Dendrite structure

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Summary

Dendrites extend from the cell body of the neuron and are specialized for processing synaptic information. Dendritic arbors assume diverse forms, branching in characteristic spatial domains where they receive specific synaptic inputs. Synaptic inputs occur directly on the shaft of some dendrites, but others have dendritic spines or specialized enlargements that host synapses. These specializations also occur in many different forms related to both local connectivity and the need for compartmentalization of molecular signaling. The use of three-dimensional reconstructions from serial section electron microscopy (3DEM) has shown that these dendritic synaptic specializations differ widely in dimensions, distribution, and intracellular composition. The shape and composition of dendrites and their synaptic specializations are influenced throughout life by genes, environment, learning, memory, and neuropathological conditions. Therefore, understanding the structure of dendrites is essential to understanding their function.

Introduction

What is the purpose of dendrites? How are their diverse shapes related to neuronal function? Why are specialized compartments formed at the sites of synaptic contacts? The Spanish neuroanatomist Santiago Ramón y Cajal posed, and to a remarkable extent answered, these questions more than 100 years ago, as summarized in his compendium *Histology of the Nervous System of Man and Vertebrates* (Ramón y Cajal, 1995). He used a variety of experimental approaches, especially the technique developed by the Italian neuroanatomist Camillo Golgi to stain individual neurons. From this work, he proposed that axons and dendrites do not anastomose in continuity between different neurons (Ramón y Cajal, 1954) as was originally suggested by Golgi (Golgi, 1908). The importance of the Golgi technique, together with Ramón y Cajal's astute analyses, won them the Nobel prize in physiology or medicine in 1906 (Shepherd, 1991). Their Norwegian contemporary, Fridtjof Nansen, took a comparative approach, also using the Golgi technique to study the structure of the nervous system in diverse organisms from crayfish to hagfish and mammals, to conclude that the complexity of the dendritic processes and the so-called "dotted substance" (neuropil), where axons and dendrites communicate, is "more complicated and extensive the higher an animal is mentally developed" (Nansen, 1887). Similarly, Ramón y Cajal argued that phylogenetic differences in specific neuronal morphologies support the relationship between dendritic complexity and the number of connections. For example, the complexity of dendritic arbors increases with increasingly complex nervous systems on different types of vertebrate neurons, including cerebellar Purkinje cells, cortical pyramidal cells, and mitral cells of the olfactory bulb (see Chapter 2 for more phylogenetic differences). Although the work of Ramón y Cajal favored what became known as the neuron doctrine, namely that communication occurred at junctions between discrete neurons,

definitive proof awaited the advent of the electron microscope and the demonstration of a synaptic cleft between the presynaptic axon and the postsynaptic dendrite (Palade and Palay, 1954; De Robertis and Bennett, 1955).

Modern approaches to the evaluation of the properties of dendrites, axons, and glia provide reasons to reconsider the strictly unidirectional, axo-dendritic patterns of synaptic activation championed in the original neuron doctrine (Shepherd, 1991; Guillery, 2005; Kruger and Otis, 2007). As Ramón y Cajal and Nansen correctly surmised, dendrites usually comprise the receptive surfaces of a neuron, while axons usually deliver signals from other neurons. However, dendrites can also be output devices (see Chapter 21). For example, dendrites make reciprocal synapses having both pre- and postsynaptic components on corresponding pairs of dendrites in the retina, olfactory bulb, lateral geniculate nucleus, some cortical neurons, and some peripheral sensory neurons (Price and Powell, 1970; Lieberman, 1973; Sloper and Powell, 1978; Ellias and Stevens, 1980). Conversely, axons also provide receptive surfaces, forming axo-axonic synapses in many places throughout nervous systems (Cuello, 1983). Furthermore, dendrites, axons, and glia all form gap junctions—bidirectional sites of communication through channels that are arranged so as to span the adjacent membranes and bridge the extracellular space (Bennett and Zukin, 2004). While such dendro-dendritic, axo-axonic, and gap junction-mediated communications are relatively rare compared with axo-dendritic communication, they nevertheless play important roles in both direct neuron-to-neuron communication and modulation of that communication.

More complex dendritic arbors have the potential to host more synapses. A neuron with a roughly spherical cell body has a very limited surface area for receiving inputs. By extending dendrites, the neuron increases its surface area without excessively increasing cell volume. For example, the dendrites of cat spinal motoneurons have a volume of 300,000 μm^3 and provide a surface area of 370,000 μm^2 for synaptic input (Ulfhake and Kellerth, 1981). To provide an equivalent surface area, a spherical cell body would have to have a diameter of 342 μm and a volume of more than 20,900,000 μm^3, a factor of 70 greater than the dendrite-bearing motoneurons.

The convolution of the cell surface into a dendritic arbor also facilitates the packing of a larger number of neurons in close proximity and extends their reach to more axons. However, the expanded dendritic arbor does not necessarily correspond one-to-one with increased synapse density. For example, only 20% of the axons within reach of or touching a dendrite of a hippocampal pyramidal cell actually form a synapse on that dendrite; furthermore, the summed surface area occupied by those synapses is only about 10% of the total dendritic surface area (Harris and Stevens, 1988a, 1989; Mishchenko et al., 2010). Thus, 3DEM findings suggest that enhancing synaptic connectivity is probably not the primary function of dendrites, their spines, and other synaptic specializations (Peters and Kaiserman-Abramof, 1970; Swindale, 1981; Gray, 1982).

