CHAPTER

1

Neurons

By using the silver impregnation method developed by Golgi (1873), Ramon y Cajal studied neurons, and their connections, in the nervous system of numerous species. Based on his own work (1888) and that of others (e.g. Forel, His, Kölliker and Lenhossék) he proposed the concept that neurons are isolated units connected to each other by contacts formed by their processes: 'The terminal arborizations of neurons are free and are not joined to other terminal arborizations. They make contacts with the cell bodies and protoplasmic processes of other cellular elements.'

As proposed by Cajal, neurons are independent cells making specific contacts called *synapses*, with hundreds or thousands of other neurons sometimes greatly distant from their cell bodies. The neurons connected together form circuits, and so the nervous system is composed of neuronal networks which transmit and process information. In the nervous system there is another class of cells, the glial cells, which surround the various parts of neurons and cooperate with them. Glial cells are discussed in Chapter 2.

Neurons are *excitable* cells. Depending on the information they receive, neurons generate electrical signals and propagate them along their processes. This capacity is due to the presence of particular proteins in their plasma membrane which allow the selective passage of ions: the ion channels.

Neurons are also *secretory* cells. Their secretory product is called a *neurotransmitter*. The release of a neurotransmitter occurs only in restricted regions, the synapses. The neurotransmitter is released in the extracellular space. The synaptic secretion is highly focalized and directed specifically on cell regions to which the neuron is connected. The synaptic secretion is then different (with only a few exceptions) from other secretory cells, such as from hormonal and exocrine cells which respectively release their secretory products into the general circulation (endocrine secretion) or the external environment (exocrine secretion). Synapses are discussed in Chapter 6.

Neurons are *quiescent* cells. When lesioned, most neurons cannot be replaced, since they are postmitotic cells. Thus, they renew their constituents during their entire life, involving the precise targeting of mRNAs and proteins to particular cytoplasmic domains or membrane areas.

1.1 NEURONS HAVE A CELL BODY FROM WHICH EMERGE TWO TYPES OF PROCESSES: THE DENDRITES AND THE AXON

Although neurons present varied morphologies, they all share features that identify them as neurons. The cell body or *soma* gives rise to processes which give the neuron the regionalization of its functions, its polarity and its capacity to connect to other neurons, to sensory cells or to effector cells.

1.1.1 The somatodendritic tree is the neuron's receptive pole

The soma of the neuron contains the nucleus and its surrounding cytoplasm (or *perikaryon*). Its shape is variable: pyramidal soma for pyramidal cells in the cerebral cortex and hippocampus; ovoid soma for Purkinje cells in the cerebellar cortex; granular soma for small multipolar cells in the cerebral cortex, cerebellar cortex and hippocampus; fusiform soma for neurons in the pallidal complex; and stellar or multipolar soma for motoneurons in the spinal cord (**Figure 1.1**).

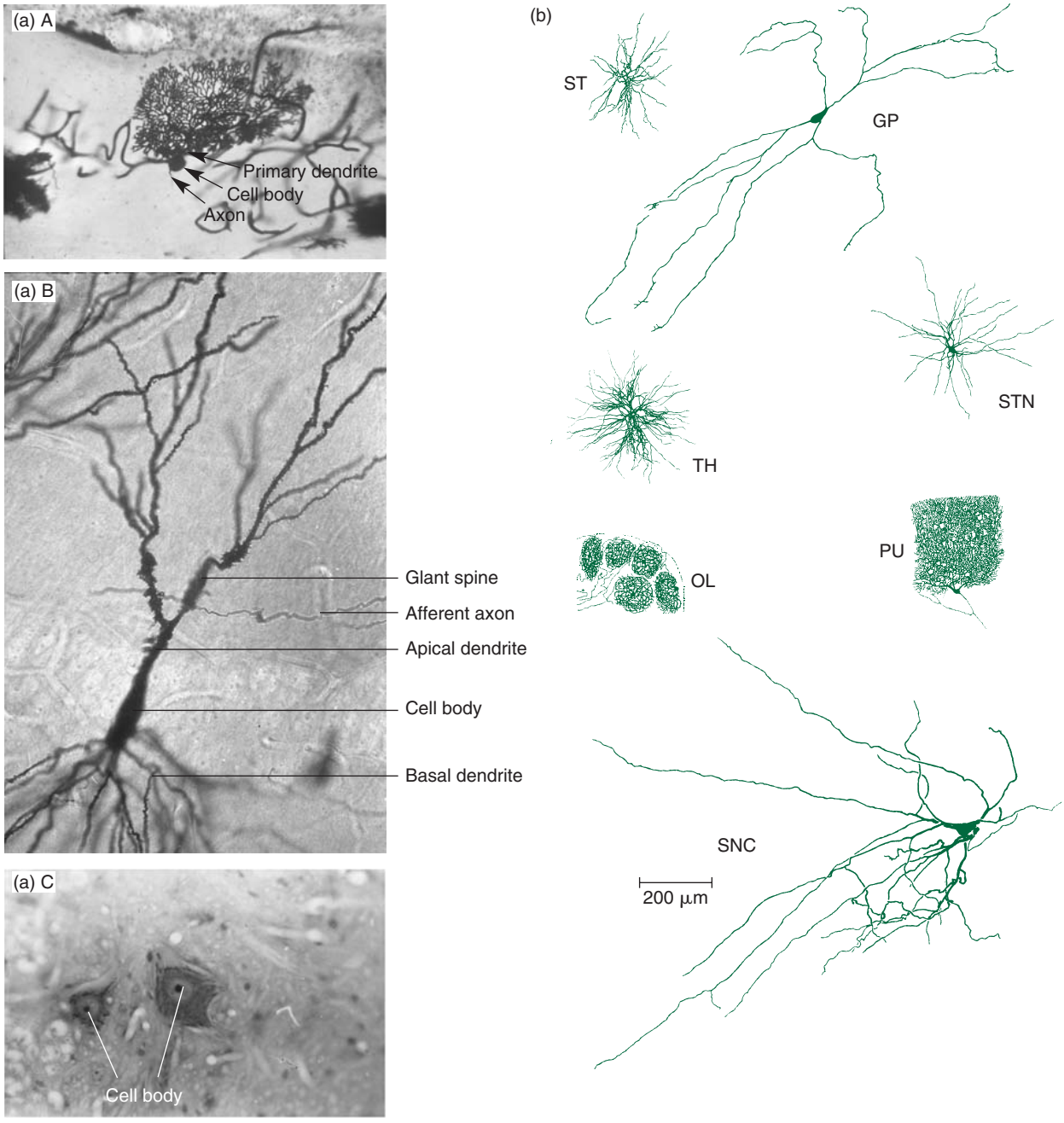

FIGURE 1.1 The neurons of the central nervous system present different dendritic arborizations.
(a) Photomicrographs of neurons in the central nervous system as observed under the light microscope. A – Purkinje cell of the cerebellar cortex; B – pyramidal cell of the hippocampus; C – soma of a motoneuron of the spinal cord. Golgi (A and B) and Nissl (C) staining. The Golgi technique is a silver staining which allows observation of dendrites, somas and axon emergence. The Nissl staining is a basophile staining which displays neuronal regions (soma and primary dendrites) containing Nissl bodies (parts of the rough endoplasmic reticulum). **(b)** Camera lucida drawings of neurons in the central nervous system of primates, revealed by the Golgi silver impregnation technique and reconstructed from serial sections: ST, medium spiny neuron of the striatum; GP, neuron of the globus pallidus; TH, thalamocortical neuron; STN, neuron of the subthalamic nucleus; OL, neurons of the inferior olivary complex; PU, Purkinje cell of the cerebellar cortex; SNC, dopaminergic neuron of the substantia nigra pars compacta. All these neurons are illustrated at the same magnification. Photomicrographs by Olivier Robain (aA and aB) and Paul Derer (aC). Drawings by Jérôme Yelnik, except OL and PU by Ramon Y Cajal (1911).

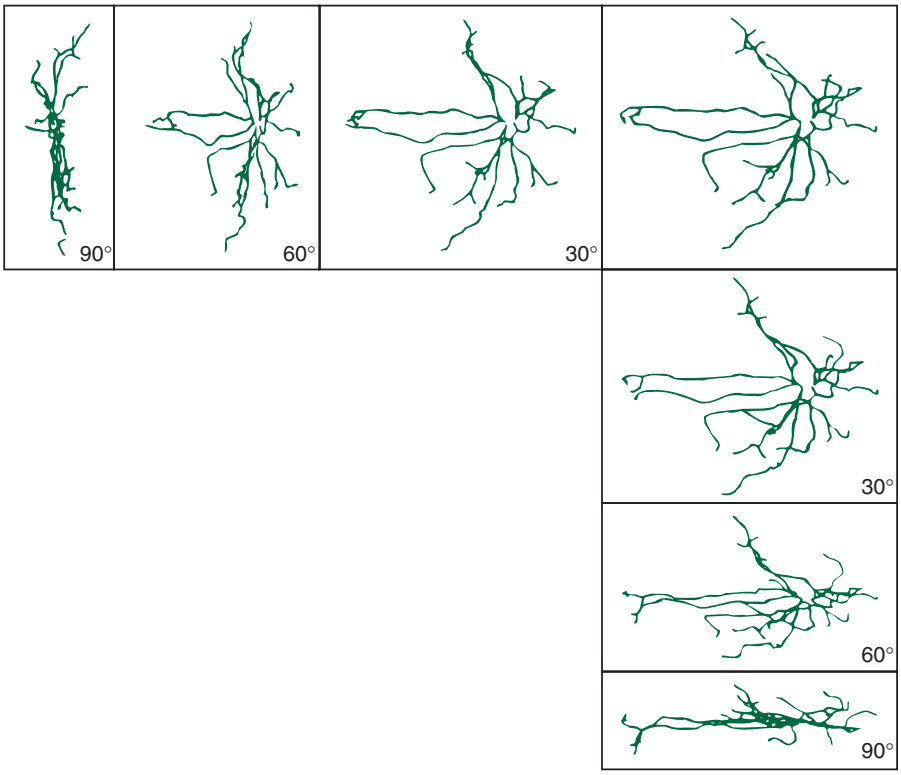

FIGURE 1.2 Tridimensional illustration of a dendritic arborization.
Computer drawing of a neuron of the subthalamic nucleus injected intracellularly with horseradish peroxidase (HRP) and reconstructed in three dimensions from serial sections. At 0°, the dendritic arborization of this neuron is represented in its principal plane; i.e. in the plane where it has its largest surface. In this plane, the dendritic field is almost circular (859 μm long and 804 μm wide). 30°, 60° and 90° rotations from the principal plane around the horizontal (horizontal column) and vertical (vertical column) axis show that the dendritic field has a flattened ovoidal form (230 μm thick). From Hammond C and Yelnik J (1983) Intracellular labelling of rat subthalamic nucleus with horseradish peroxidase: computer analysis of dendrites and characterization of axon arborization. *Neuroscience* **8**, 781–790, with permission.

