she showed rapidly increasing memory impairments; she could not find her way about her home, she dragged objects to and fro, hid herself, or sometimes thought that people were out to kill her, then she would start to scream loudly.
>
> During institutionalization her gestures showed a complete helplessness. She was disoriented as to time and place. From time to time she would state that she did not understand anything, that she felt confused and totally lost. Sometimes she considered the coming of the doctor as an official visit and apologized for not having finished her work, but other times she would start to yell in the fear that the doctor wanted to operate on her; or there were times that she would send him away in complete indignation, uttering phrases that indicated her fear that the doctor wanted to damage her woman's honor. From time to time she was completely delirious, dragging her blankets and sheets to and fro, calling for her husband and daughter, and seeming to have auditory hallucinations. Often she would scream for hours and hours in a horrible voice.
>
> Mental regression advanced quite steadily. After four and a half years of illness the patient died. She was completely apathetic in the end, and was confined to bed in a fetal position. (Bick et al., 1987, pp. 1–2.)

Following her death, Alzheimer examined the woman's brain under the microscope. He made particular note of changes in the "neurofibrils," elements of the cytoskeleton that can be stained by a silver solution.

> The Bielschowsky silver preparation showed very characteristic changes in the neurofibrils. However, inside an apparently normal-looking cell, one or more single fibers could be observed that became prominent through their striking thickness and specific impregnability. At a more advanced stage, many fibrils arranged parallel showed the same changes. Then they accumulated forming dense bundles and gradually advanced to the surface of the cell. Eventually, the nucleus and cytoplasm disappeared, and only a tangled bundle of fibrils indicated the site where once the neuron had been located.
>
> As these fibrils can be stained with dyes different from the normal neurofibrils, a chemical transformation of the fibril substance must have taken place. This might be the reason why the fibrils survived the destruction of the cell. It seems that the transformation of the fibrils goes hand in hand with the storage of an as yet not closely examined pathological product of the metabolism in the neuron. About one-quarter to one-third of all the neurons of the cerebral cortex showed such alterations. Numerous neurons, especially in the upper cell layers, had totally disappeared. (Bick et al., 1987, pp. 2–3.)

The severity of the dementia in Alzheimer's disease is well correlated with the number and distribution of what are now commonly known as *neurofibrillary tangles*, the "tombstones" of dead and dying neurons (Figure A). Indeed, as Alzheimer speculated, tangle formation in the cerebral cortex very likely causes the symptoms of the disease. Electron microscopy reveals that the major components of the tangles are *paired helical filaments*, long fibrous proteins braided together like strands of a rope (Figure B). It is now understood that these filaments consist of the microtubule-associated protein *tau*.

Tau normally functions as a bridge between the microtubules in axons, ensuring that they run straight and parallel to one another. In Alzheimer's disease, the tau detaches from the microtubules and accumulates in the soma. This disruption of the cytoskeleton causes the axons to wither, thus impeding the normal flow of information in the affected neurons.

travel long distances to communicate with different parts of the nervous system. Occasionally, an axon collateral returns to communicate with the same cell that gave rise to the axon or with the dendrites of neighboring cells. These axon branches are called *recurrent collaterals*.

The diameter of an axon is variable, ranging from less than 1 μm to about 25 μm in humans and to as large as 1 mm in squid. This variation in axon size is important. As will be explained in Chapter 4, the speed

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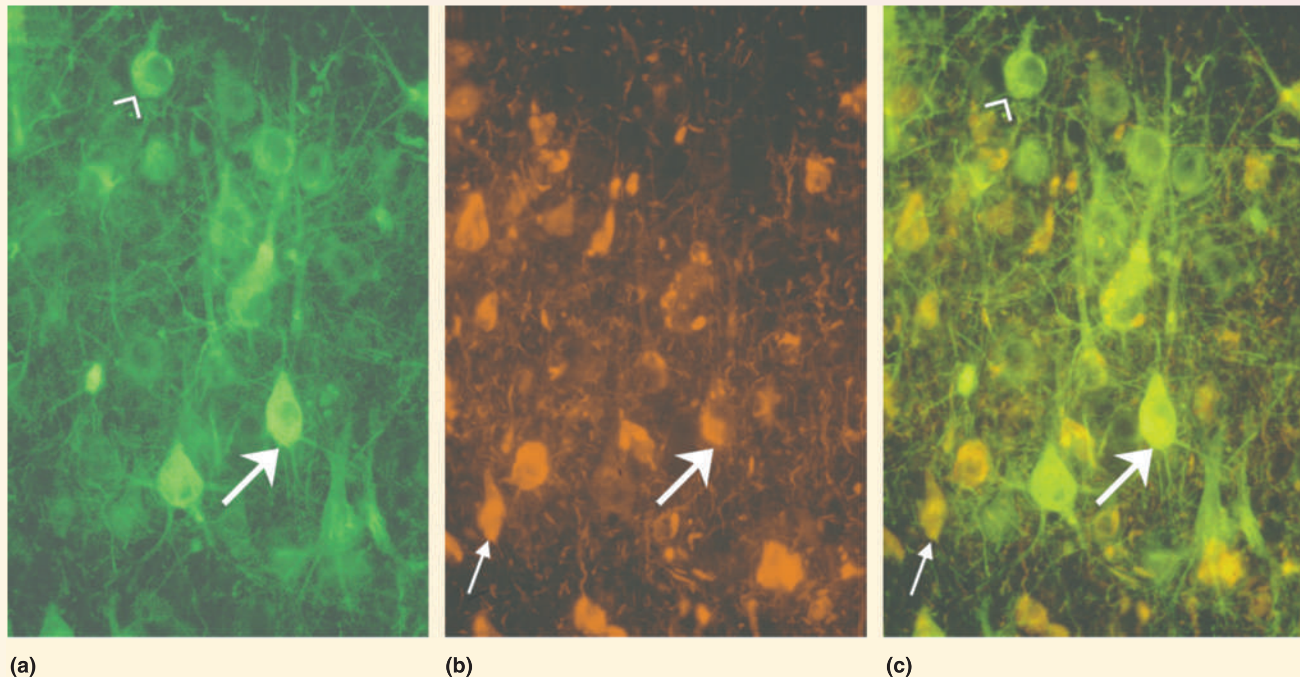

Figure A
Neurons in a human brain with Alzheimer's disease. Normal neurons contain neurofilaments but no neurofibrillary tangles. **(a)** Brain tissue stained by a method that makes neuronal neurofilaments fluoresce green, showing viable neurons. **(b)** The same region of the brain stained to show the presence of tau within neurofibrillary tangles, revealed by red fluorescence. **(c)** Superimposition of images in parts **a** and **b**. The neuron indicated by the arrowhead contains neurofilaments but no tangles and therefore is healthy. The neuron indicated by the large arrow has neurofilaments but also has started to show accumulation of tau and therefore is diseased. The neuron indicated by the small arrow in parts **b** and **c** is dead because it contains no neurofilaments. The remaining tangle is the tombstone of a neuron killed by Alzheimer's disease. (Source: Courtesy of Dr. John Morrison and modified from Vickers et al., 1994.)

What causes such changes in tau? Attention has focused on another protein that accumulates in the brain of Alzheimer's patients, called *amyloid*. Alzheimer's disease research is moving very fast, but the consensus today is that the abnormal secretion of amyloid by neurons is the first step in a process that leads to neurofibrillary tangle formation and dementia. Currently, hope for therapeutic intervention focuses on strategies to reduce the depositions of amyloid in the brain. The need for effective therapy is urgent: In the United States alone, more than 5 million people are afflicted with this tragic disease.