Dendrites have a relatively local reach compared with the axon. The axon, emerging either from the soma or from a proximal dendrite, may extend to distant targets a meter or more away from the cell body (e.g., motoneurons and corticospinal projection neurons in large mammals). In contrast, dendrites are rarely longer than 1–2 mm, even on the largest neurons, and are often much smaller (Table 1.1). The diameter of dendrites at their origin from the cell body is proportional to that of the cell body itself. Dendrites taper and ramify in proportion to their size, such that the total length and number of branches are also correlated with dendrite caliber. Thus, larger neurons typically have both larger cell bodies and more extensive dendritic fields. Compare and contrast, for example, the dendritic arbor of a Purkinje cell that integrates input from multiple cell types with the tiny granule cell that typically forms just four small dendritic branches (on average), each ending in a claw that forms synapses (Fig. 1.1).

Table 1.1 Typical dimensions of dendrites for a few types of neuron

Neuron	Average soma diameter (μm)	Number of dendrites at soma	Proximal dendrite diameter (μm)	Number of branch points	Distal dendrite diameter (μm)	Dendrite extent* (μm)	Total dendritic length (μm)
Cerebellar granule cell (cat)	7	4	1	0	0.2–2	15	60
Starburst amacrine cell (rhesus)	9	1	1	40	0.2–2	120	—
Dentate gyrus granule cell (rat)	14	2	3	14	0.5–1	300	3,200
CA1 pyramidal cell (rat)	21						11,900
basal dendrites		5	1	30	0.5–1	130	5,500
stratum radiatum		1	3	30	0.25–1	110	4,100
stratum lacunosum-moleculare				15	0.25–1	500	2,300
Cerebellar Purkinje cell (guinea pig)	25	1	3	440	0.8–2.2	200	9,100
Principal cell of globus pallidus (human)	33	4	4	12	0.3–0.5	1,000	7,600
Meynert cell of visual cortex (macaque)	35						15,400
basal dendrites		5	3	—	—	250	10,200
apical dendrites		1	4	15	2–3	1,800	5,200
Spinal α-motoneuron (cat)	58	11	8	120	0.5–1.5	1,100	52,000

*The average distance from the cell body to the tips of the longest dendrites.

Sources: Palay (1978), Ulfhake and Kellerth (1981), Ito (1984), Yelnik et al. (1984), Mariani (1990), Claiborne et al. (1990), Rapp et al. (1994), Bannister and Larkman (1995a).
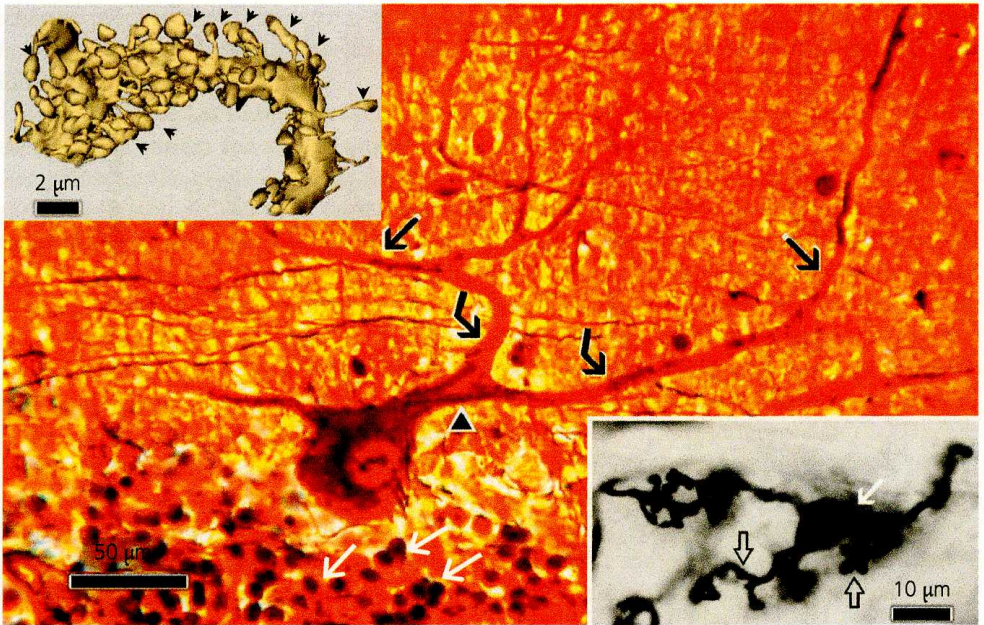
Fig. 1.1 Dendrites often look like tree branches, and their name derives from the Greek word for tree, *dendron*. In this image of the cerebellar cortex of a mouse impregnated with silver using the Bielschowski method, a thick primary dendrite (triangle, main image) extends from the upper right of the cell body of a Purkinje neuron. The primary dendrite branches into secondary (curved arrows) and tertiary (straight arrows) dendrites within the plane of the section. Terminal dendritic branchlets are not visible with this method. In the upper inset 3DEM reveals the high density of protrusions, known as dendritic spines (small arrows). Beneath the large Purkinje cell can be seen a layer of smaller granule cell bodies (white arrows). In the lower inset higher magnification of a Golgi-impregnated granule cell (white arrow) reveals that this neuron possesses just a few, short dendrites ending in claw-like formations (open arrows).