One function of the soma is to ensure the synthesis of many of the components required for the structure and function of a neuron. Indeed, the soma contains all the organelles responsible for the synthesis of macromolecules. Most neurons in the central nervous system cannot further divide or regenerate after birth, and the cell body must maintain the structural integrity of the neuron throughout the individual's entire life. Moreover, the soma receives numerous synaptic contacts from other neurons and constitutes, with the dendrites, the main receptive area of neurons (see **Figure 1.5** and Section 6.2).The neurons have one or several processes emerging from the cell body and arborizing more or less profusely. The two types of neuronal processes are the dendrites and the axon (**Figures 1.1** and **1.3**). This division is based on morphological, ultrastructural, biochemical and functional criteria.

The dendrites, when they emerge from the soma, are simple perikaryal extensions, the primary dendrites. On average, between one and nine primary dendrites emerge from the soma and then divide successively to give a dendritic tree with specific characteristics (number of branches, volume, etc.) for each neuronal population (**Figures 1.1** and **1.2**). The dendrites are morphologically distinguishable from axons by their irregular outline, by their diameter which decreases along their branchings, by the acute angles between the branches, and by their ultrastructural characteristics (**Figures 1.1**, **1.3** and **1.7**). The irregular outline of dendrites is related to the presence of numerous appendices of various shapes and dimensions at their surface. The most frequently observed are the dendritic spines which are lateral expansions with ovoid heads binding to the dendritic branches by a peduncle that is variable in length (**Figure 1.3**). Some neurons are termed 'spiny' because there are between 40,000 and 100,000 spines on the surface of their dendrites (e.g. pyramidal neurons of the cerebral cortex and hippocampus, the medium-sized neurons of the striatum, and the Purkinje cells of the cerebellar cortex). However, other neurons with only

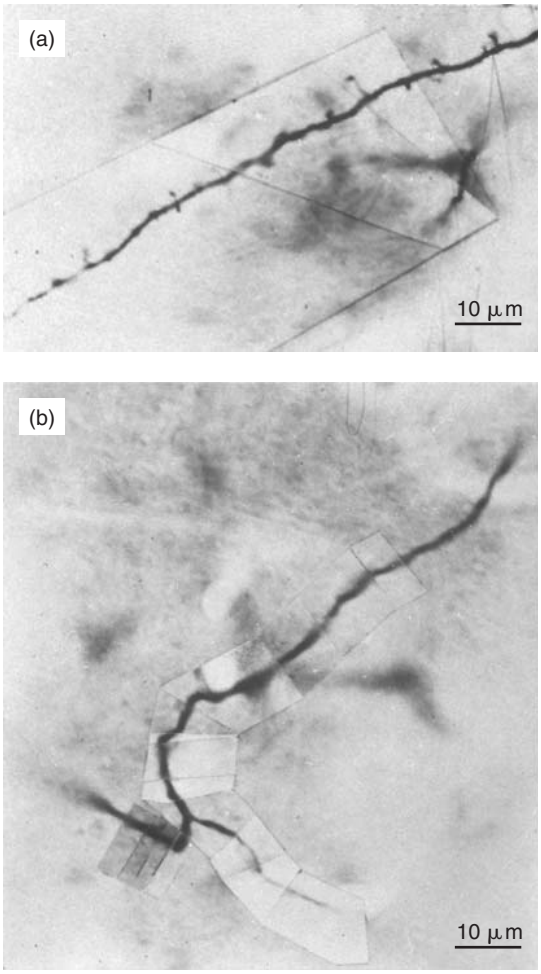

FIGURE 1.3 Dendrite and axon of a rat subthalamic nucleus neuron.
(a) A distal dendrite: dendritic spines of various shapes are present on its surface. **(b)** The axon: it has a smooth surface and gives off an axonal collateral. The processes of this neuron are stained by an intracellular injection of horseradish peroxidase. To follow the dendrites and axon along their trajectories, each figure is a photomontage of numerous photomicrographs of serial sections. From Hammond C and Yelnik J (see Figure 1.2), with permission.

a few spines on their dendritic surface are termed 'smooth' (e.g. neurons of the pallidal complex) (**Figure 1.1**). The transition from the cell body to proximal dendrites is gradual, and the cytoplasmic architectures of proximal dendrites and the cell body are similar. In particular, the endoplasmic reticulum and ribosomes are almost as abundant in the proximal dendrites as in the cell body. Moreover, even distal dendrites contain ribosomes and endoplasmic reticula.

Dendrites and soma receive numerous synaptic contacts from other neurons and constitute the main receptive area of neurons (see **Figure 1.5** and Section 6.2). In response to afferent information, they generate electrical signals such as postsynaptic potentials (EPSPs or IPSPs; see **Figure 1.5** left 1, 2) or calcium action potentials, and integrate the afferent information. Chapters 8–10 look at the mechanisms underlying the excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials generated in the postsynaptic membrane in response to transmitter release. Chapters 13–16 discuss how these postsynaptic responses are integrated along the somato-dendritic tree. Although dendrites are generally a receptive zone, there are certain exceptions: some dendrites are connected with other dendrites and act as a transmitter area by releasing neurotransmitters (see **Figure 6.2d**).

1.1.2 The axon and its collaterals are the neuron's transmitter pole

The axon is morphologically distinct from dendrites in having a smooth appearance and a uniform diameter along its entire extent, and by its ultrastructural characteristics (**Figures 1.3**, **1.6** and **1.7**). Axons are narrow from their origin, and do not usually contain ribosomes or endoplasmic reticula. The transition from the cell body to axon is distinct; the region of the cell body from which an axon originates is called the axon hillock and it tapers off to the axonal initial segment, where action potentials begin. Although most parts of the cell body are rich in endoplasmic reticula, the axon hillock is not. At the axon initial segment, the plasma membrane has thick underlying structures, and there is a specialized bundle of microtubules. In some neurons the axon emerges at the level of a primary dendrite.

The axon is not a single process; it is divided into one or several collaterals which form right-angles with the main axon. Some collaterals return toward the cell body area; these are recurrent axon collaterals. The axon and its collaterals may be surrounded by a sheath, the myelin sheath. Myelin is formed by glial cells (see Sections 2.2 and 2.3). The length of an axon varies. Certain neurons in the central nervous system have axons that project to one or several structures of the central nervous system that are more or less distant from their cell bodies (**Figure 1.4**), whereas other neurons have short axons (a few microns in length) that are confined to the structure where their cell bodies are located; these are interneurons or local circuit neurons (see **Figure 1.13**).

Thus projection (Golgi type I) neurons and local-circuit (Golgi type II) neurons can be differentiated. In Golgi type I neurons, the length of the axon is variable: certain projection neurons are directed to one structure only (e.g. corticothalamic neurons; see **Figure 1.14**) whereas other projection neurons have numerous axon collaterals which project to several cerebral structures (**Figure 1.4**).

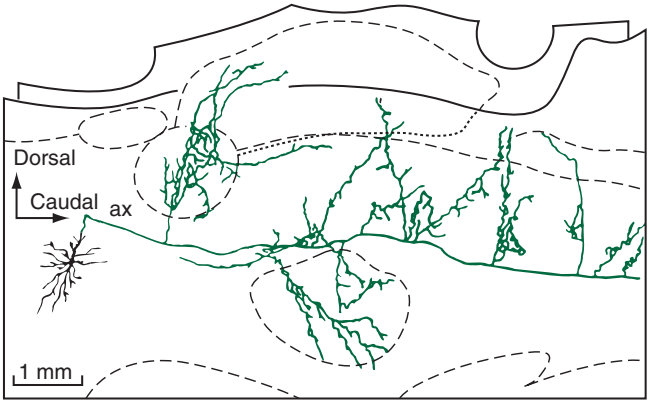

FIGURE 1.4 Neuron of the cat reticular formation (brainstem) showing a complex axonal arborization.
This reticulospinal neuron has been stained by intracellular injection of peroxidase and drawn in a parasagittal plane obtained from serial sections. The axon (ax, black) gives off numerous collaterals along its rostrocaudal trajectory, making contacts with different neuronal populations (delimited by broken lines). Scale: 7 mm = 1 μm. From Grantyn A (1987) Reticulo-spinal neurons participating in the control of synergic eye and head movement during orienting in the cat. *Exp. Brain Res.* **66**, 355–377, with permission.

The axon and axonal collaterals in certain neurons end in a terminal arborization, i.e. numerous thin branches whose extremities, the synaptic boutons, make synaptic contacts with target cells (see **Figure 6.3**). In other neurons the axon and its collaterals have enlargements or varicosities which contact target cells along their way: these are 'boutons en passant' (see **Figures 6.14** and **6.15b**). It can be noted that both types of boutons are called *axon terminals*, although 'boutons en passant' are not the real endings of the axon.

The main characteristic of axons is their capacity to trigger sodium action potentials and to propagate them over considerable distances without any decrease in their amplitude (**Figure 1.5** left 3). Action potentials are generated at the initial segment level in response to synaptic information transmitted by the somatodendritic tree. Then they propagate along the axon and its collaterals toward the axon terminals (synaptic boutons or boutons en passant). When action potentials reach the axon terminals these trigger calcium action potentials (**Figure 1.5** left 4) which may cause the release of the neurotransmitter(s) contained in axon terminals in a specific compartment, the synaptic vesicles. This secretion is localized only at the synaptic contacts. Overall, the axon is considered as the transmitter pole of the neuron.

Chapter 4 discusses the mechanisms underlying the abrupt, large and transient depolarizations called (sodium) action potentials, and how they are triggered and propogated. Chapters 4, 5 and 7 look at how sodium action potentials trigger calcium action potentials, the entry of calcium in synaptic terminals and the secretion of transmitter molecules.

Certain regions – such as the initial segment, nodes of Ranvier (zones between two myelinated segments; see **Figure 1.5**) and axon terminals – can also be receptive areas (a postsynaptic element) of synaptic contacts from other neurons (see Section 6.2).

1.2 NEURONS ARE HIGHLY POLARIZED CELLS WITH A DIFFERENTIAL DISTRIBUTION OF ORGANELLES AND PROTEINS

The somatodendritic tree is the neuron's receptive pole, whereas the axon and its collaterals are the neuron's transmitter pole. Neurons are highly polarized cells. Cellular morphology and accurate organelles and protein distribution lay the basis to this polarization. The organelles and cytoplasmic elements present in neurons are the same organelles found in other cell types. However, some elements such as cytoskeletal elements are more abundant in neurons. The nonhomogeneous distribution of organelles in their soma and processes is one of the most distinguishing characteristics of neurons.

1.2.1 The soma is the main site of macromolecule synthesis

The soma contains the same organelles and cytoplasmic elements that exist in other cells: cellular nucleus, Golgi apparatus, mitochondria, polysomes, cytoskeletal elements and lysosomes. The soma is the main site of synthesis of macromolecules since it is the one compartment containing all the required organelles.

Compared with other types of cells, the neuron differs at the nuclear level and more specifically at the chromatin and nucleolus levels. The chromatin is light and sparsely distributed: the nucleus is in interphase. Indeed, in humans, most neurons cannot divide after birth since they are postmitotic cells. The nucleolus is the site of ribosomal synthesis and ribosomes are essential for translating messenger RNA (mRNA) into proteins. The large size of the nucleolus indicates a high level of protein synthesis in neurons.