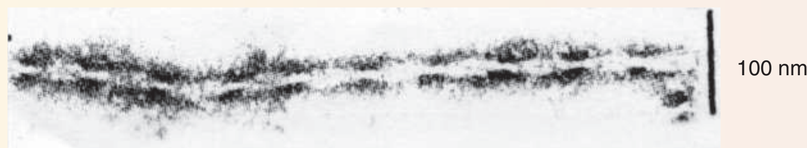

Figure B
Paired helical filaments of a tangle.
(Source: Goedert, 1996, Fig. 2b.)

of the electrical signal that sweeps down the axon—the *nerve impulse*—depends on the axonal diameter. The thicker the axon, the faster the impulse travels.

The Axon Terminal. All axons have a beginning (the axon hillock), a middle (the axon proper), and an end. The end is called the **axon terminal** or **terminal bouton** (French for "button"), reflecting the fact that it usually

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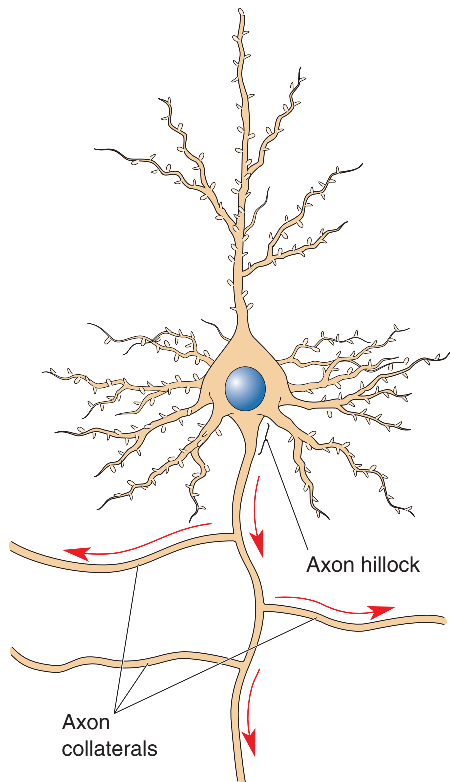
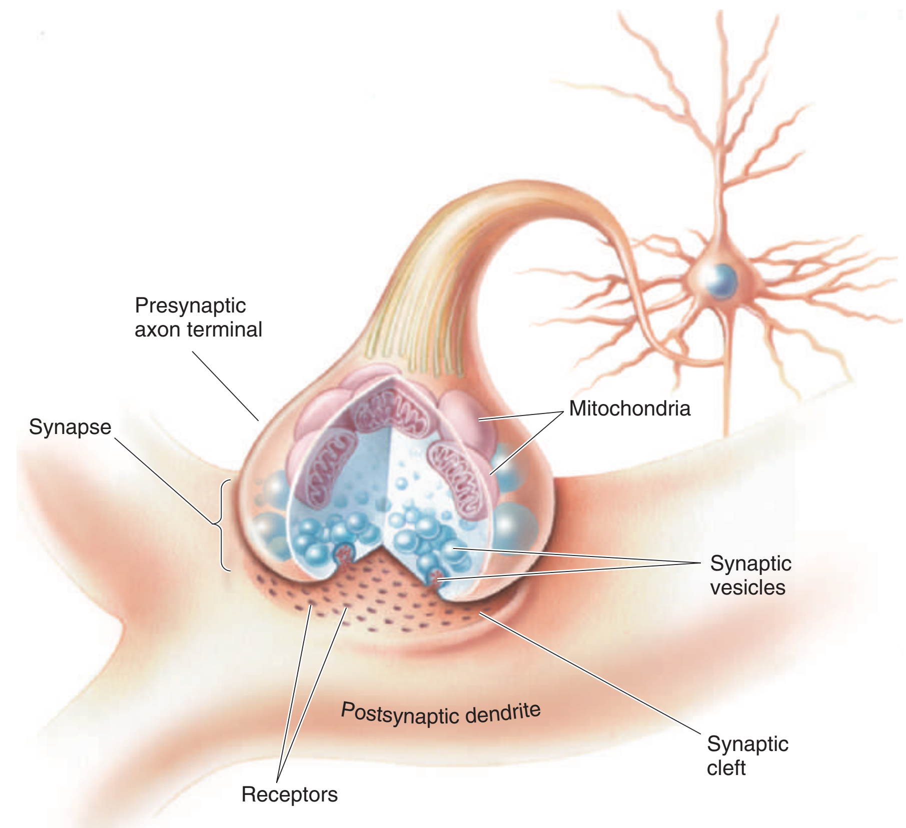


▲ FIGURE 2.15
The axon and axon collaterals. The axon functions like a telegraph wire to send electrical impulses to distant sites in the nervous system. The arrows indicate the direction of information flow.

appears as a swollen disk (Figure 2.16). The terminal is a site where the axon comes in contact with other neurons (or other cells) and passes information on to them. This point of contact is called the **synapse**, a word derived from the Greek, meaning "to fasten together." Sometimes axons have many short branches at their ends, and each branch forms a synapse on dendrites or cell bodies in the same region. These branches are collectively called the **terminal arbor**. Sometimes axons form synapses at swollen regions along their length and then continue on to terminate elsewhere (Figure 2.17). Such swellings are called *boutons en passant* ("buttons in passing"). In either case, when a neuron makes synaptic contact with another cell, it is said to innervate that cell, or to provide **innervation**.

The cytoplasm of the axon terminal differs from that of the axon in several ways:

1. Microtubules do not extend into the terminal.
2. The terminal contains numerous small bubbles of membrane, called **synaptic vesicles**, that measure about 50 nm in diameter.
3. The inside surface of the membrane that faces the synapse has a particularly dense covering of proteins.
4. The axon terminal cytoplasm has numerous mitochondria, indicating a high energy demand.



▲ FIGURE 2.16
The axon terminal and the synapse. Axon terminals form synapses with the dendrites or somata of other neurons. When a nerve impulse arrives in the presynaptic axon terminal, neurotransmitter molecules are released from synaptic vesicles into the synaptic cleft. Neurotransmitter then binds to specific receptor proteins, causing the generation of electrical or chemical signals in the postsynaptic cell.

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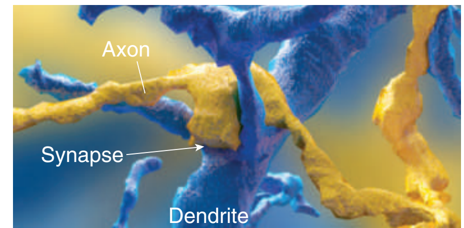
The Synapse. Although Chapters 5 and 6 are devoted entirely to how information is transferred from one neuron to another at the synapse, we'll preview the process here. The synapse has two sides: *presynaptic* and *postsynaptic* (see Figure 2.16). These names indicate the usual direction of information flow from "pre" to "post." The presynaptic side generally consists of an axon terminal, whereas the postsynaptic side may be a dendrite or the soma of another neuron. The space between the presynaptic and postsynaptic membranes is called the **synaptic cleft**. The transfer of information at the synapse from one neuron to another is called **synaptic transmission**.

At most synapses, information in the form of electrical impulses traveling down the axon is converted in the terminal into a chemical signal that crosses the synaptic cleft. On the postsynaptic membrane, this chemical signal is converted again into an electrical one. The chemical signal, called a **neurotransmitter**, is stored in and released from the synaptic vesicles within the terminal. As we will see, different neurotransmitters are used by different types of neurons.

This electrical-to-chemical-to-electrical transformation of information makes possible many of the brain's computational abilities. Modification of this process is involved in memory and learning, and synaptic transmission dysfunction accounts for certain mental disorders. The synapse is also the site of action for many toxins and for most psychoactive drugs.