In this chapter we consider the functional significance of the diversity of dendritic arbor structures together with diversity in the structure and subcellular composition of individual dendrites, dendritic spines, and other synaptic specializations. We show that dendrites contain subcellular structures including smooth endoplasmic reticulum, microtubules, endosomes, Golgi apparatus, polyribosomes, and others that can rapidly direct resources to sites of synaptic activation and plasticity. We end with an analysis of ultrastructural changes that occur in synaptic specializations during long-term potentiation, a cellular mechanism of learning, and various neuropathological conditions.

Dendritic arbors

Both the geometry (Fig. 1.2 and Table 1.2) and the density of dendritic branching (Figs 1.2 and 1.3) define the dendritic arbor and are important for understanding connectivity in the nervous system. Dendritic arbors are shaped to receive inputs in particular spatial domains. At one extreme, a dendritic arbor connects a single remote target to the rest of the neuron, providing a highly
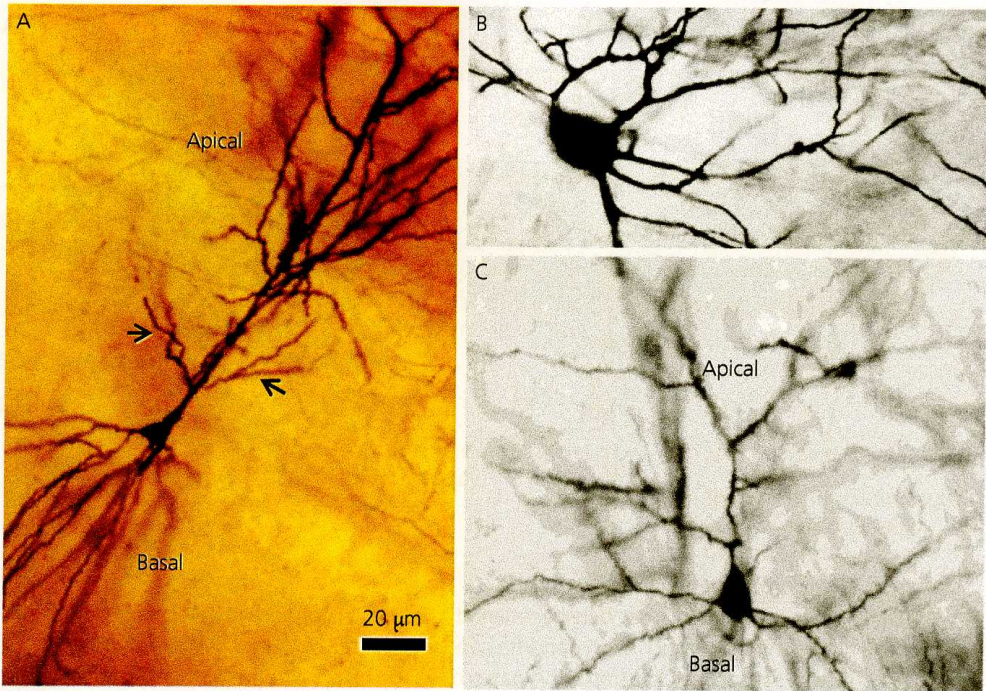
Fig. 1.2 Neurons impregnated with silver by the Golgi method. (**A**) A pyramidal neuron from hippocampal area CA1 of the rat, in which basal and apical dendrites ramify in separate conical domains. Between these two denser domains, a few dendrites extend obliquely (arrows) from the apical dendrite as it passes through stratum radiatum. (**B**) A rat thalamic projection neuron exhibits many primary dendrites extending from the cell body with a spherical radiation. (**C**) A pyramidal neuron from the parietal area of mouse cerebral cortex has a sparsely branching apical dendrite and a few dendrites that extend almost horizontally from the base of the cell body. All three images have been scaled to match the magnification and scale bar in A.

selective arborization. At the other extreme, dendritic branches occupy most of the domain in a *space-filling* arborization. The majority of dendritic arborizations lie between these extremes and are considered to be *sampling* arborizations. Here we describe various geometrical patterns as a basis for understanding the consequences of diversity in dendritic arborization on synaptic connectivity.