1.2.2 The dendrites contain free ribosomes and synthesize some of their proteins

In dendrites can be found smooth endoplasmic reticulum, elongated mitochondria, free ribosomes or

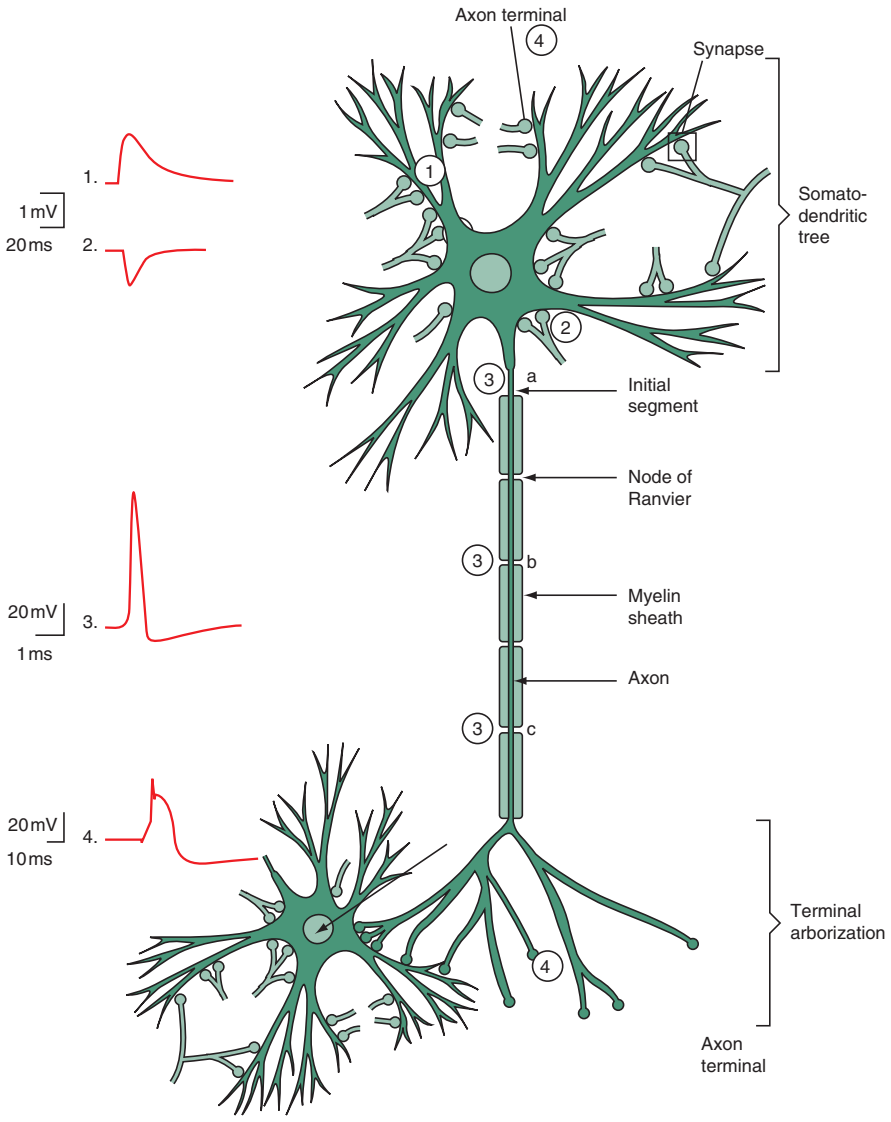

FIGURE 1.5 Comprehensive schematic drawing of neuron polarity.
The somatodendritic compartment of a neuron receives a large amount of information from other neurons that establish synapses with it. At each synapse level, the neuron generates postsynaptic potentials in response to the released neurotransmitter (1, EPSP; 2, IPSP). These postsynaptic potentials propagate and summate in the somatodendritic compartment, then they propagate to the initial segment of the axon where they generate (or not) action potential(s) (3a). The action potentials propagate along the axon (3b, 3c) and its collaterals up to the axon terminals where they evoke (or not) the entry of calcium (4) and neurotransmitter release. Note the different voltage and time calibrations.

polysomes, and cytoskeletal elements including microtubules which are oriented parallel to the long axis of the dendrites (but they lack neurofilaments) (**Figure 1.6**).

By using the hook procedure, microtubules have been shown to have two orientations in proximal dendrites: half of them are oriented with the plus-ends distal to the cell body, and the other half has the plus-ends proximal to the cell body. This is very different from the orientation in distal dendrites and axons (**Figure 1.7**), which is uniform. Moreover, one microtubule-associated protein (MAP), the high-molecular-weight MAP2 protein and more precisely the MAP2A and MAP2B, are more common to dendrites than to axons. For this reason MAP2A or MAP2B antibodies coupled to fluorescent molecules are useful for labelling dendrites, particularly for dendrite identification in cell cultures.

mRNA trafficking and local protein synthesis in dendrites

The dendritic compartment contains many ribosomes whereas an axon has considerably fewer ribosomes.

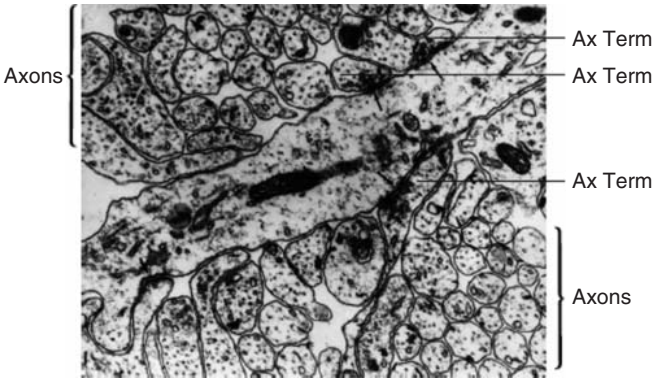

FIGURE 1.6 Photomicrograph of a tissue section of the central nervous system at the hippocampal level.
This shows the ultrastructure of a dendrite, numerous axons and their synaptic contacts (observation under the electron microscope). The apical dendrite of a pyramidal neuron contains mitochondria, microtubules, ribosomes and smooth endoplasmic reticulum. It is surrounded by fascicles of unmyelinated axons with mitochondria and microtubules but no ribosomes. The axon's trajectory is perpendicular to the section plane. Three synaptic boutons (Ax Term) with synaptic vesicles make synaptic contacts (arrows) with the dendrite. Photomicrograph by Olivier Robain.

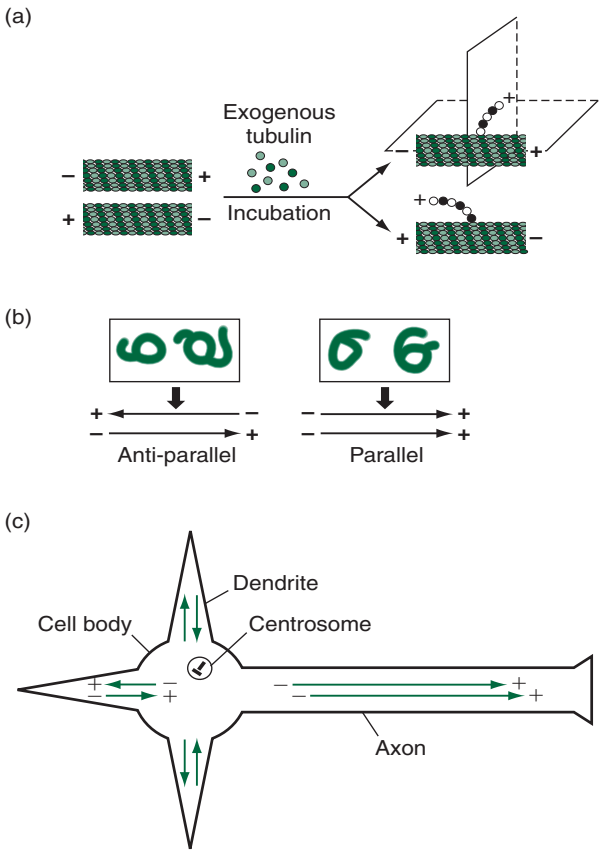

FIGURE 1.7 Microtubule polarity in neuronal processes.
(a) The polarity of microtubules is defined by the hook procedure. Neurons in culture are permeabilized in the presence of taxol to stabilize microtubules. Monomers of tubulin, purified from brain extracts, are added in the extracellular medium. Several minutes after, transversal cuts are performed at the level of dendrites or at the level of an axon. Slices are treated for electron microscopy. Hook-like structures are observed. They result from exogenous tubulin polymerization at the surface of endogenous microtubules. Hooks are always oriented toward the plus-end of microtubules. **(b)** When hooks, at the electron microscopic level, have mixed orientations (clockwise and anticlockwise), this means that endogenous microtubules are antiparallel (left). Uniformly oriented hooks (right) indicate that endogenous microtubules are parallel. **(c)** Orientation of microtubules in dendrites and the axon. Drawing (a) by Lotfi Ferhat. Drawing (b) adapted from Sharp DJ, Wenqian Yu, Ferhat L *et al.* (1997) Identification of a microtubule-associated motor protein essential for dendrite differentiation. *J. Cell. Biol.* **138**, 833–843. Drawing (c) adapted from Baas PW, Deitch JS, Black MM, Banker GA (1988) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc. Natl Acad. Sci. USA* **85**, 8335–8339, with permission.

One particular feature of dendrites, compared with axons, is the presence of synapse-associated polyribosome complexes (SPRCs); these are clusters of polyribosomes and associated membranous cisterns that are selectively localized beneath synapses (more precisely, beneath postsynaptic sites), at the base of dendritic spines when spines are present.

What is the origin of this selective distribution of ribosomes in neurons? This question is particularly important since this compartmentalization leads to different properties of dendrites and axons: dendrites can locally synthesize some of their proteins, whereas axons would synthesize very few of them, if any.

Whereas most proteins destined for dendrites and dendritic spines are conveyed from the cell body, a subset of mRNAs are transported into dendrites to support local protein synthesis. Such a local dendritic protein synthesis requires that a particular subset of mRNAs synthesized in the nucleus is transported into the dendrites up to the polysomes where they are translated.