▲ FIGURE 2.17
A bouton en passant. An axon (colored yellow) makes a synapse on a dendrite (colored blue) as they cross. This synapse was reconstructed from a series of images made using an electron microscope. (Source: Courtesy of Dr. Sebastian Seung, Princeton University, and Kris Krug, Pop Tech.)

Axoplasmic Transport. As mentioned, one feature of the cytoplasm of axons, including the terminal, is the absence of ribosomes. Because ribosomes are the protein factories of the cell, their absence means that the proteins of the axon must be synthesized in the soma and then shipped down the axon. Indeed, in the mid-nineteenth century, English physiologist Augustus Waller showed that axons cannot be sustained when separated from their parent cell body. The degeneration of axons that occurs when they are cut is now called *Wallerian degeneration.* Because it can be detected with certain staining methods, Wallerian degeneration is one way to trace axonal connections in the brain.

Wallerian degeneration occurs because the normal flow of materials from the soma to the axon terminal is interrupted. This movement of material down the axon is called **axoplasmic transport**. This was first demonstrated directly by the experiments of American neurobiologist Paul Weiss and his colleagues in the 1940s. They found that if they tied a thread around an axon, material accumulated on the side of the knot closest to the soma. When the knot was untied, the accumulated material continued down the axon at a rate of 1–10 mm per day.

This was a remarkable discovery, but it is not the whole story. If all material moved down the axon by this transport mechanism alone, it would not reach the ends of the longest axons for at least half a year—too long a wait to feed hungry synapses. In the late 1960s, methods were developed to track the movements of protein molecules down the axon into the terminal. These methods entailed injecting the somata of neurons with radioactive amino acids. Recall that amino acids are the building blocks of proteins. The "hot" amino acids were assembled into proteins, and the arrival of radioactive proteins in the axon terminal was timed to calculate the rate of transport. This *fast axoplasmic transport* (so named to distinguish it from *slow axoplasmic transport* described by Weiss) occurred at a rate as high as 1,000 mm per day.

Much is now known about how fast axoplasmic transport works. Material is enclosed within vesicles, which then "walk down" the microtubules of the axon. The "legs" are provided by a protein called *kinesin*, and

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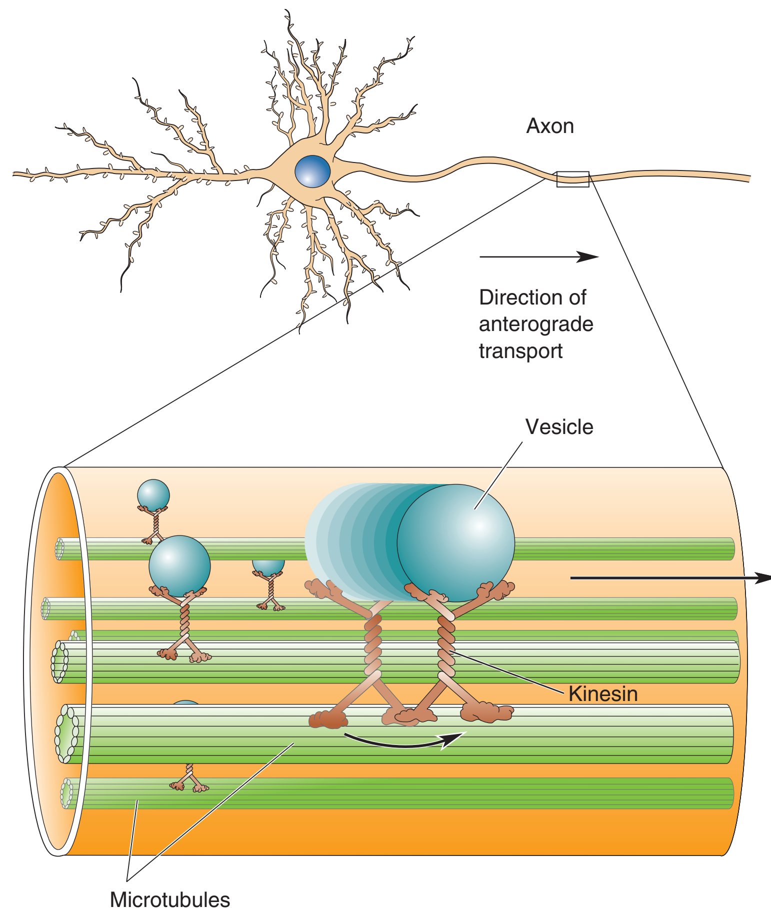

▲ FIGURE 2.18
A mechanism for the movement of material on the microtubules of the axon. Trapped in membrane-enclosed vesicles, material is transported from the soma to the axon terminal by the action of the protein kinesin, which "walks" along microtubules at the expense of ATP.

the process is fueled by ATP (Figure 2.18). Kinesin moves material only from the soma to the terminal. All movement of material in this direction is called **anterograde transport**.

In addition to anterograde transport, there is a mechanism for the movement of material up the axon from the terminal to the soma. This process is believed to provide signals to the soma about changes in the metabolic needs of the axon terminal. Movement in this direction, from terminal to soma, is called **retrograde transport**. The molecular mechanism is similar to anterograde transport, except the "legs" for retrograde transport are provided by a different protein, *dynein*. Both anterograde and retrograde transport mechanisms have been exploited by neuroscientists to trace connections in the brain (Box 2.5).

Dendrites

The term *dendrite* is derived from the Greek for "tree," reflecting the fact that these neurites resemble the branches of a tree as they extend from the soma. The dendrites of a single neuron are collectively called a **dendritic tree**; each branch of the tree is called a *dendritic branch*. The wide variety of shapes and sizes of dendritic trees are used to classify different groups of neurons.

Because dendrites function as the antennae of the neuron, they are covered with thousands of synapses (Figure 2.19). The dendritic membrane

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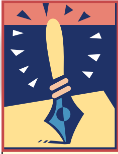

BOX 2.5 OF SPECIAL INTEREST

Hitching a Ride with Retrograde Transport

Fast anterograde transport of proteins in axons was shown by injecting the soma with radioactive amino acids. The success of this method immediately suggested a way to trace connections in the brain. For example, to determine where neurons in the eye send their axons elsewhere in the brain, the eye was injected with radioactive proline, an amino acid. The proline was incorporated into proteins in the somata that were then transported to the axon terminals. By use of a technique called *autoradiography*, the location of radioactive axon terminals could be detected, thereby revealing the extent of the connection between the eye and the brain.

Researchers subsequently discovered that retrograde transport could also be exploited to work out connections in the brain. Strangely enough, the enzyme horseradish peroxidase (HRP) is selectively taken up by axon terminals and then transported retrogradely to the soma. A chemical reaction can then be initiated to visualize the location of the HRP in slices of brain tissue after the experimental animal is euthanized. This method is commonly used to trace connections in the brain (Figure A).

Some viruses also exploit retrograde transport to infect neurons. For example, the oral type of herpesvirus enters axon terminals in the lips and mouth and is then transported back to the parent cell bodies. Here the virus usually remains dormant until physical or emotional stress occurs (as on a first date), at which time it replicates and returns to the nerve ending, causing a painful cold sore. Similarly, the rabies virus enters the nervous system by retrograde transport through axons in the skin. However, once inside the soma, the virus wastes no time in replicating madly, killing its neuronal host. The virus is then taken up by other neurons within the nervous system, and the process repeats itself again and again, usually until the victim dies.