Adendritic neurons have no dendrites and usually have a single branched axon arising from the soma (Table 1.2). Examples of adendritic neurons occur in the trigeminal, nodose, and dorsal root ganglia. These neurons conduct sensory input from the periphery to the central nervous system. Although GABAergic and glutamatergic axons have been found to surround the soma of these
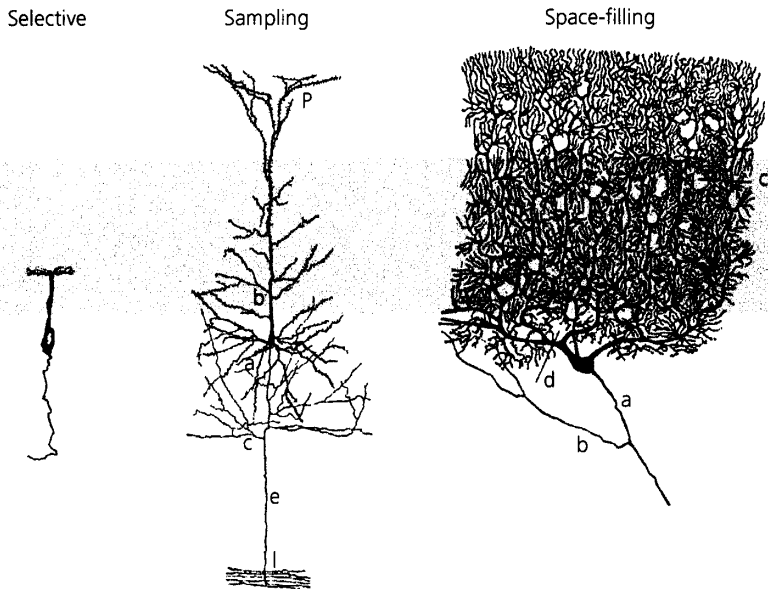
Selective   Sampling   Space-filling

Fig. 1.3 Differences in dendritic arbor density reflect differences in connectivity. At one extreme are selective arborizations in which each dendrite connects the cell body to a single remote target. An olfactory sensory cell illustrates this pattern. At the other extreme lie the space-filling arborizations in which the dendrites cover a region, as with the cerebellar Purkinje cell. Intermediate arbor densities are referred to as sampling arborizations, as demonstrated by a pyramidal cell from cerebral cortex.

The material in this figure has been adapted from Ramón y Cajal's *Histology of the Nervous System of Man and Vertebrates*, translated by Neely Swanson and Larry W. Swanson pp. 52–4, figures 7, 8, and 9 © 1995, Oxford University Press.

adendritic neurons (Sholl, 1953, 1967; Stoyanova et al., 1998), to our knowledge no electron microscopy studies have been done to determine whether these processes make synapses on them.

Dendritic arbors of many slender neurons found throughout the brain have a *spindle radiation*, with two sparsely branching dendrites emerging from opposite poles of the soma. Examples include Lugaro cells of the cerebellar cortex (Palay and Chan-Palay, 1974) and bipolar cells of the cerebral cortex, which are usually interneurons expressing calretinin in combination with neuropeptides (Cauli et al., 2014). This arborization would perform sparse sampling of two distinct input domains.

Stellate neurons of the central nervous system have a dendritic arbor with a *spherical* or *partial spherical radiation* depending on their position with respect to the boundaries of the region in which they reside. The dendrites of the cerebellar granule cells have spherical radiations that make synapses selectively at their claw-like ends (Fig. 1.1). Other examples include spinal cord neurons and principal neurons in non-laminated nuclei such as the inferior olive, pontine nuclei, striatum, and thalamus. Interneurons are defined as having axons that terminate locally within a particular brain region, and many interneurons have dendritic arbors with spherical radiations. Although attempts have been made to describe in general terms the variety of stellate arbor types

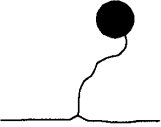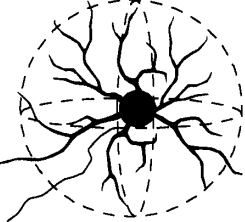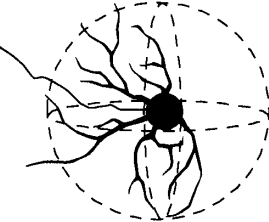**Table 1.2** Some characteristic dendritic arborization patterns
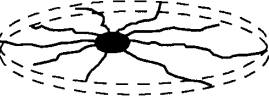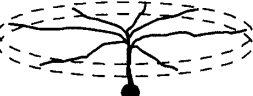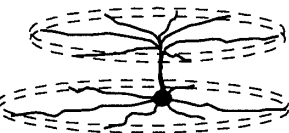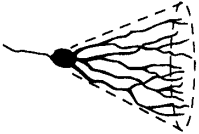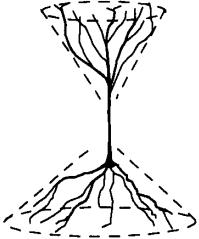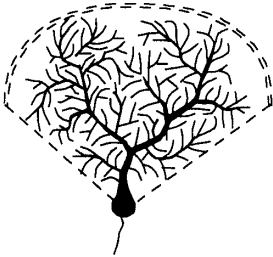
Pattern	Characteristics	Examples
Adendritic		
	Cell body lacks dendrites	Dorsal root ganglion cells
		Sympathetic ganglion cells
Spindle radiation		
	Two dendrites emerge from opposite poles of the cell body and have few branches	Lugaro cells
		Bipolar cells of cortex
Spherical radiation		
Stellate		
	Dendrites radiate in all directions from cell body	Spinal neurons
		Neurons of subcortical nuclei (e.g., inferior olive, pons, thalamus, striatum)
		Cerebellar granule cells
Partial		
	Dendrites radiate from cell body in directions restricted to a part of a sphere	Neurons at edges of "closed" nuclei (e.g., Clarke's column, inferior olive, vestibular nuclei)
Laminar radiation		
Planar		
	Dendrites radiate from cell body in all directions within a thin domain	Retinal horizontal cells
Offset		
	Plane of radial dendrites offset from cell body by one or more stems	Retinal ganglion cells
Multi		
	Cell has multiple layers of radial dendrites	Retinal amacrine cells