In cultured hippocampal neurons, RNA labelled with tritiated uridin is shown to be transported at a rate of 250–500 μm per day. This transport is blocked by metabolic poisons and the RNA in transit appears to be bound to the cytoskeleton, since much of it remains following detergent extraction of the cells. Studies using video microscopy techniques and cell-permeant dyes which fluoresce on binding to nucleic acids have permitted observation of the movement of RNA-containing granules along microtubules in dendrites. These studies suggest that mRNAs are transported as part of a larger structure. The visualized RNA particles colocalize with poly(A) mRNA, the 60S ribosomal subunit, suggesting that the granules may represent translational units or complexes (**Figure 1.8**). Therefore, this energy-dependent transport seems to be associated with the dendrite cytoskeleton as also shown by the delocalization of

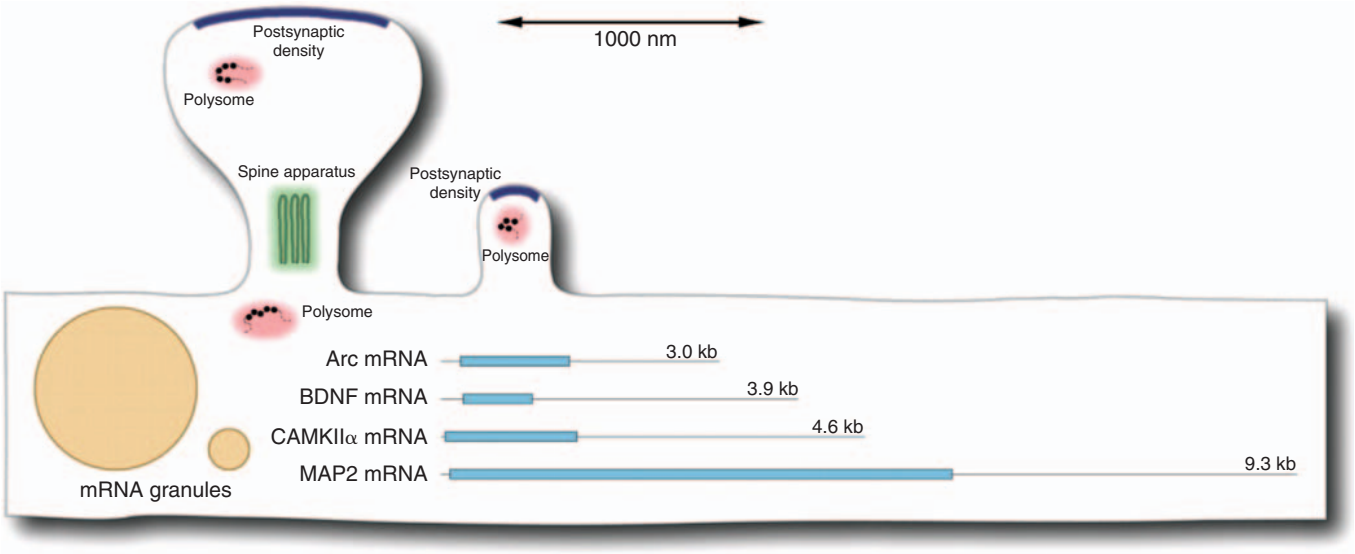

FIGURE 1.8 Approximate sizes of representative dendritic mRNAs and translational elements at synaptic sites on dendrites.
The drawing illustrates the approximate size range of spine synapses that would be found in rat forebrain structures such as the hippocampus and cerebral cortex. The lines represent the approximate length of representative dendritic mRNAs if they were straightened out. Shading indicates the length and position of the coding region. Adapted from Schuman EM, Dynes JL, Steward O (2006) Synaptic regulation of dendritic mRNAs. *J. Neurosci.* **26**, 7143–7146.

mRNA granules in response to colchicin (a drug which blocks microtubule polymerization).

To visualize mRNA translocation in live neurons studies used nucleic acid stains and green fluorescent protein fused to RNA-binding proteins. It showed that mRNAs are transported in the form of large granules containing mRNAs, RNA-binding proteins, ribosomes, and translational factors (RNA-containing granules) in a rapid (average speed, 0.1 μm/s), bidirectional, and microtubule dependent manner.

The exact mechanisms underlying the targeting of newly synthesized mRNAs to dendrites – which includes transport (i.e. recognition of particular mRNAs within a granule and movement along microtubules) and docking (shift from a microtubule-based transport to a cytoskeletal-based anchor) – are not yet clear. However, RNA-containing granules that are transported to dendrites bind to the C-terminal tail of the conventional kinesin KIF5 as a large detergent resistant, RNase-sensitive granule (see **Figure 1.11** and Section 1.3).

The mRNAs present in dendrites encode proteins of different functional types. Among the mRNAs detected in dendrites by *in situ* hybridization (see Appendix 6.2) are mRNAs that encode certain cytoskeletal proteins (as the high-molecular-weight MAP2), a kinase (the α subunit of calcium/calmodulin-dependent protein kinase II), an integral membrane protein of the endoplasmic reticulum (the inositol trisphosphate receptor), calcium-binding proteins, certain units of neurotransmitter receptors (the NR1 subunit of the NMDA receptor) as well as other proteins of unknown function. Moreover, within dendrites, different mRNAs are localized in different domains and different mRNAs are localized in the dendrites of different neuron types. In summary, it has become clear that certain mRNAs are transported in dendrites in large macromolecular complexes, the granules (>1000 S). These granules are transported by a kinesin KIF5, which binds RNA-binding proteins by a recognition motif in its tail domain.

The relatively large amount of RNA transported into dendrites raises the question of why neurons need this supply. Targeting of mRNAs to dendritic synthetic machinery located at the base of dendritic spines could occur, for example, in response to synaptic information and trigger local protein synthesis that would be responsible for the stability of the synaptic transmission or the modulation of it by changing, for example, the subunits or the number of receptors to the neurotransmitter in the postsynaptic membrane (this can occur during plasticity and may produce long-lasting changes in synaptic strength) (see Chapter 18).

1.2.3 The axon, to a large extent, lacks the machinery for protein synthesis

The axoplasm contains thin elongated mitochondria, numerous cytoskeletal elements and transport vesicles. It is devoid of ribosomes associated to the reticulum

but may contain ribonucleoprotein complexes especially during development. Nevertheless, axons cannot restore the vast majority of the macromolecules from which they are made; neither can they ensure alone the synthesis of the neurotransmitter(s) that they release since they are unable to synthesize proteins (such as enzymes). This problem is resolved by the existence of a continuous supply of macromolecules from the cell body to the axon through anterograde axonal transport (see Section 1.3).

Another major difference between dendrites and axons is the orientation of microtubules. By using the hook procedure (see **Figure 1.7**) it has been shown that the polarity of microtubules is uniform in the axon, meaning that all their plus-ends point away from the cell body, toward the axon terminals. The polarity of the microtubules is relevant for transport properties (see Section 1.3). Moreover, one MAP, the Tau protein, is more common to axons than to dendrites. Tau antibodies coupled to fluorescent molecules are useful for labelling axons, particularly for axon identification in cell cultures.

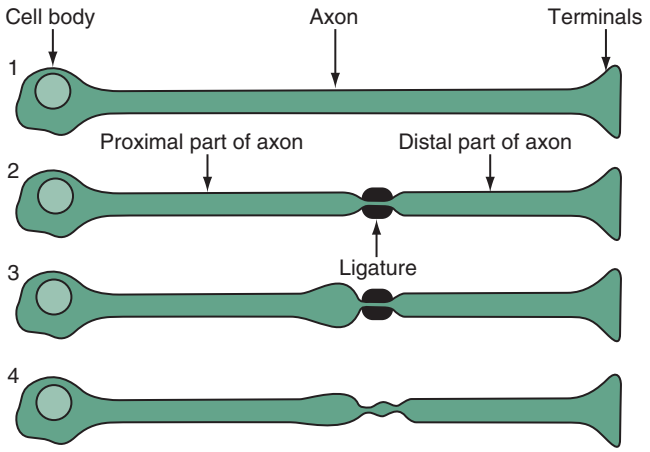

FIGURE 1.9 Experiment by Weiss and others demonstrating anterograde axonal transport.
Schematic of a chicken motoneuron (1). When a ligature is placed on the axon (2) an enlargement of the axon's diameter above the ligature is noted after several weeks (3). When this ligature is removed, the enlargement progressively disappears (4). From Weiss P, Hiscoe HB (1948) Experiments on the mechanism of nerve growth. *J. Exp. Zool.* **107**, 315–396, with permission.

1.3 AXONAL TRANSPORT ALLOWS BIDIRECTIONAL COMMUNICATION BETWEEN THE CELL BODY AND THE AXON TERMINALS

Axonal transport is the movement of subcellular structures (such as vesicles, mitochondria, etc.) and proteins (like those of the cytoskeleton) from the cell body to axonal sites (nodes of Ranvier, presynaptic release sites, etc.) and from axon terminals to the cell body.

1.3.1 Demonstration of axonal transport

Weiss and Hiscoe (1948) first demonstrated the existence of material transport in growing axons (during development) as in mature axons. Their work consisted of placing a ligature on the chicken sciatic nerve, and then examining the change in diameter of the axons over several weeks. They showed that these neurons became enlarged in their proximal part and presented degenerative signs in their distal part (**Figure 1.9**). The authors suggested that material from the cell body had accumulated above the ligature and ensured the survival of the distal part.

Later Lubinska *et al*. (1964) elaborated the concept of anterograde and retrograde transport. These authors placed two ligatures on a dog sciatic nerve, isolated part of the nerve and divided it into short segments in order to analyze their acetylcholinesterase content. This enzyme is responsible for acetylcholine degradation and was used here as a marker. They showed that it accumulates at the level of both ligatures. This result therefore suggested the existence of two types of transport: an anterograde transport (from cell body to terminals) and a retrograde transport (from terminals to cell body). Moreover, it appeared that both types of transport are distributed along the entire extent of the axon.

We presently know of three types of axonal transport: fast (anterograde and retrograde), slow (anterograde) and mitochondrial.

1.3.2 Fast anterograde axonal transport is responsible for the movement of membranous organelles from cell body towards axon terminals, and allows renewal of axonal proteins

Fast anterograde axonal transport consists in the movement of vesicles along the axonal microtubules at a rate of 200–400 mm per day (i.e. 2–5 μm/s). These transport vesicles, which are 40–60 nm in diameter, emerge from the Golgi apparatus in the cell body (**Figure 1.10a**). They transport, among other things, proteins required to renew plasma membrane and internal axonal membranes, neurotransmitter synthesis enzymes and neurotransmitter precursors when the neurotransmitter is a peptide. This transport is independent of the type of axon (central, peripheral, etc.).