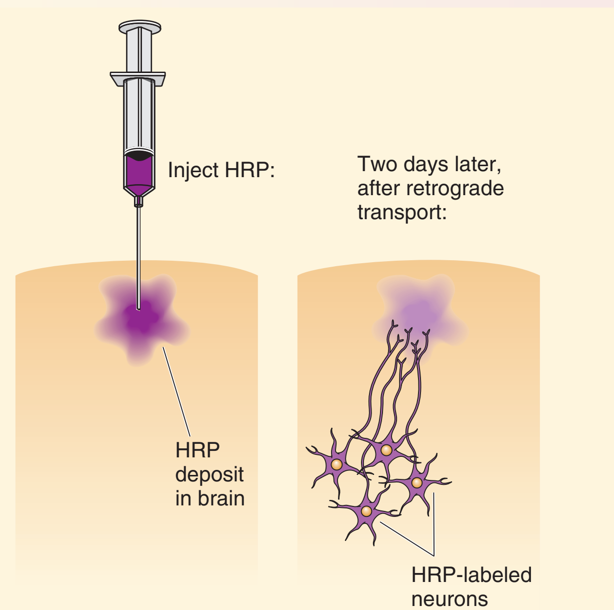

Figure A

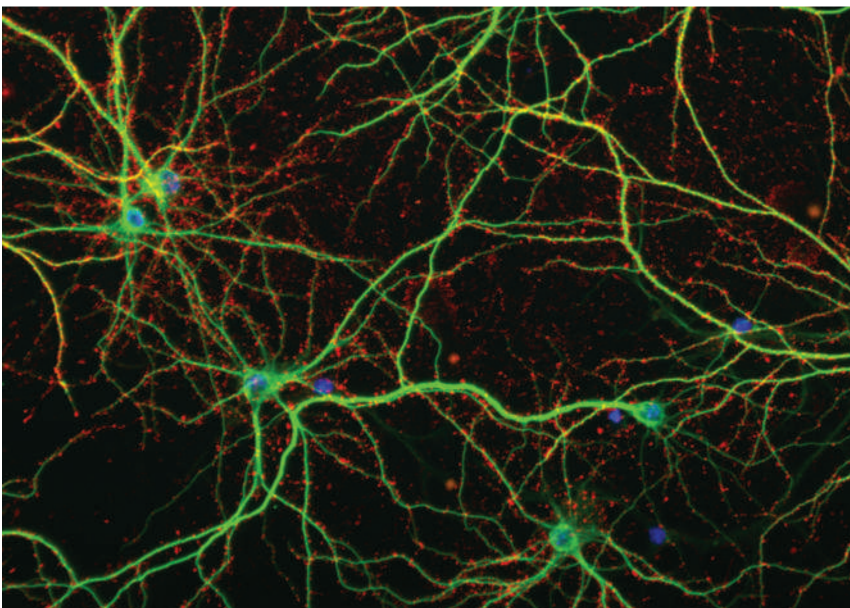

FIGURE 2.19
Dendrites receiving synaptic inputs from axon terminals. Neurons have been made to fluoresce green using a method that reveals the distribution of a microtubule-associated protein. Axon terminals have been made to fluoresce orange-red using a method to reveal the distribution of synaptic vesicles. The nuclei are stained to fluoresce blue. (Source: Dr. Asha Bhakar, Massachusetts Institute of Technology.)

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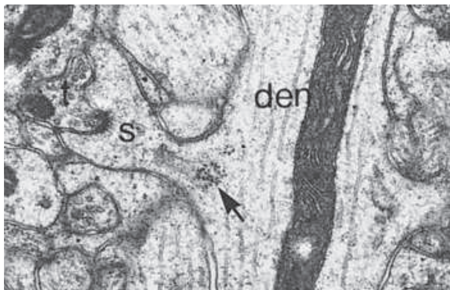
▲ FIGURE 2.20
Dendritic spines. This is a computer reconstruction of a segment of dendrite, showing the variable shapes and sizes of spines. Each spine is postsynaptic to one or two axon terminals. (Source: Harris & Stevens, 1989, cover image.)



▲ FIGURE 2.21
Postsynaptic polyribosomes. This electron micrograph shows a dendrite (den) with a cluster of polyribosomes (arrow) at the base of a dendritic spine (s) receiving a synapse from an axon terminal (t). (Source: Courtesy of Dr. Oswald Steward, University of California, Irvine.)

under the synapse (the *postsynaptic* membrane) has many specialized protein molecules called **receptors** that detect the neurotransmitters in the synaptic cleft.

The dendrites of some neurons are covered with specialized structures called **dendritic spines** that receive some types of synaptic input. Spines look like little punching bags that hang off the dendrite (Figure 2.20). The unusual morphology of spines has fascinated neuroscientists ever since their discovery by Cajal. They are believed to isolate various chemical reactions that are triggered by some types of synaptic activation. Spine structure is sensitive to the type and amount of synaptic activity. Unusual changes in spines have been shown to occur in the brains of individuals with cognitive impairments (Box 2.6).

For the most part, the cytoplasm of dendrites resembles that of axons. It is filled with cytoskeletal elements and mitochondria. One interesting difference is that polyribosomes can be observed in dendrites, often right under spines (Figure 2.21). Research has shown that synaptic transmission can actually direct local protein synthesis in some neurons. In Chapter 25, we will see that synaptic regulation of protein synthesis is crucial for information storage by the brain.

CLASSIFYING NEURONS

It is likely that we will never understand how each of the 85 billion neurons in the nervous system uniquely contributes to the function of the brain. But what if we could show that all the neurons in the brain can be categorized and that within each category all neurons function identically? The complexity of the problem might then be reduced to understanding the unique contribution of each category rather than each cell. With this hope, neuroscientists have devised schemes for classifying neurons.

Classification Based on Neuronal Structure

Efforts to classify neurons began in earnest with the development of the Golgi stain. These classification schemes, based on the morphology of dendrites, axons, and the structures they innervate, are still in wide use.

Number of Neurites. Neurons can be classified according to the total number of neurites (axons and dendrites) that extend from the soma (Figure 2.22). A neuron with a single neurite is said to be **unipolar**. If there are two neurites, the cell is **bipolar**, and if there are three or more, the cell is multipolar. Most neurons in the brain are **multipolar**.

Dendrites. Dendritic trees can vary widely from one type of neuron to another. Some have inspired names with flourish, like "double bouquet cells" or "chandelier cells." Others have more utilitarian names, such as "alpha cells." Classification is often unique to a particular part of the brain. For example, in the cerebral cortex (the structure that lies just under the surface of the cerebrum), there are two broad classes: **stellate cells** (star shaped) and **pyramidal cells** (pyramid shaped) (Figure 2.23).

Neurons can also be classified according to whether their dendrites have spines. Those that do are called **spiny**, and those that do not are called **aspinous**. These dendritic classification schemes can overlap. For example, in the cerebral cortex, all pyramidal cells are spiny. Stellate cells, on the other hand, can be either spiny or aspinous.

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BOX 2.6 OF SPECIAL INTEREST

Intellectual Disability and Dendritic Spines

The elaborate architecture of a neuron's dendritic tree reflects the complexity of its synaptic connections with other neurons. Brain function depends on these highly precise synaptic connections, which are formed during the fetal period and are refined during infancy and early childhood. Not surprisingly, this very complex developmental process is vulnerable to disruption. Intellectual disability is said to have occurred if a disruption of brain development results in subaverage cognitive functioning that impairs adaptive behavior.

According to standardized tests, intelligence in the general population is distributed along a bell-shaped (Gaussian) curve. By convention, the mean intelligence quotient (IQ) is set at 100. About two-thirds of the total population falls within 15 points (one standard deviation) of the mean, and 95% of the population falls within 30 points (two standard deviations). People with intelligence scores below 70 are considered to be intellectually disabled if their cognitive impairment affects their ability to adapt their behavior to the setting in which they live. Some 2–3% of humans fit this description.