Table 1.2 (continued) Some characteristic dendritic arborization patterns

Pattern	Characteristics	Examples
Cylindrical radiation		
	Dendrites ramify from a central soma or dendrite in a thick cylindrical (disk-shaped) domain	Pallidal neurons
		Reticular neurons
Conical radiation		
	Dendrites radiate from cell body or apical stem within a cone or paraboloid	Granule cells of dentate gyrus and olfactory bulb
		Primary dendrites of mitral cells of olfactory bulb
		Semilunar cells of piriform cortex
Biconical radiation		
	Dendrites radiate in opposite directions from the cell body	Bitufted, double bouquet, and pyramidal cells of cerebral cortex
		Vertical cells of superior colliculus
Fan radiation		
	One or a few dendrites radiate from cell body in a flat fan shape	Cerebellar Purkinje cells

(Ramón-Moliner, 1968), classification often comes down to individual characteristics. For example, in the ventral cochlear nucleus, descriptive morphologies of the dendritic arbor include spherical bushy, globular bushy, stellate, bushy multipolar, elongate, octopus, and giant (Ostapoff et al., 1994). These descriptors are not readily applicable to stellate neurons in other areas of the brain where, for example, in the cerebral cortex the primary distinguishing characteristic of the many stellate interneurons is not their dendritic arbors but rather their axonal arborization pattern (Jones, 1975). Overall, the morphology of stellate dendritic arbors suggests that they perform selective or sparse sampling of axonal inputs that congregate locally within the neuropil of a circumscribed region.

Dendritic arbors with a *laminar radiation* spread in arbitrary directions from the cell body but are restricted to a *planar* region, as seen, for example, in horizontal cells of the retina (Kolb et al., 1994) and in some interneurons of the hippocampus (Parra et al., 1998). Nearly 20 kinds of retinal ganglion cells are distinguished by their planar dendritic arborization patterns that are *offset* by one or more dendritic stems (Fukuda et al., 1984; Sterling and Demb, 2004; Wingate et al., 1992). These dendrites can ramify into one or more layers to access multiple types of afferents and provide either a selective or sampling arborization that gives rise to distinct physiological functions.

Multi-laminar radiation characterizes the dendritic arborizations of retinal amacrine cells. At least 26 different types of amacrine cell can be identified based on their dendritic arborization and retinal tiling patterns alone (Mariani, 1990; Kolb et al., 1992; MacNeil and Masland, 1998). As with ganglion cells, the morphological differences in the extent of the dendritic arbors of amacrine cells also denote differences in the computational properties of these neurons.

Some dendritic arbors have a strictly *cylindrical radiation*. For example, in the globus pallidus of primates, the dendrites of the large pallidal neurons have a cylindrical radiation that fills spatial domains approximately 1,000–1,500 μm in diameter and 250 μm thick (Yelnik et al., 1984). These dendrites run parallel to the boundaries of the globus pallidus and thus perpendicular to incoming striatal axons, such that each neuron receives a broad distribution of input in their dense-sampling to space-filling arbors.

Many types of granule cells have a dendritic arbor with a *conical radiation*, where the dendrites radiate from one side of the neuron in a conical or paraboloidal fashion. This selective but dense sampling pattern characterizes granule cells in the hippocampal dentate gyrus, for example, where axonal input is strictly layered. Here, the primary excitatory input from the lateral entorhinal cortex synapses in the outer third, input from the medial entorhinal cortex synapses in the middle third, and from the contralateral hippocampus in the inner third of the dendritic arbor (Steward, 1976; Amaral et al., 1990; Claiborne et al., 1990).

The dendritic arbors of pyramidal cells often have two distinct conical arbors, one at the apex and the other at the base of the pyramid-shaped cell body. This configuration corresponds to a *biconical radiation* and may be characterized by different afferents contacting the basal versus apical domains. Furthermore, the length of an apical dendrite of a cortical pyramidal cell depends on how far the cell body is from the outermost layer in which it ramifies its apical tuft. Cells very near the outermost layer do not usually have an apical stem at all, since one is not required to reach the appropriate axons (Ramón y Cajal, 1995). Other pyramidal cells may have three distinct spatial domains, including the apical and basal cones as well as a central cylinder. This pattern occurs, for example, in the large pyramidal cells of hippocampal area CA1 (Fig. 1.2A). There the apical tuft arborizes in stratum lacunosum-moleculare to receive perforant path input from entorhinal cortex. The middle cylindrical arbor in stratum radiatum receives the Schaffer collaterals from CA3 pyramidal cells. The basal cone extends into stratum oriens where it receives afferents from a more proximal part of CA3 (Amaral and Witter, 1989). A similar pattern is frequently seen among other neocortical pyramidal cells (Feldman and Peters, 1978; Prieto and Winer, 1999).