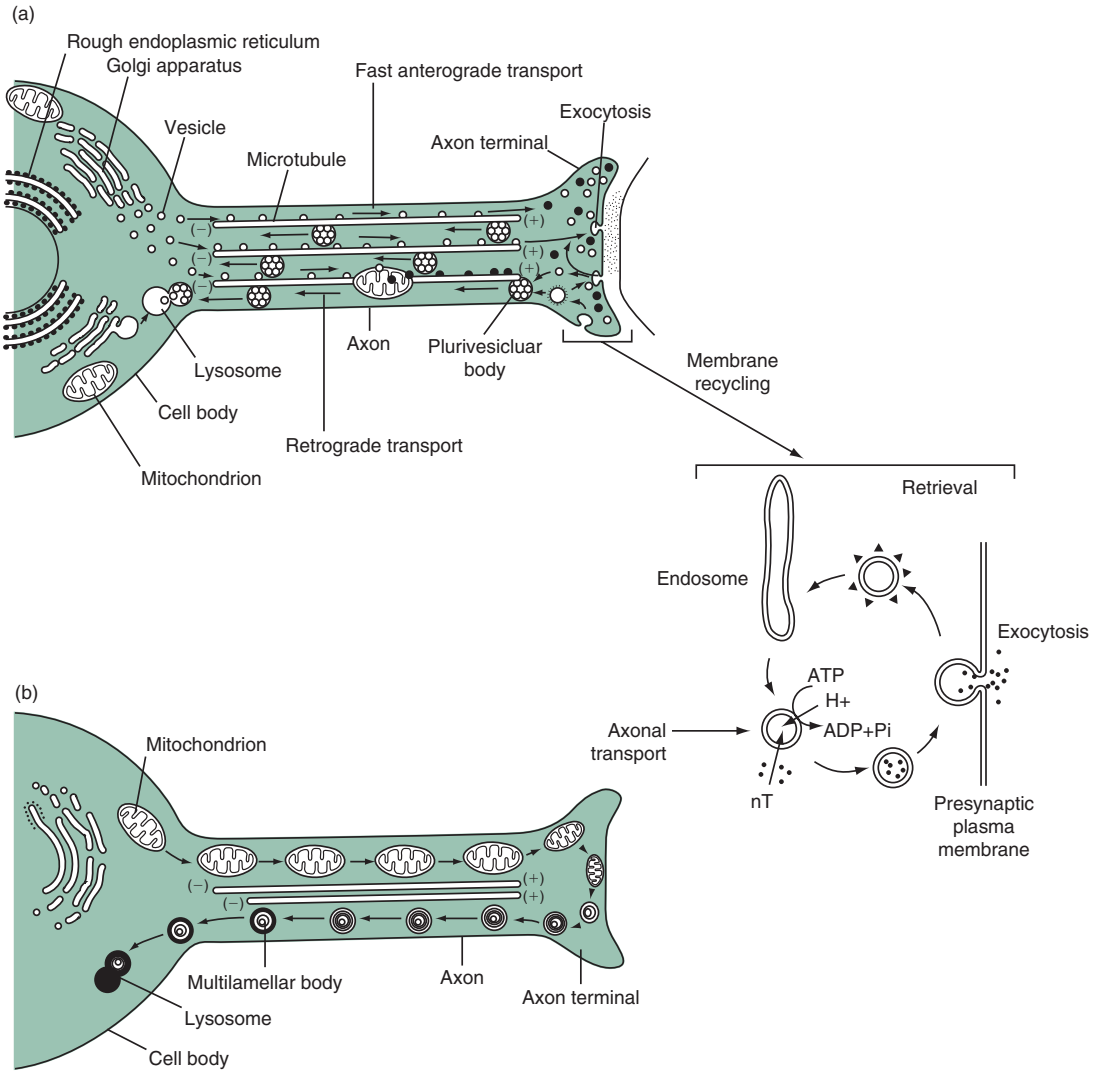

FIGURE 1.10 Fast axonal transport.
(a) Schematic of fast anterograde axonal transport (anterograde movement of vesicles) and retrograde axonal transport (retrograde movement of plurivesicular bodies). These two transports use microtubules as substrate. The detail shows recycling of small synaptic vesicles. Vesicles synthetized in the cell body and transported to the axon terminals are loaded with cytoplasmic neurotransmitter and targeted to the presynaptic plasma membrane. In response to Ca^{2+} entry, they fuse with the plasma membrane, release their content into the synaptic cleft (exocytosis); then they are recycled via an endosomal compartment. **(b)** Schematic of mitochondrial transport. Note that the neuron representation is extremely schematic since axons do not give off *one* axon terminal. Drawing (a) adapted from Allen R (1987) Les trottoirs roulants de la cellule. *Pour la Science*, April, 52–66; and Südhof TC, Jahn R (1991) Proteins of synaptic vesicles involved in exocytosis and membrane recycling. *Neuron* **6**, 665–677, with permission. Drawing (b) adapted from Lasek RJ, Katz M (1987) Mechanisms at the axon tip regulate metabolic processes critical to axonal elongation. *Prog. Brain Res.* **71**, 49–60, with permission.

The most currently used preparation

The squid's giant axon is most commonly used for these observations since its axoplasm can easily be extruded and a translucent cylinder of axoplasm devoid of its membrane is thus obtained. This living extruded axon keeps its transport properties for several hours. The absence of plasma membrane allows a precise control of the experimental conditions and entry into the axoplasm of several components that cannot usually pass through the membrane barrier *in vivo* (e.g. antibodies). The improvement of video techniques applied to light microscopy allowed the first observations of the movement of a multitude of small particles along the microtubules in a living extruded axon.

Identification of the moving organelles and their substrates

Analysis of the particles that accumulate on each side of the 1.0–1.5-mm long isolated frozen segments of the squid axon has permitted the identification of moving organelles in axons. Correlation between video and electron microscopy images of these axonal segments has shown that the particles moving anterogradely on video images are small vesicles. Indeed, when a purified fraction of small labelled vesicles (with fluorescent dyes) is placed in an extruded axon, these vesicles and also native vesicles are transported essentially in the anterograde direction.

Evidence demonstrating the implication of microtubules in fast anterograde transport came from experiments with antimitotic agents (colchicin, vinblastin) which prevent the elongation of microtubules and block this transport. Finally, video techniques have also demonstrated that the vesicles are associated to microtubules by arms of 25–30 nm length (**Figure 1.11a**).

The role of ATP and kinesin

By analogy with actin–myosin movements in muscle cells, scientists tried to isolate in neurons an ATPase (the enzyme responsible for the hydrolysis of ATP) associated with microtubules and able to generate the movement of vesicles. To demonstrate molecular components responsible for interactions between vesicles and microtubules, the vesicle–microtubule complex system has been reconstituted *in vitro*: isolated vesicles from squid giant axons are added to a preparation of purified microtubules and placed on a glass coverslip. These vesicles occasionally move in the presence of ATP. If an extract of solubilized axoplasm is then added to this system the number of transported vesicles is considerably increased.

In order to determine the factor present in the solubilized fraction responsible for vesicle movement, a non-hydrolyzable ATP analogue has been used: the 5′-adenylyl imidophosphate (AMP-PNP). In the presence of AMP-PNP, the vesicles associate with the microtubules but then stop. In these conditions, vesicles are bound to the microtubules and also, consequently, to the transport factor. When an overdose of ATP is added to this vesicle–microtubule complex isolated by centrifugation, the AMP-PNP is removed and so vesicles are released and the transport factor is solubilized. Kinesin has been thus isolated and purified. It is a soluble microtubule-associated ATPase that couples ATP hydrolysis to unidirectional movement of vesicles along the microtubule. As we have already seen, in axons, all microtubules are oriented, their plus-end being distally located from the cell body. It has been shown that kinesin moves vesicles in one direction only: from the minus-end toward the plus-end. All these results show that kinesin is responsible for anterograde transport. In mammals, kinesin is a homodimer composed of two identical heavy chains associated with two light chains. These form a 80 nm rod-like molecule consisting of two globular head domains (formed by the heavy chains), a stalk domain and a tail domain (formed by the light chains) (**Figure 1.11a,b**). Kinesin is a microtubule-associated protein (MAP) belonging to the family of mechanochemical ATPases. In proposed mechanism models, the arms observed between vesicles and microtubules *in vitro* would be kinesin. The head transiently binds to microtubules whereas the tail would be, directly or indirectly, associated to membranous organelles. The head binds to and dissociates from a microtubule through a cycle of ATP hydrolysis.

The effects of mutations of the kinesin heavy-chain gene (*khc*) on the physiology and ultrastructure of *Drosophila* larval neurons have been studied. Motoneuron activity and corresponding synaptic (junctional) excitatory potentials of the muscle cells they innervate were recorded in control and mutant larvae in response to segmental nerve stimulation. The mutations dramatically reduced the evoked motoneuron activity and synaptic responses. The synaptic responses were reduced even when the terminals were directly stimulated. However, there was no apparent effect on the number of axons in the nerve bundle or the number of synaptic vesicles in the nerve terminal cytoplasm. These observations show that kinesin mutations impair the function of action potential propagation and neurotransmitter release at nerve terminals. Thus kinesin appears to be required for axonal transport of material other than synaptic vesicles: for example, vesicles containing ion channels such as Na^+ channels delivered to Ranvier nodes and Ca^{2+} channels delivered to presynaptic membranes. These vesicles, called ‘cargoes’, are linked to kinesin. The observation that mutation of kinesin heavy chain had no effect on the number of synaptic vesicles within nerve terminals would obviously not be expected if conventional kinesin were the universal anterograde axonal transport motor.

Plus-end vesicle motors

Since the original discovery of kinesin, a large family of proteins (kinesin superfamily proteins or KIFs) with homology to kinesin’s motor domain has been discovered. The kinesin superfamily is a large gene family of microtubule-dependent motors with 45 members identified at present in mice and humans. The 45 murine and human KIF genes have been classified