Intellectual disability has many causes. The most severe forms are associated with genetic disorders, such as a condition called *phenylketonuria* (*PKU*). The basic abnormality is a deficit in the liver enzyme that metabolizes the dietary amino acid phenylalanine. Infants born with PKU have an abnormally high level of the amino acid in the blood and brain. If the condition goes untreated, brain growth is stunted and severe intellectual disability results. Another example is *Down syndrome*, which occurs when the fetus has an extra copy of chromosome 21, thus disrupting normal gene expression during brain development.

Another cause of intellectual disability is problems during pregnancy that can include a maternal infection, for example with German measles (rubella), and malnutrition. Children born to alcoholic mothers frequently have *fetal alcohol syndrome* comprising a constellation of developmental abnormalities that include intellectual disability. Other causes of intellectual disability are asphyxia of the infant during childbirth and environmental impoverishment—the lack of good nutrition, socialization, and sensory stimulation—during infancy.

Although some forms of intellectual disability have very clear physical correlates (e.g., stunted growth; abnormalities in the structure of the head, hands, and body), most cases have only behavioral manifestations. The brains of these individuals appear grossly normal. How, then, do we account for the profound cognitive impairment? An important clue came in the 1970s from the research of Miguel Marin-Padilla, working at Dartmouth College, and Dominick Purpura, working at the Albert Einstein College of Medicine in New York City. Using the Golgi stain, they studied the brains of intellectually disabled children and discovered remarkable changes in dendritic structure. The dendrites of low-functioning children had many fewer dendritic spines, and the spines that they did have were unusually long and thin (Figure A). The extent of the spine changes was well correlated with the degree of intellectual disability.

Dendritic spines are an important target of synaptic input. Purpura pointed out that the dendritic spines of intellectually disabled children resemble those of the normal human fetus. He suggested that intellectual disability reflects the failure of normal circuits to form in the brain. In the three decades since this seminal work was published, it was established that normal synaptic development, including maturation of the dendritic spines, depends critically on the environment during infancy and early childhood. An impoverished environment during an early critical period of development can lead to profound changes in the circuits of the brain. However, there is some good news. Many of the deprivation-induced changes in the brain can be reversed if intervention occurs early enough. In Chapter 23, we will take a closer look at the role of experience in brain development.

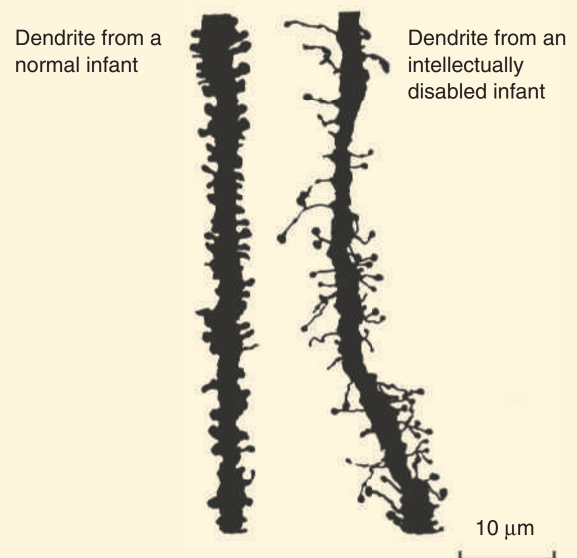

Figure A
Normal and abnormal dendrites. (Source: Purpura, 1974, Fig. 2A.)

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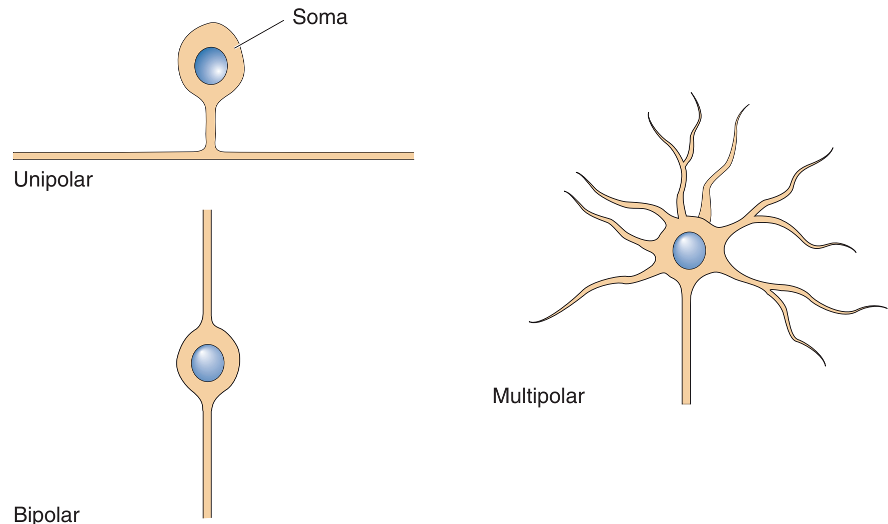

▲ FIGURE 2.22
Classification of neurons based on the number of neurites.

Connections. Information is delivered to the nervous system by neurons that have neurites in the sensory surfaces of the body, such as the skin and the retina of the eye. Cells with these connections are called **primary sensory neurons**. Other neurons have axons that form synapses with the muscles and command movements; these are called **motor neurons**. But most neurons in the nervous system form connections only with other neurons. In this classification scheme, these cells are called **interneurons**.

Axon Length. Some neurons have long axons that extend from one part of the brain to the other; these are called *Golgi type I neurons*, or *projection neurons*. Other neurons have short axons that do not extend beyond the vicinity of the cell body; these are called *Golgi type II neurons*, or *local circuit neurons*. In the cerebral cortex, for example, pyramidal cells usually have long axons that extend to other parts of the brain and are therefore Golgi type I neurons. In contrast, stellate cells have axons that never extend beyond the cerebral cortex and are therefore Golgi type II neurons.

Classification Based on Gene Expression

We now understand that most differences between neurons ultimately can be explained at the genetic level. For example, differences in gene expression cause pyramidal cells and stellate cells to develop different shapes. Once a genetic difference is known, that information can be used to create transgenic mice that allow detailed investigation of neurons in this class. For example, a foreign gene encoding a fluorescent protein can be introduced and placed under the control of a cell type–specific gene promoter. **Green fluorescent protein** (usually simply abbreviated as **GFP**), encoded by a gene discovered in jellyfish, is used commonly in neuroscience research. When illuminated with the appropriate wavelength of light, the GFP fluoresces bright green, allowing visualization of the neuron in which it is expressed. Genetic engineering methods are now commonly used for measuring and manipulating the functions of neurons in different categories (Box 2.7).

We have known for some time that one important way neurons differ is the neurotransmitter they use. Neurotransmitter differences arise because of differences in the expression of proteins involved in transmitter

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synthesis, storage, and use. Understanding these genetic differences enables a classification of neurons based on their neurotransmitters. For example, the motor neurons that command voluntary movements all release the neurotransmitter *acetylcholine* at their synapses; these motor cells are therefore also classified as *cholinergic*, meaning that they express the genes that enable use of this particular neurotransmitter. Collections of cells that use the same neurotransmitter make up the brain's neurotransmitter systems (see Chapters 6 and 15).

▲ FIGURE 2.23
Classification of neurons based on dendritic tree structure. Stellate cells and pyramidal cells, distinguished by the arrangement of their dendrites, are two types of neurons found in the cerebral cortex.

GLIA

Although most of this chapter is about neurons, as justified by the current state of knowledge, some neuroscientists consider glia the "sleeping giants" of neuroscience. Indeed, we continue to learn that glia contribute much more importantly to information processing in the brain than has been historically appreciated. Nevertheless, the data continue to indicate that glia contribute to brain function mainly by supporting neuronal functions. Although their role may be subordinate, without glia, the brain could not function properly.