The *fan radiation* is basically an elaboration of the conical radiation that is more space-filling, and is flatter. This pattern is best characterized by the dendritic arbor of cerebellar Purkinje cells that synapses with a large fraction of the parallel fiber axons that pass through it (Palay and Chan-Palay, 1974; Harvey and Napper, 1991).

This summary of common arborization patterns is far from exhaustive. Many other arbors can be characterized by elaborations or combinations of the basic patterns outlined in Table 1.2. Mitral cells of the olfactory bulb, for example, can exhibit a number of variations, such as laminar radiation of secondary dendrites from the soma or a branch in the apical stem, giving rise to two

separate dendritic tufts (Kishi et al., 1982). The apical stem of pyramidal cells in CA1 may likewise bifurcate midway through stratum radiatum, giving rise to a pair of conical tufts (Bannister and Larkman, 1995a). Some types of neocortical pyramidal cells have an essentially stellate or planar arbor around the cell body, rather than a conical arbor of basal dendrites (Ramón y Cajal, 1995; Prieto and Winer, 1999).

Several approaches have been developed for estimating the density of dendritic arborizations (Uylings and van Pelt, 2002; Scorcioni et al., 2004). The simplest scheme is to count the number of branch points in the entire dendritic arbor (Table 1.1). The *centrifugal method* identifies dendrites by their branching order, with primary dendrites emerging from the cell soma, their first branches being classified as secondary branches, and with increasing order until the tips are reached; the number of dendrite segments of each order characterizes arbor branching (Fig. 1.4). Sholl analysis is the most widely used centrifugal analysis method (Sholl, 1953, 1967), and modern, computer-assisted analyses are available that are well documented and referenced (see http://fiji. sc/Sholl_Analysis). Such schemes show how branched a neuron is but do not measure the degree to which the branches fill the space of the dendritic arbor.

The *fractal dimension* is an estimate of the degree to which an arborization fills its spatial domain. From basic geometry, linear objects have a dimension of one; planar objects have a dimension of two; solid objects, such as a sphere, have a dimension of three; and *fractal* objects fill a fraction of the space in which they are embedded. Dendritic arbors are not fractal objects in the strict mathematical sense, but the concept of a fractal dimension is useful for quantifying their space-filling properties (Smith et al., 1989; Kniffki et al., 1994; Panico and Sterling, 1995; Fernández and Jelinek, 2001). Selective arborizations have fractal dimensions close to one whereas sampling arborizations have fractal dimensions greater than one but less than the dimension of the spatial domain in which they arborize. Space-filling arborizations have fractal dimensions closer to the dimension
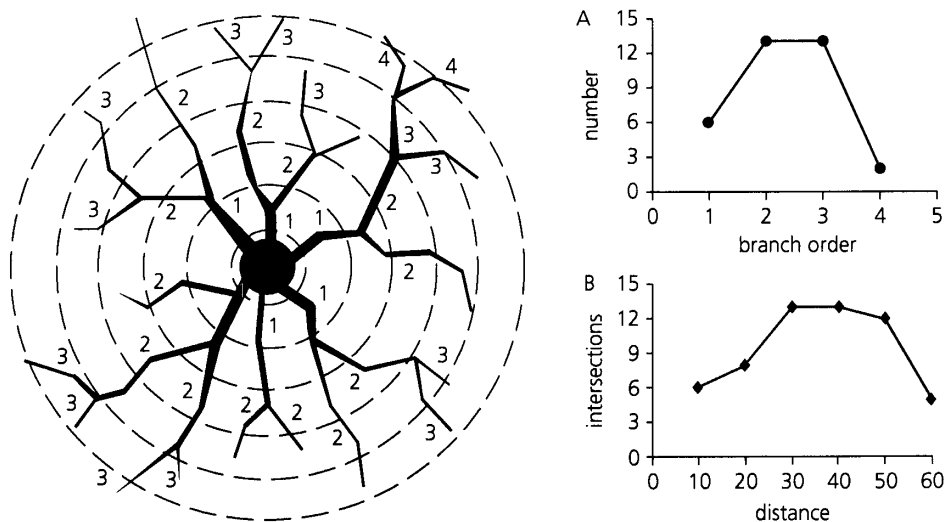

Fig. 1.4 Methods for quantifying dendritic arbors. (**A**) A plot of the number of branches of each order using the centrifugal method of branch ordering. (**B**) A Sholl plot with counts of the number of intersections the dendritic tree makes with circles of increasing radius from the center of the soma. When three-dimensional data are available, concentric spheres are used.

of the geometrical domain they occupy, namely close to two for planar domains and closer to three for solid domains such as spheres. As with Sholl analysis, there are modern, computer-assisted approaches available to perform fractal analyses of dendritic arbors that are well documented and referenced (see http://rsb.info.nih.gov/ij/plugins/fraclac/fraclac.html).