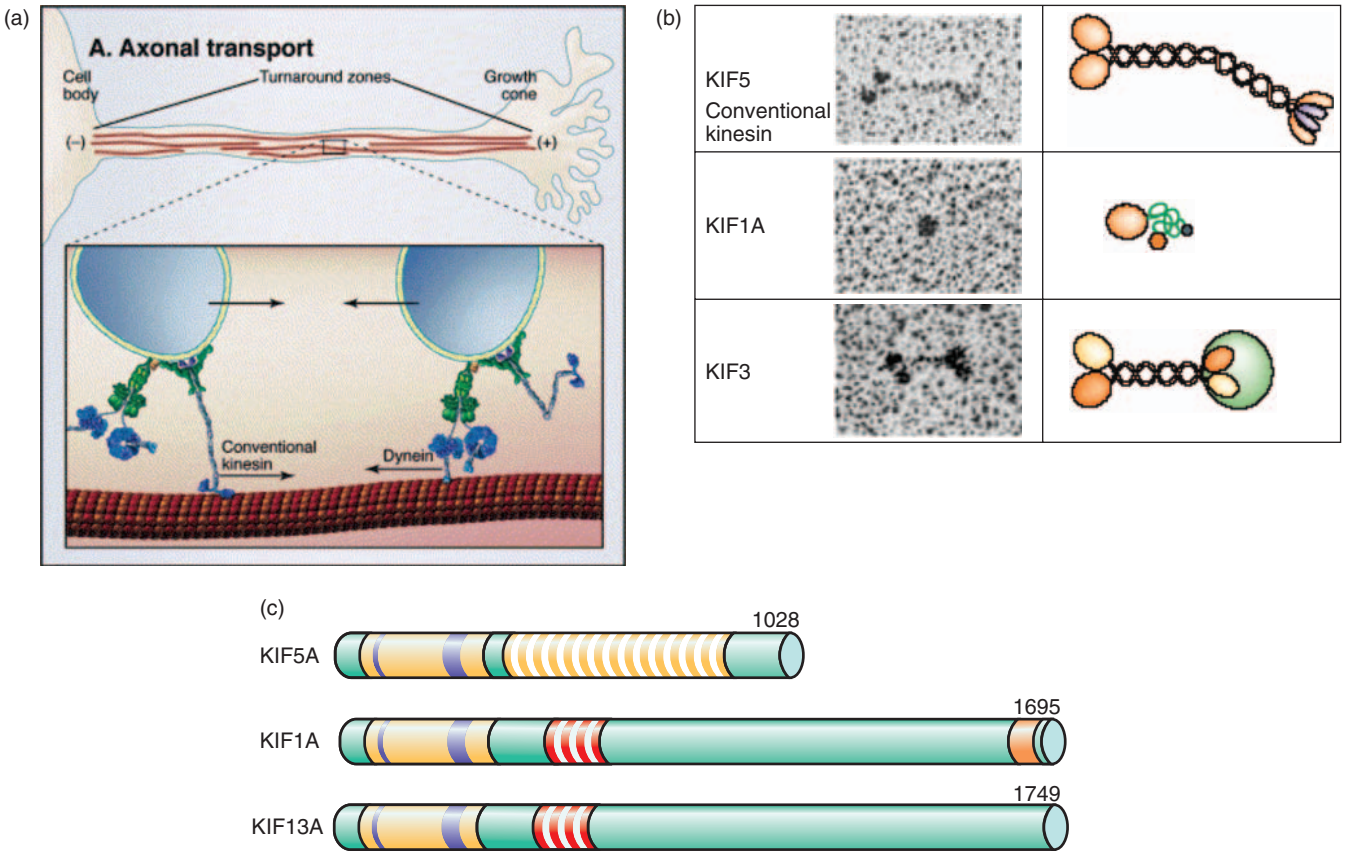

FIGURE 1.11 The motors of fast anterograde and retrograde axonal transport.
(a) Kinesin motors carry cargo (membrane organelles in the axon) along a unipolar array of microtubule towards the plus-ends. Dynein is carried along with this anterograde cargo in a repressed form, and reversals in the direction of movement are infrequent. At a 'turnaround' zone at the tip of these structures, dynein would be activated and kinesin repressed, and the processed cargo then can be transported back towards the cell body. The opposite activation/inactivation of the motors is believed to occur at the base near the cell body. **(b)** Some members of kinesin superfamily proteins (KIFs) observed by low angle rotary shadowing (left column). Diagrams, constructed on the basis of electron microscopy or predicted from the analysis of their primary structures, are shown on the right (the larger orange ovals in each diagram indicate motor domains). KIF5 (orange) forms a homodimer and kinesin light chains (blue) associate at the carboxyl C-terminus to form fanlike ends. KIF1A is monomeric and globular. KIF3 forms heterodimers. (**c**) The domain structures of the same KIFs. The motor domains are shown in orange, the ATP-binding consensus sequence by a thin purple line, the microtubule-binding consensus sequence by a thick purple line, the dimerization domains by yellow stripes, the forkhead-associated domains by red stripes and pleckstrin homology domains by orange stripes. The number of amino acids in each molecule is shown on the right. All these KIFs have their motor domains in the amino (N) terminus and are therefore N-kinesins. Part (a) adapted from Vale RD (2003) The molecular motor toolbox for intracellular transport. *Cell* **112**, 467–480, with permission. Part (b) adapted from Hirokawa N (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science* **279**, 519–526, with permission. Part (c) adapted from Hirokawa N and Takemura R (2005) Molecular motors and mechanisms of directional transport in neurons. *Nat. Rev. Neurosci*. **6**, 201–214, with permission.

into three types on the basis of the positions of their motor domains: the amino (N)-terminal motor, middle motor and carboxy (C)-terminal motor types (referred to as N-kinesins, M-kinesins and C-kinesins, respectively). All KIFs have a globular motor domain that shows high degrees of homology and contains a microtubule-binding sequence and an ATP-binding sequence, but, outside the motor domain, each KIF has a unique sequence (**Figure 1.11b,c**). The diversity of these cargo-binding domains explains how KIFs can transport numerous different cargoes. Kinesins have either a monomeric (KIF1A, KIF1B), a homodimeric (KIF5, KIF2) or a heterodimeric (KIF3B and KIF3C with KIF3A) structure. The 'classical kinesin' corresponds to KIF5. Many KIFs are expressed primarily in the nervous system, but KIFs are also expressed in other tissues and participate in various types of intracellular transport.

Most KIFs are plus-end motors that transport cargoes from the minus-end of microtubules toward their plus-end, i.e. from the cell body toward axon terminals. The motor domain is necessary and sufficient for ATP-driven movement along microtubules. The hypothesis is that each KIF member is targeted to a specific cargo population, allowing the trafficking of the different neuronal compartments to be regulated independently. While there is some functional redundancy among members of the kinesin superfamily, there is also a remarkable degree of cargo specialization. Many members of the kinesin superfamily have been identified as motors for specific cellular cargoes.

1.3.3 Retrograde axonal transport returns old membrane constituents, trophic factors, exogenous material to the cell body

Retrograde axonal transport allows debris elimination and could represent a feedback mechanism for controlling the metabolic activity of the soma. The vesicles or cargoes transported retrogradely are larger (100–300 nm) than those transported anterogradely. Structurally they are prelysosomal structures, multivesicular or multilamellar bodies (**Figure 1.10**). In the squid extruded axoplasm, vesicles move on to each filament in both directions and frequently cross each other without apparent collisions or interactions.

Do filaments used for the fast transport of vesicles form a complex made up of several distinct filaments where certain filaments would be implicated in fast anterograde and others in retrograde transport? By using a monoclonal antibody raised against α-tubulin (a specific component of microtubules) it has been demonstrated that all the filaments implicated in anterograde or retrograde axonal transport contain α-tubulin. Moreover, by using a toxin-binding actin (and so consequently binding microfilaments) it was shown that filaments used for fast anterograde transport or retrograde transport were devoid of actin in their structure. Thus it appeared that filaments used for the movement of vesicles in both directions are microtubules.

The minus-end motor(s)

Morphometric analysis of the arms between retrograde vesicles (pluricellular bodies) and microtubules demonstrated that these are similar to arms between anterograde vesicles and microtubules. Studies looking to find a factor different from, but homologous to, kinesin and responsible for retrograde transport were undertaken. This factor present in axoplasm homogenate might be lost during kinesin purification procedures since no retrograde vesicles movement was observed *in vitro* with kinesin. Cytoplasmic dynein (also called MAP1C) has been thus isolated. It is a microtubule-associated protein with an ATPase activity (see **Figures 1.11a** and **1.12**).

Cytoplasmic dynein is a large and complex molecule, composed of two heavy chains, and multiple intermediate, light intermediate, and light chains to yield a 2 million Da protein complex. The two heavy chains, each around 500 kDa, fold to form globular heads on relatively flexible stalks that dimerize at their ends. Most of the other subunits of the complex are associated with the base of the molecule. Each of the globular heads forms a motor domain, while the base of the complex functions primarily to bind to an associated protein complex, dynactin, as well as participating in direct or indirect associations with cargo (**Figure 1.12**).

In vitro, cytoplasmic dynein alone is sufficient to drive microtubule gliding. However, within the cell a second protein complex, dynactin, is required for most of cytoplasmic dynein's motile functions (**Figure 1.12**). Dynactin is also a large protein complex (1 MDa) with a distinct structure, composed of 11 distinct subunits.

What mechanism regulates the direction of vesicle movement?

It can be hypothesized that kinesin and dynein are bound to only one type of vesicle, specific receptors present at their surface recognizing only one of the two motors. Or both motors might be located on the different vesicles, and by a regulation mechanism only one type is active and so transport takes place in only one direction. Anterogradely transported vesicle populations isolated from squid axoplasm have been shown to carry both motors, a kinesin and dynein. Therefore, during anterograde movement dynein would be repressed whereas kinesin would be repressed during retrograde movement (**Figure 1.11a**).

Functions of retrograde transport

The removal of misfolded or aggregated protein is a key problem in neurons. Cytoplasmic dynein's role as a retrograde motor makes it an ideal candidate for 'taking out the trash' in the cell, returning misfolded or degraded proteins from the cell periphery to the cell centre for recycling and/or degradation. Evidence for such a role has come from the analysis of aggresome formation, in which the formation of perinuclear aggregates of misfolded protein was found to be dynein-dependent. Further, dynein has been implicated as the motor driving vesicles from late endosomes to

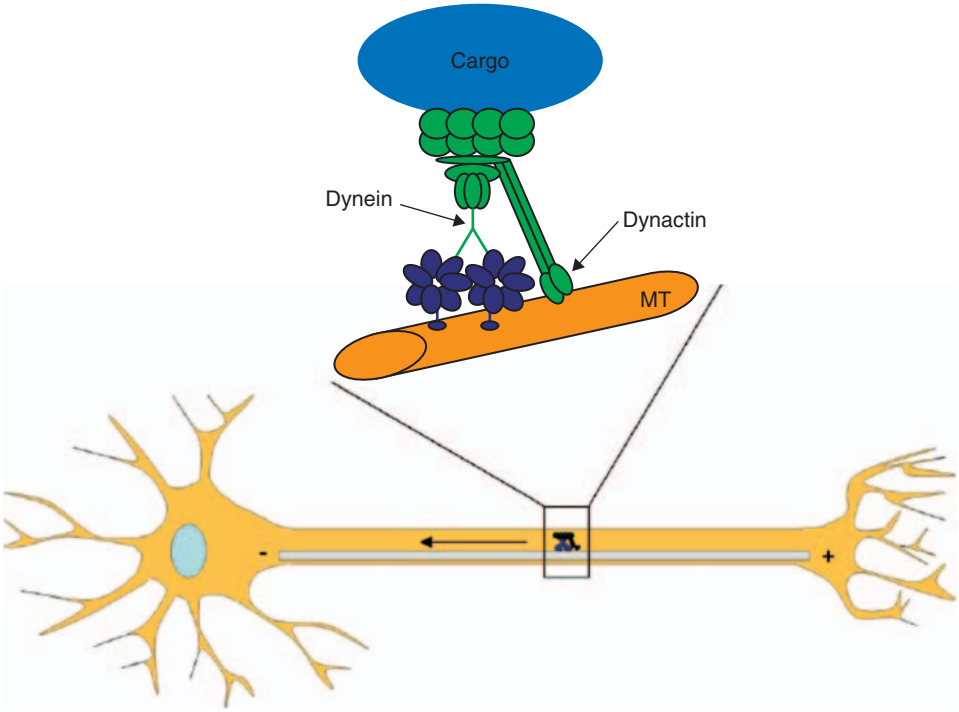

FIGURE 1.12 Cytoplasmic dynein and dynactin drive retrograde axonal transport in motor neurons. Dynein and its activator dynactin are critical for the transport of neurotrophins and material targeted for degradation from the distal regions of the neurons, including the synapse, to the cell body. This transport occurs along microtubules (MT), which are oriented with their minus ends (-) towards the cell body and their plus ends (+) toward the synapse. Cytoplasmic dynein and dynactin are large, multimeric protein complexes that function together to produce minus-end-directed motility. Adapted from Levy R and Holzbaur LF (2006) Cytoplasmic dynein/dynactin function and dysfunction in motor neurons. *Int. J. Devl. Neurosci.* **24**, 103–111, with permission.

lysosomes, as well as driving the minus end-directed motility of lysosomes along microtubules. Retrograde axonal transport allows the return of membrane molecules to cell bodies, where they are degraded by acidic hydrolases found in lysosomes.