Astrocytes

The most numerous glia in the brain are called **astrocytes** (Figure 2.24). These cells fill most of the spaces between neurons. The space that remains between neurons and astrocytes in the brain is only about 20 nm wide. Consequently, astrocytes probably influence whether a neurite can grow or retract.

An essential role of astrocytes is regulating the chemical content of this *extracellular space*. For example, astrocytes envelop synaptic junctions in the brain (Figure 2.25), thereby restricting the spread of neurotransmitter molecules that have been released. Astrocytes also have special proteins in their membranes that actively remove many neurotransmitters from the synaptic cleft. A recent and unexpected discovery is that astrocytic membranes also possess neurotransmitter receptors that, like the receptors on neurons, can trigger electrical and biochemical events inside the glial cell. Besides regulating neurotransmitters, astrocytes also tightly control the extracellular concentration of several substances that could interfere with proper neuronal function. For example, astrocytes regulate the concentration of potassium ions in the extracellular fluid.

Myelinating Glia

Unlike astrocytes, the primary function of **oligodendroglial** and **Schwann cells** is clear. These glia provide layers of membrane that insulate axons. Boston University anatomist Alan Peters, a pioneer in the electron microscopic study of the nervous system, showed that this wrapping, called **myelin**, spirals around axons in the brain (Figure 2.26). Because the axon fits inside the spiral wrapping like a sword in its scabbard, the name *myelin sheath* describes the entire covering. The sheath is interrupted periodically, leaving a short length where the axonal membrane is exposed. This region is called a **node of Ranvier** (Figure 2.27).

We will see in Chapter 4 that myelin serves to speed the propagation of nerve impulses down the axon. Oligodendroglia and Schwann cells differ in their location and some other characteristics. For example, oligodendroglia are found only in the central nervous system (brain and spinal cord), whereas Schwann cells are found only in the peripheral nervous

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BOX 2.7 **BRAIN FOOD**

Understanding Neuronal Structure and Function with Incredible Cre

One type of cell in the body can be distinguished from another by the unique pattern of genes it expresses as proteins. Similarly, different classes of neurons in the brain can be identified based on which genes are expressed. With modern methods of genetic engineering, knowledge that a gene is uniquely expressed in one type of neuron can help determine the contributions of this cell type to brain function.

Let's consider as an example the neurons that uniquely express the gene encoding the protein choline acetyltransferase (ChAT). ChAT is an enzyme that synthesizes the neurotransmitter acetylcholine. It is only expressed in "cholinergic neurons" that use acetylcholine because only these neurons have the transcription factors that act on this gene's promoter. If we insert into a mouse's genome a transgene engineered to be under the control of the same promoter, this foreign transgene will also be expressed only in cholinergic neurons. If the transgene expresses the enzyme Cre recombinase, derived from a bacterial virus, we can compel these cholinergic neurons to give up their secrets in myriad ways. Let's examine how.

Cre recombinase recognizes short DNA sequences called *loxP sites* that can be inserted on either side of another gene. The DNA between the loxP sites is said to be *floxed*. The Cre recombinase functions to cut out, or excise, the gene between the loxP sites. By breeding the "Cre mouse" with the "floxed mouse," one can generate mice in which a gene is deleted only in one particular type of neuron.

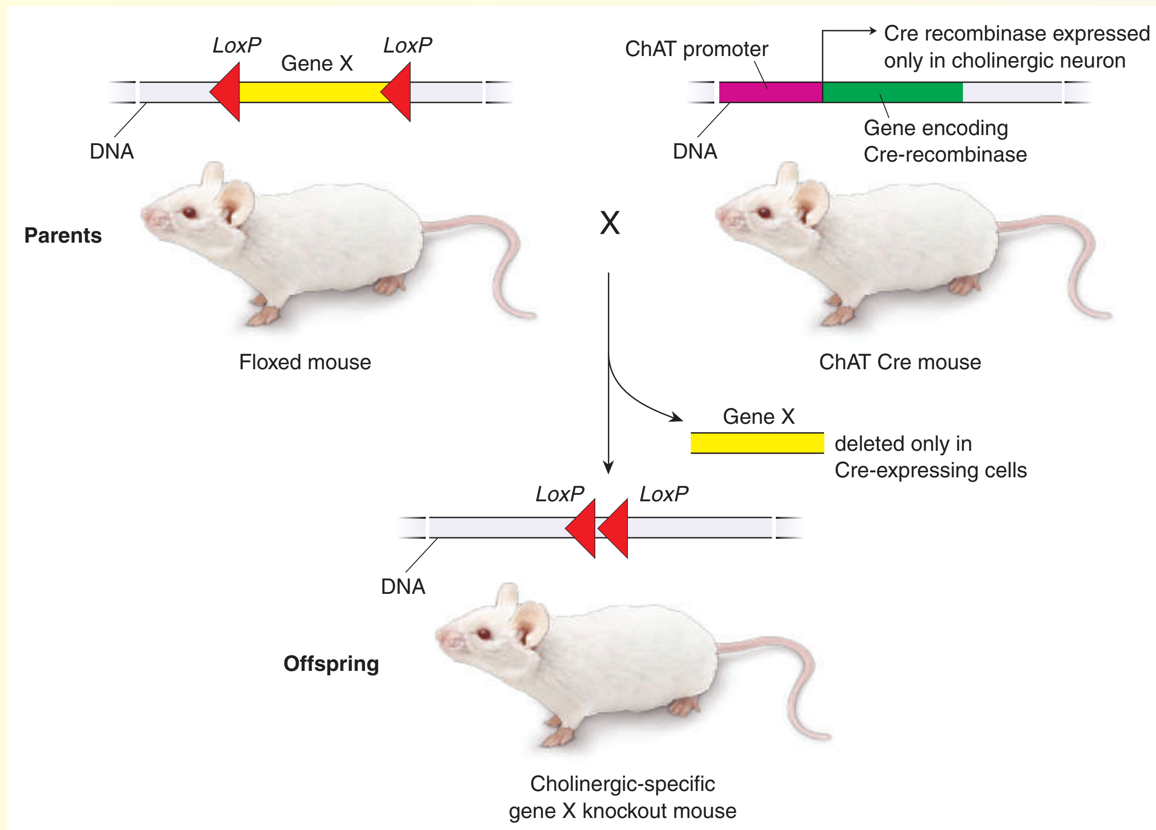

Figure A
Creating a mouse with a gene knockout only in cholinergic neurons is accomplished by crossing a floxed mouse with the gene of interest (gene X) flanked by loxP sites with another mouse in which Cre recombinase is under the control of the ChAT promoter. In the offspring, gene X is cut out only in the cells expressing Cre, namely, the cholinergic neurons.

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In a simple example, we can ask how cholinergic neurons react to the deletion of another gene they normally express; let's call this gene X. To answer this question, we cross mice that have had gene X floxed (the "floxed mice") with our mice that express Cre under the control of the ChAT promoter (the "ChAT-Cre mice"). In the offspring, the floxed gene is removed only in those neurons that express Cre; that is, only in the cholinergic neurons (Figure A).

We can also use Cre to cause expression of novel transgenes in cholinergic neurons. Normally, expression of a transgene requires that we include a promoter sequence upstream of the protein-coding region. Transcription of the transgene fails to occur if a stop sequence is inserted between this promoter and the protein-coding sequence. Now consider what happens if we generate a transgenic mouse with this stop sequence flanked by loxP sites. Crossing this mouse with our ChAT-Cre mouse generates offspring in which the transgene is expressed *only* in cholinergic neurons because the stop sequence has been removed only in these neurons (Figure B).