Some examples illustrate the effect of the branching pattern and fractal dimension on connectivity. Pyramidal neurons have selective arborizations in a three-dimensional volume with fractal dimensions of about 1.4–1.5 relative to 3.0 that would fill the volume they occupy (Porter et al., 1991; Scorcioni et al., 2004). In contrast, retinal ganglion cells have essentially two-dimensional arbors, with a fractal dimension of about 1.5 (Wingate et al., 1992; Fernández and Jelinek, 2001). Fractal dimensions of the dendritic arbors of Purkinje cells range from a value of 1.13 in lampreys up to 1.86 in humans, showing an increase with phylogeny to a nearly complete coverage of the two-dimensional area they occupy (Takeda et al., 1992). To understand how the differences in fractal dimension relate to differences in connectivity, consider the retinal ganglion cell, which has a *sampling* planar arbor covering 25,000 μm^2 and a fractal dimension of 1.5 but receives only 2,000 synapses (Sterling and Demb, 2004). In contrast, a Purkinje cell with a *space-filling* planar arbor covering an area of 50,000 μm^2 with a fractal dimension above 1.5 receives about 160,000 synapses (Smith et al., 1989; Harvey and Napper, 1991). Somewhere in between, the dendritic arbors of hippocampal CA1 pyramidal cells receive about 20,000–30,000 synapses (Shepherd and Harris, 1998).

These analyses suggest that the complexity of the dendritic arbor reflects a propensity for connectivity; however, the actual connectivity also depends on the axonal arborization pattern and the direction of axonal projections relative to the dendritic arbor. For example, parallel fiber axons are orthogonal to the dendritic trees of Purkinje cells, permitting only a few synapses per axon per dendritic arbor (Palay and Chan-Palay, 1974; Harris and Stevens, 1988a; Xu-Friedman et al., 2001). In contrast, the climbing fiber from the inferior olive arborizes within the plane of the dendritic arbor of a single Purkinje cell, wrapping itself around the dendrite and forming many synapses (Harvey and Napper, 1991; Xu-Friedman et al., 2001). Similarly, pyramidal cells may receive many synapses from a single axon which runs parallel to a dendritic segment or a few synapses from axons which traverse its dendrites perpendicularly (Sorra and Harris, 1993). Thus, dendritic arbors provide a rich array of patterns that provide capacity for both specificity and diversity in connectivity. The pattern of the dendritic arbor is also sensitive to experience (Volkmar and Greenough, 1972; Greenough et al., 1973; Chang and Greenough, 1982). How particular arbor morphologies affect physiology, behavior, and the capacity for learning is examined in other chapters.

Intracellular structure of dendrites

Dendrites contain numerous subcellular structures that provide local resources at a distance from the soma. These structures include the Golgi apparatus, rough endoplasmic reticulum with ribosomes, free polyribosomes, smooth endoplasmic reticulum, mitochondria, cytoskeletal elements, smooth vesicles, and organelles of the endosomal pathways. These structures provide dendrites with the resources needed to respond rapidly to local changes in synaptic efficacy.

Electron microscopy reveals that the contents of large proximal dendrites are similar to those of the cell soma (Fig. 1.5A). The *Golgi apparatus* (Fig. 1.5A) and the *rough endoplasmic reticulum* (Fig. 1.5B) extend well into proximal dendrites. These also form mobile units called Golgi outposts that occur particularly at dendritic branch points but can also move into the thinner more distal dendrites (Horton and Ehlers, 2003, 2004; Horton et al., 2005; Cui-Wang et al., 2012). The Golgi apparatus is involved in posttranslational modifications of proteins that are synthesized
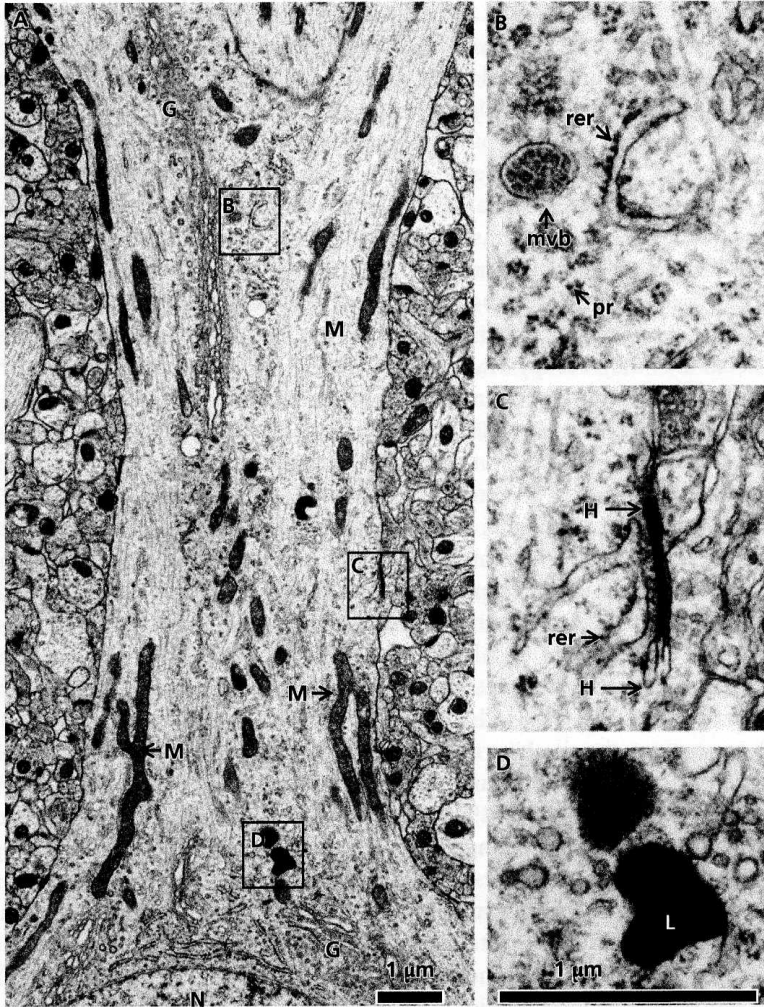
Fig. 1.5 Organelles extend from soma into the apical dendrite of an adult rat CA1 pyramidal cell:
(**A**) nucleus (N), Golgi apparatus (G), mitochondria (M, branch points indicated by an arrow); (**B**) detail
of rough endoplasmic reticulum (rer), polyribosomes (pr), multivesicular body (mvb); (**C**) two arrows
mark the extent of a hypolemmal cisterna of the endoplasmic reticulum (H) that also has rer in close
proximity; (**D**) lysosome (L).