Retrograde axonal transport is not only a means of transporting cellular debris for their elimination, but also a way of communicating information from the axon terminals to the soma. The retrogradely transported molecules would inform the cell body about activities taking place at the axon terminal level, or they may even have a neurotrophic action on the neuron. One key role for cytoplasmic dynein and dynactin in neurons is in retrograde signalling, specifically the transport of neurotrophic factors from synapse to cell body. Neurotrophins are a family of small molecules, such as the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), and the neurotrophic factor NT3, that are secreted by target tissues, and then bind to receptor tyrosine kinases (Trk receptors) on the surface of the neuron. The neurotrophin/Trk receptor complex is then internalized (taken up by endocytosis) transported to the cell body where it initiates signaling cascades that regulate cell growth and survival.

Moreover, it allows the transport of tetanus toxin or cholera toxin macromolecules that are taken up by axon terminals and have a toxic effect on the cell body. These toxins, as well as horseradish peroxidase (HRP), an enzyme taken up by the axon terminals, are used in research studies for the retrograde labelling of neuronal pathways.

In conclusion, cargoes are transported in either the antero- or retrograde direction, depending on whether plus- or minus-end motors are active on their surface. Cargoes destined for the nerve terminal, such as synaptic vesicles or their precursors, are transported by plus-end motors; while cargoes targeted for the cell body, such as vesicles containing neurotrophin-receptor complexes, are transported by minus-end motors. In axons, oriented microtubules establish a 'road map' inside the neuron to motors that are linked to particular intracellular cargoes.

1.3.4 Slow anterograde axonal transport moves cytoskeletal proteins and cytosoluble proteins

The cytoskeleton (microtubules, neurofilaments and microfilaments) and cytosolic proteins (intermediate metabolic enzymes including glycolysis enzymes) are transported anterogradely along axons at a slow rate of about 0.002–0.1 μm/s (0.17–8.6 mm/day). In the elongating axon (i.e. during development or regeneration) the function of the slow transport is to supply axoplasm required for axonal growth. In mature neurons its function is to renew continuously the total proteins present in the axon and axon terminals and to act as a substrate for the anterograde and retrograde axonal transport. To appreciate fully the structural achievement of this transport, one must put the size of cell bodies and axons into relation. The neuronal cell bodies (10–50 μm diameter) are connected by axons that can be over 1 m length (the axonal diameter is 1 to 25 μm). This is a factor of 100,000 difference. As there is relatively little protein synthesis in the axon, the proteins that comprise the microtubules, neurofilaments and microfilaments must be actively transported from the cell body into and down the length of the axon.

To understand the mechanisms involved in slow axonal transport, several questions can be raised: (i) in which state are cytoskeletal proteins transported in the axons: as soluble proteins or as polymers? (ii) in which axonal region(s) is the cytoskeleton (i.e. the complex network of filaments) assembled? The following are, in chronological order, the diverse hypotheses that have been proposed:

The different cytoskeletal elements are assembled and connected by bridges in the cell body

They then progress as a whole (a matrix) in the axon. However, studies have demonstrated that crossbridges between the different cytoskeletal elements are weak and unstable. Moreover, numerous cytoskeletal discontinuities exist along the axon as seen in the nodes of Ranvier. Thus, the hypothesis of the continuous transport of a stable matrix of assembled cytoskeletal elements explaining the ultrastructure of the axon is now known to be false.

The cytoskeletal proteins are transported in a soluble form or as isolated fibrils and assembled during their progression

Lasek and his colleagues proposed that the microtubules and other cytoskeletal elements in slow transport are moved as polymer by sliding. When they are assembled some become stationary and would be renewed onsite. This hypothesis came from pulse-labelling studies and particularly those coupled with photobleaching experiments. Purified subunits of cytoskeletal proteins (tubulin or actin) coupled to a fluorescent dye molecule are introduced into living neurons in culture by injection into their soma. The observation with fluorescent microscopy shows that these labelled subunits are gradually incorporated into the polymer pool of the corresponding cytoskeletal proteins (microtubules and microfilaments) throughout the axon. A highly focused light source is then used to extinguish or bleach the fluorescence of the molecules contained within a discrete axonal segment (about 3 μm long). The fate of the bleached zone is followed over a period of hours. The bleached zone does not move along the axon or widen and recovers a low level of fluorescence within seconds. This latter effect is ascribed to the diffusion of free fluorescent subunits from the neighbouring fluorescent regions into the bleached region. These observations suggested that microtubules and microfilaments are essentially stationary and are exchanging subunits.

The transport of microtubules and neurofilaments is bidirectional, intermittent, asynchronous, and occurs at the fast rate of known motors

However, when Wang and Brown widened the parameters of the live-cell imaging paradigm, such that a much longer bleached zone (about 30 μm in length) was created, and the zone was imaged every several seconds rather than minutes, they found that the transport of microtubules is bidirectional, intermittent, highly asynchronous, and at the fast rates of known motors (average rates of 1 μm/s) such as cytoplasmic dynein and the kinesin superfamily. These observations indicate that microtubules are propelled along axons by fast motors. The average moving microtubule length is around 3 μm.

The rapid, infrequent, and highly asynchronous nature of the movement may explain why the axonal transport of tubulin has eluded detection in so many other studies. In addition, these results offer an explanation for the slow rate of tubulin transport documented in the early kinetic studies: it reflects an average rate of fast movements and non-movements. The overall rate of microtubules movement is slow because the microtubules spend only a small proportion of their time moving.

Similarly, the initial studies of neurofilaments transport using radiographic labelling suggested a velocity of 0.25–3 mm/day which is slower than any speed produced by known molecular motors. Recent studies using green-fluorescent-protein (GFP)-tagged neurofilament

subunits and real-time confocal microscopy show more accurately that the conventional fast axonal transport also applies to neurofilaments. Peak velocities of 2 μm/s occur anterogradely and retrogradely and are interrupted by prolonged resting phases resulting in the overall slow transport originally described.

1.3.5 Axonal transport of mitochondria allows the turnover of mitochondria in axons and axon terminals

Mitochondria are prominent members of the cast of axonally transported organelles. They are essential for the function of all aerobic cells, including neurons. They produce ATP, buffer cytosolic calcium and sequester apoptotic factors. Like many other neuronal organelles, mitochondria are thought to arise mainly in the neuronal cell body, but their transport is distinctive. In postmitotic neurons, mitochondria are delivered to and remain in areas of the axon where metabolic demand is high, such as initial segments, nodes of Ranvier and synapses. How do mitochondria achieve these distributions in the axon?

The mitochondria recently formed in the cell body are transported anterogradely in axons up to axon terminals at a rate of 10–40 mm per day. A retrograde movement of mitochondria showing degenerative signs is also observed (see **Figure 1.10b**). Specific inhibition of kinesin-1 stops most mitochondrial movement in *Drosophila melanogaster* motor axons. Also time-lapse imaging of GFP-tagged mitochondria in *Drosophila* axons has shown that kinesin-1 mutations cause a profound reduction in the retrograde transport of mitochondria. In addition, the plus-end motor KIF1Bα has been shown to be associated with mitochondria with subcellular fractionation, and purified KIF1Bα can transport mitochondria along microtubules *in vitro*. Thus, kinesin-1 (KIF5A, KIF5B, and KIF5C) and KIF1Bα (**Figure 1.11b**) transport mitochondria in the anterograde direction.

1.4 NEURONS CONNECTED BY SYNAPSES FORM NETWORKS OR CIRCUITS

1.4.1 The circuit of the withdrawal medullary reflex

Sensory stimuli (including visual, auditive, tactile, gustative, olfactive, proprioceptive, and nociceptive stimuli) are detected by specific sensory receptors and transmitted to the central nervous system (encephalon and spinal cord) by networks of neurons. These stimuli are analyzed at the encephalic level. They can also evoke movements such as motor reflexes on their way to higher central structures.

Thus, when a noxious stimulus (i.e. a stimulus provoking tissue damage, for example pricking or burning) is applied to the skin of the right foot, it induces a withdrawal reflex consisting of the removal of the affected foot (contraction of flexor muscles of the right inferior limb) to protect itself against this stimulus. The noxious stimulus activates nociceptors which are the peripheral endings of primary sensory neurons whose cell bodies are located, in this case, where injury is located at the body level – in dorsal root ganglia. Action potentials are then generated (or not, if the intensity of the noxious stimulus is too small) in primary sensory neurons and propagate to the central nervous system (spinal cord). Local circuit neurons of the dorsal horn of the spinal cord (**Figure 1.13a**) relay the sensory information. Sensory information is thus transmitted to motoneurons (neurons innervating skeletal striated muscles and located in the ventral horn) through a complex network of local circuit neurons (Golgi type II neurons) which have either an excitatory or an inhibitory effect. It results on the stimulus side (ipsilateral side) in an activation of the flexor motoneurons (F) and an inhibition of the extensor motoneurons (E): the right inferior limb is being withdrawn (is in flexion). The opposite limb is extended to maintain posture.

This pathway illustrates peculiarities present in numerous other circuits.

- *Divergence of information*. Primary sensory information is distributed to several types of neurons in the medulla: local circuit neurons connected to motoneurons that innervate posterior limb muscles and also projection neurons that relay sensory informations to higher centres where they are analyzed.
- *Convergence of information*. Motoneurons receive sensory informations via local circuit neurons and also descending motor information via descending neurons whose cell bodies are located in central motor regions (motor commands elaborated at the encephalic level) (**Figure 1.13a**).
- *Anterograde inhibition* (feedforward inhibition). A neuron inhibits another neuron by the activation of an inhibitory interneuron (**Figure 1.13b**).
- *Recurrent inhibition* (feedback inhibition). A neuron inhibits itself by a recurrent collateral of its own axon which synapses on an inhibitory interneuron. The inhibitory interneuron establishes synapses on the motoneuron (**Figure 1.13c**). This recurrent inhibition allows for rapid cessation of the motoneuron's activity.