If we design this transgene to encode a fluorescent protein, we can use fluorescence to examine the structure and connections of these cholinergic neurons. If we design this transgene to express a protein that fluoresces only when impulses are generated by the neurons, we can monitor the activity of the cholinergic neurons by measuring light flashes. If we design this transgene to express a protein that kills or silences the neuron, we can see how brain function is altered in the absence of cholinergic neurons. The possible manipulations of cholinergic neurons through this feat of genetic engineering are limited only by the imagination of the scientist.

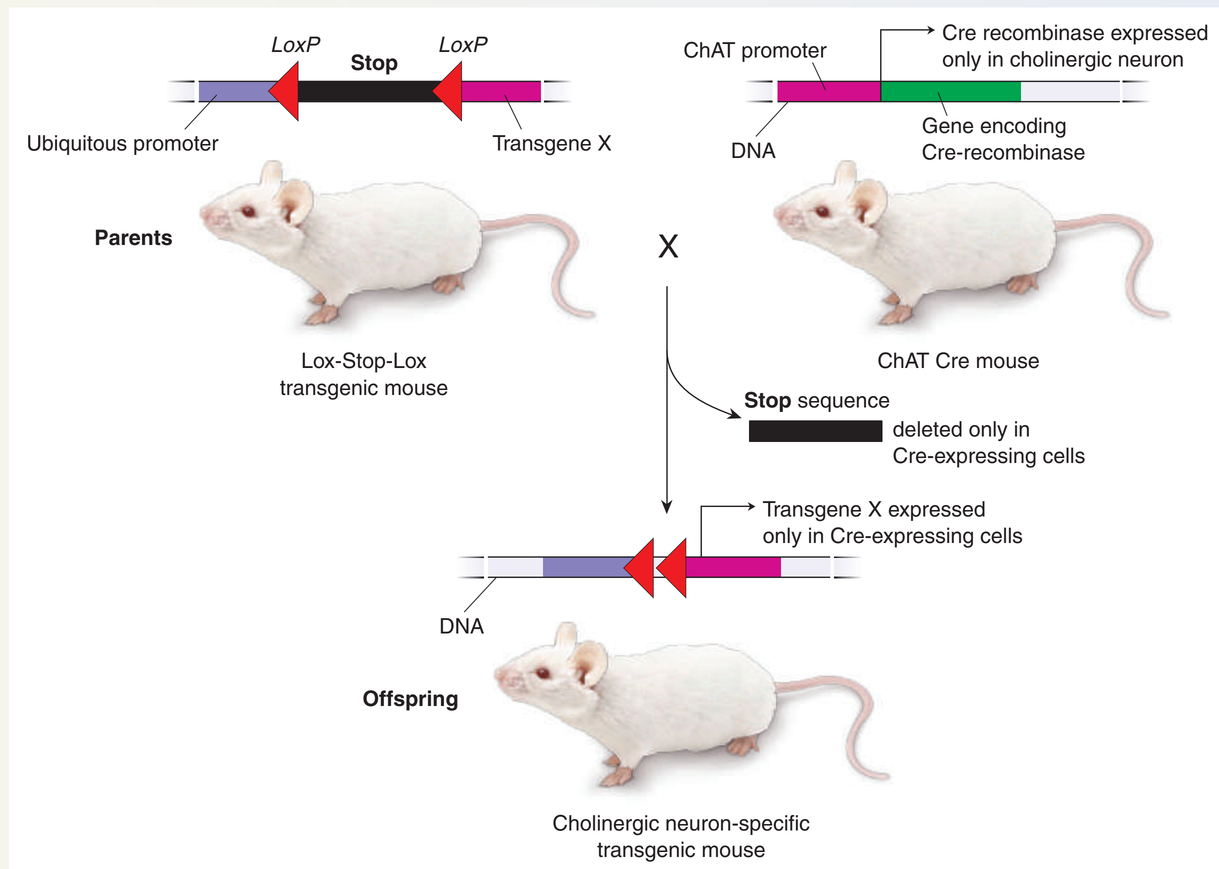

Figure B

A transgene of interest (transgene X) can also be expressed exclusively in cholinergic neurons. First, a mouse is created in which expression of the transgene is prevented by insertion of a floxed stop sequence between a strong, ubiquitous promoter and the coding region of the gene. Crossing this mouse with the ChAT-Cre mouse results in offspring in which the stop sequence has been deleted only in cholinergic neurons, allowing expression of the transgene only in these neurons.

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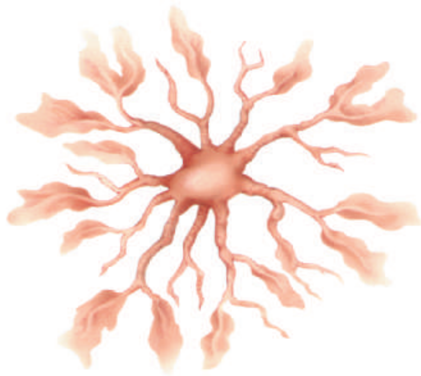
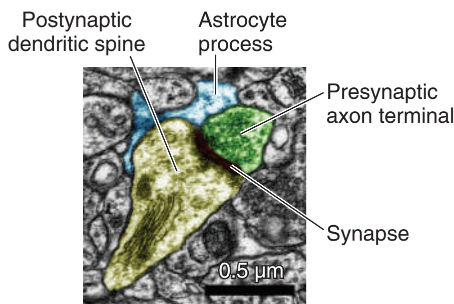
system (parts outside the skull and vertebral column). Another difference is that one oligodendroglial cell contributes myelin to several axons, whereas each Schwann cell myelinates only a single axon.

Other Non-Neuronal Cells

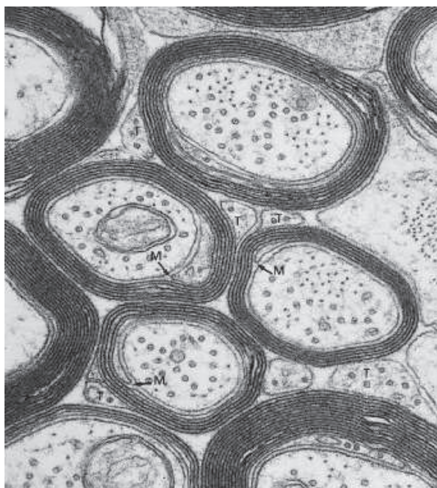
Even if we eliminated every neuron, every astrocyte, and every oligodendroglial cell, other cells would still remain in the brain. First, special cells called **ependymal cells** line fluid-filled ventricles within the brain and play a role in directing cell migration during brain development. Second, a class of cells called **microglia** function as phagocytes to remove debris left by dead or degenerating neurons and glia. Microglia have attracted much interest recently, as they appear to be involved in remodeling synaptic connections by gobbling them up. As we saw in Box 2.3, microglia can migrate into the brain from the blood, and disruption of this microglial invasion can interfere with brain functions and behavior. Finally, in addition to glial and ependymal cells, the brain also has vasculature: arteries, veins, and capillaries that deliver via the blood essential nutrients and oxygen to neurons.



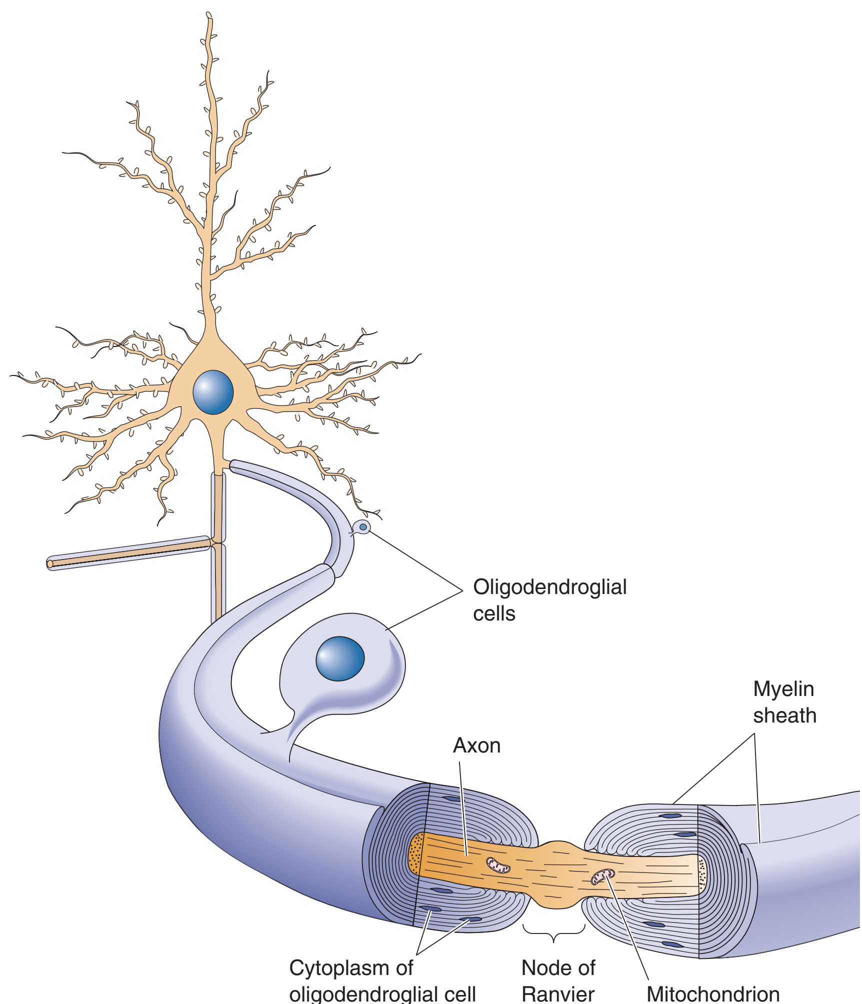
▲ FIGURE 2.24
An astrocyte. Astrocytes fill most of the space in the brain that is not occupied by neurons and blood vessels.



▲ FIGURE 2.25
Astrocytes envelop synapses. An electron micrograph of a thin slice through a synapse showing the presynaptic axon terminal and the postsynaptic dendritic spine (colored green) and an astrocyte process (colored blue) that wraps around them and restricts the extracellular space. (Source: Courtesy of Drs. Cagla Eroglu and Chris Risher, Duke University.)



▲ FIGURE 2.26
Myelinated optic nerve fibers cut in cross section. (Source: Courtesy of Dr. Alan Peters.)



▲ FIGURE 2.27
An oligodendroglial cell. Like the Schwann cells found in the nerves of the body, oligodendroglia provide myelin sheaths around axons in the brain and spinal cord. The myelin sheath of an axon is interrupted periodically at the nodes of Ranvier.

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CONCLUDING REMARKS

Learning the structural characteristics of the neuron provides insight into how neurons and their different parts work because structure correlates with function. For example, the absence of ribosomes in the axon correctly predicts that proteins in the axon terminal are provided from the soma via axoplasmic transport. A large number of mitochondria in the axon terminal correctly predicts a high energy demand. The elaborate structure of the dendritic tree appears ideally suited for receiving incoming information, and indeed, this is where most of the synapses are formed with the axons of other neurons.

From the time of Nissl, the rough ER has been recognized as an important feature of neurons. What does this tell us about neurons? Recall that rough ER is a site of the synthesis of proteins destined to be inserted into the membrane. We will next see how the various proteins in the neuronal membrane give rise to the unique capabilities of neurons to transmit, receive, and store information.

KEY TERMS

Introduction
neuron (p. 24)
glial cell (p. 24)

The Neuron Doctrine
histology (p. 25)
Nissl stain (p. 25)
cytoarchitecture (p. 25)
Golgi stain (p. 26)
cell body (p. 26)
soma (p. 26)
perikaryon (p. 26)
neurite (p. 26)
axon (p. 26)
dendrite (p. 26)
neuron doctrine (p. 27)

The Prototypical Neuron
cytosol (p. 29)
organelle (p. 29)
cytoplasm (p. 29)
nucleus (p. 29)
chromosome (p. 29)
DNA (deoxyribonucleic acid) (p. 29)
gene (p. 29)
gene expression (p. 29)
protein (p. 29)
protein synthesis (p. 29)
mRNA (messenger ribonucleic acid) (p. 29)
transcription (p. 29)
promoter (p. 31)
transcription factor (p. 31)
RNA splicing (p. 31)
amino acid (p. 32)
translation (p. 32)
genome (p. 32)
genetic engineering (p. 32)
knockout mice (p. 33)
transgenic mice (p. 33)
knock-in mice (p. 33)
ribosome (p. 36)
rough endoplasmic reticulum (rough ER) (p. 36)
polyribosome (p. 36)
smooth endoplasmic reticulum (smooth ER) (p. 36)
Golgi apparatus (p. 36)
mitochondrion (p. 36)
ATP (adenosine triphosphate) (p. 38)
neuronal membrane (p. 38)
cytoskeleton (p. 38)
microtubule (p. 38)
microfilament (p. 39)
neurofilament (p. 39)
axon hillock (p. 39)
axon collateral (p. 39)
axon terminal (p. 41)
terminal bouton (p. 41)
synapse (p. 42)
terminal arbor (p. 42)
innervation (p. 42)
synaptic vesicle (p. 42)
synaptic cleft (p. 43)
synaptic transmission (p. 43)
neurotransmitter (p. 43)
axoplasmic transport (p. 43)
anterograde transport (p. 44)
retrograde transport (p. 44)
dendritic tree (p. 44)
receptor (p. 46)
dendritic spine (p. 46)

Classifying Neurons
unipolar neuron (p. 46)
bipolar neuron (p. 46)
multipolar neuron (p. 46)
stellate cell (p. 46)
pyramidal cell (p. 46)
spiny neuron (p. 46)
aspinous neuron (p. 46)
primary sensory neuron (p. 48)
motor neuron (p. 48)
interneuron (p. 48)
green fluorescent protein (GFP) (p. 48)

Glia
astrocyte (p. 49)
oligodendroglial cell (p. 49)
Schwann cell (p. 49)
myelin (p. 49)
node of Ranvier (p. 49)
ependymal cell (p. 52)
microglial cell (p. 52)

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REVIEW QUESTIONS

1. State the neuron doctrine in a single sentence. To whom is this insight credited?
2. Which parts of a neuron are shown by a Golgi stain that are not shown by a Nissl stain?
3. What are three physical characteristics that distinguish axons from dendrites?
4. Of the following structures, state which ones are unique to neurons and which are not: nucleus, mitochondria, rough ER, synaptic vesicle, Golgi apparatus.
5. What are the steps by which the information in the DNA of the nucleus directs the synthesis of a membrane-associated protein molecule?
6. Colchicine is a drug that causes microtubules to break apart (depolymerize). What effect would this drug have on anterograde transport? What would happen in the axon terminal?
7. Classify the cortical pyramidal cell based on (1) the number of neurites, (2) the presence or absence of dendritic spines, (3) connections, and (4) axon length.
8. Knowledge of genes uniquely expressed in a particular category of neurons can be used to understand how those neurons function. Give one example of how you could use genetic information to study a category of neuron.
9. What is myelin? What does it do? Which cells provide it in the central nervous system?

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