The last two circuits described are also called *microcircuits*, since they are included in a larger circuit or *macrocircuit*. In this selected example, all the neurons

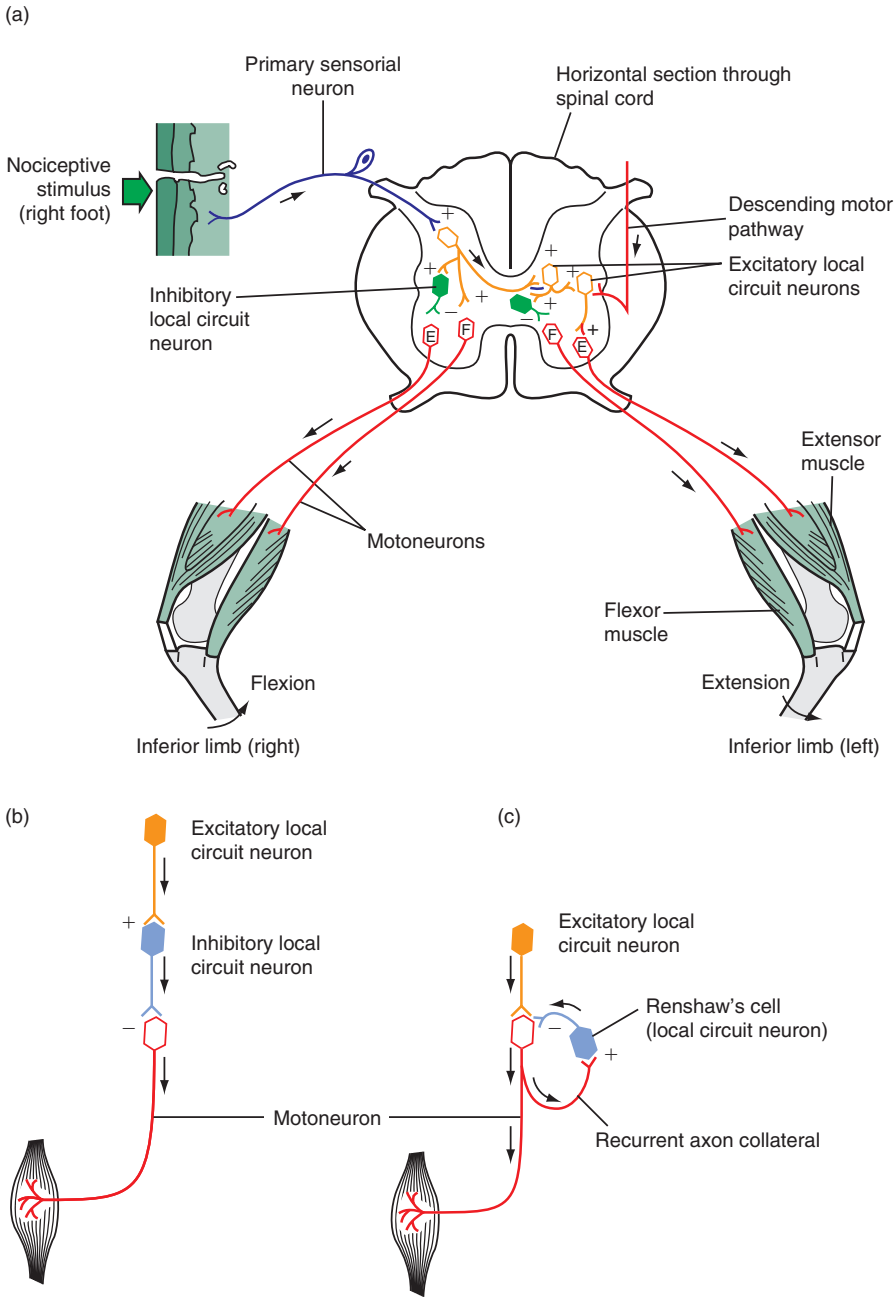

FIGURE 1.13 Withdrawal medullary reflex pathway.
(a) Schematic of a horizontal section through the spinal cord and of connections between a primary nociceptive sensory neuron, medullary local circuit neurons and ipsi- and contralateral motoneurons innervating inferior limb muscles. See text for details. **(b)** Anterograde inhibitory circuit. **(c)** Recurrent inhibitory circuit. Arrows show the direction of action potential propagation.

forming the microcircuit enable precise regulation of motoneuron activity.

1.4.2 The spinothalamic tract or anterolateral pathway is a somatosensory pathway

Noxious stimuli (temperature and sometimes touch) are detected at the skin level by free nerve endings, are transduced (or not) in action potentials and are conveyed to the somatosensory cortex via relay neurons. Information from the body reaches the dorsal horn neurons of the spinal cord, and information from the face reaches the trigeminal nuclei in the brainstem, via primary sensory neurons whose cell bodies are located in dorsal root ganglia or cranial ganglia, respectively. They relay on projection neurons located in dorsal horns or in

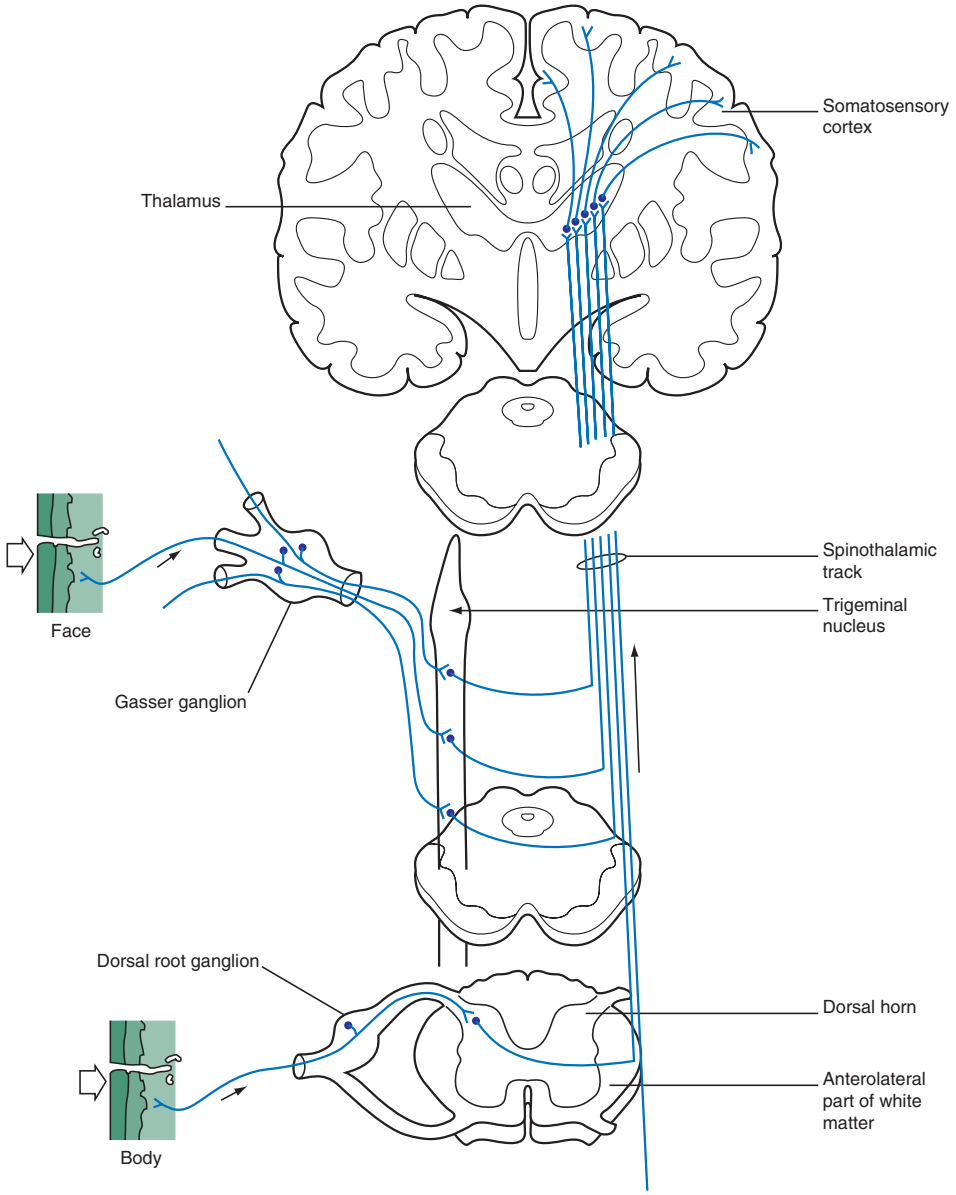

FIGURE 1.14 The spinothalamic tract or anterolateral ascending sensory pathway.
This pathway integrates and conveys sensory information such as nociception, temperature and some touch. Bottom to top: horizontal sections through the spinal cord, the pons and frontal section through the diencephalon. See text for explanations.

trigeminal nuclei which send axons to the thalamus. These axons cross the midline, form a tract in the anterolateral part of the white matter, and terminate in non-specific thalamic nuclei. Thalamic neurons then send the sensory information to cortical areas specializing in noxious perception (somatosensory cortex). At each level of synapses (dorsal horn or trigeminal nucleus, thalamus, cortex) the somatosensory information is not simply relayed, it is also processed through local microcircuits receiving afferent sensory information and descending information from higher centres which modulate incoming sensory information.

When superposing horizontal sections through the spinal cord (**Figures 1.13a** and **1.14**), it becomes clear that a noxious stimulus applied to the skin of the right inferior limb is transmitted to motoneurons where it can evoke a withdrawal reflex and also reaches the somatosensory cortex where it is analyzed. The reflex

is evoked before the consciousness of the stimulus because of the longer distance to brain areas than to the ventral horn of the spinal cord.

1.5 SUMMARY: THE NEURON IS AN EXCITABLE AND SECRETORY CELL PRESENTING AN EXTREME FUNCTIONAL REGIONALIZATION

This chapter has described how the various functions of neurons, such as their metabolism, excitability and secretion, are localized to specific regions of the neuron. The main neuronal compartments are the dendrites (more precisely postsynaptic sites), soma, axon and axon terminals (more precisely presynaptic sites). These regions are sometimes located at great distances from each other, and so neurons have to resolve the problems of communication between these regions and harmonization of their activities.

Regionalization of metabolic functions

The essential synthesis activity of a neuron is localized in its cell body, since dendrites can synthesize only some of their proteins, and axons are able to synthesize only a few. In this cell, where the axon's volume represents up to a thousand times the volume of the cell body, the structural and functional integrity of the axon and its terminals requires an important and continuous supply of macromolecules. This supply is ensured by anterograde axonal transport. In dendrites, RNA transport from the cell body to the polysomes has been demonstrated and would allow the synthesis of some of their proteins.

The degradation of cellular metabolism debris and non-neuronal elements taken up from the external environment by endocytosis (e.g. uptake of viruses) takes place in the lysosomes of the cell body. They are transported from axon terminals to the cell body via the retrograde axonal transport. Finally, to coordinate synthesis activity in the cell body with the needs of the axon terminals, the existence of a feedback mechanism (from terminals to cell body) seems essential. This could take place through retrograde axonal transport.

Anterograde transport moves newly synthesized material outward from the cell body along the axon. Retrograde transport drives the movement of organelles, vesicles, and signalling complexes from the cell periphery and distal axon back to the cell centre. Key motors for this transport include members of the kinesin superfamily and cytoplasmic dynein.

Regionalization of functions implicated in reception and transmission of electrical signals

The neuronal regions receiving synapses are mainly the dendritic (primary segments, branches and spines of dendrites) and somatic regions, but also some axonal regions. These receptive regions, called postsynaptic elements, have a restricted surface. They contain, within their plasma membrane, proteins specialized in the recognition of neurotransmitters: the neurotransmitter receptors (receptor channels and receptors coupled to G proteins). These proteins synthesized in the cell body are then transported toward the dendritic, somatic or axonal postsynaptic membranes to be incorporated. Similarly, the proteins specialized in the generation and propagation of action potentials (voltage-dependent channels) are synthesized in the soma and have to be transported and incorporated in the axonal membrane.

Regionalization of secretory function

This function is localized in regions making synaptic contacts and more generally in presynaptic regions such as axon terminals (and sometimes in dendritic and somatic regions). At the level of presynaptic structures, the neurotransmitter is stocked in synaptic vesicles and released. The secretory function implicates the presence of specific molecules and organelles in the presynaptic region: neurotransmitter synthesis enzymes, synaptic vesicles, microtubules and associated proteins, voltage-dependent channels, etc.

In conclusion, owing to its extreme regionalization and the extreme length and volume of its processes, the neuron has the challenge to deliver the proteins synthesized in the soma at the appropriate sites (targeting) at appropriate times.

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