by ribosomes on the rough endoplasmic reticulum. As discussed later, the spine apparatus has a
structure and molecular composition suggesting that it too may be a Golgi outpost that occurs in
some dendritic spines (Spacek, 1985a; Spacek and Harris, 1997; Pierce et al., 2000, 2001).

Polyribosomes are clusters of free ribosomes that occur throughout the dendritic cytoplasm
(Fig. 1.5B) and in some dendritic spines and other dendritic synaptic specializations as discussed
later. Whereas the rough endoplasmic reticulum synthesizes transmembrane proteins, free pol-
yribosomes synthesize cytoplasmic proteins. Thus, proteins can be synthesized locally in the den-
drite, although the ribosomes themselves are synthesized in the nucleolus and ribonucleic acids
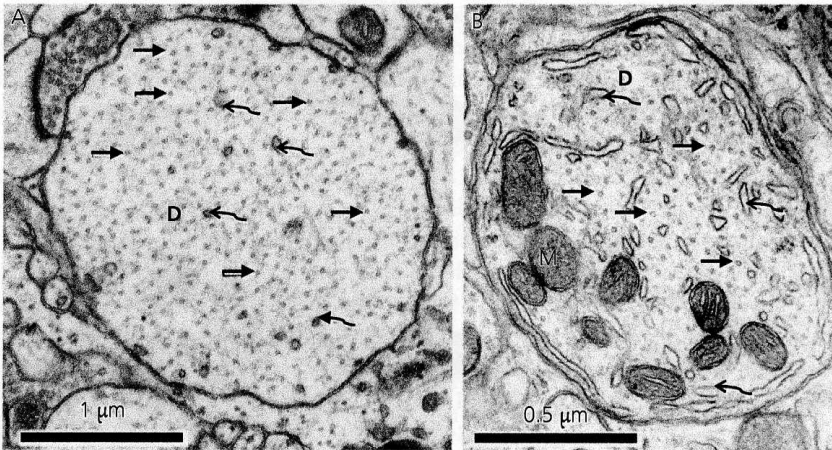
Fig. 1.6 The effect of subcellular organelles on the distribution of microtubules in the cytoplasm. (**A**) An apical dendrite (D) in the middle of hippocampal CA1 stratum radiatum from an adult rat has a regular array of microtubules (arrows) interspersed with a few, narrow tubules of SER (wavy arrows), and no mitochondria. (**B**) A secondary dendrite (D) of an adult mouse Purkinje cell with microtubules (horizontal arrows) interspersed between many elements of SER (wavy arrows) and nine mitochondrial profiles (M). Note that the magnification of part B is exactly twice that of part A.

(RNAs) are transcribed in the nucleus and both are subsequently transported into the dendrites (Bassell et al., 1998; Krichevsky and Kosik, 2001).

Smooth endoplasmic reticulum (SER) is the largest subcellular organelle, forming an essentially continuous network throughout the neuron (Harris and Stevens, 1988a, 1989; Martone et al., 1993; Spacek and Harris, 1997; Cooney et al., 2002). Hypolemmal cisternae (Fig. 1.5C) form junctions between the SER and the plasma membrane (Henkart et al., 1976) where store-operated calcium channels regulate the replenishment of calcium in the SER from the extracellular space, among other functions (Majewski and Kuznicki, 2015). In single sections, the SER usually appears as tubules or flattened cisternae with a clear interior that is bounded by a wavy membrane (Fig. 1.6). In a three-dimensional view obtained by reconstruction from serial sections the continuous reticulum becomes apparent throughout dendrites and some spines (Fig. 1.7). The SER regulates calcium locally and provides posttranslational modification and trafficking of integral membrane proteins (Higley and Sabatini, 2008; Ehlers, 2013). In the dendritic shaft, the SER forms local areas of complexity that retain and enhance the delivery of cargo to nearby synapses (Cui-Wang et al., 2012).

Mitochondria in dendrites are typically rod-shaped organelles that run parallel to the long axis of the dendrite or curve into a dendritic branch (Fig. 1.5). They vary greatly in length, with single mitochondria extending more than 10 μm while others can form a branched network more than 25 μm long (Popov et al., 2003, 2005). In thin dendrites (<0.5 μm in diameter), a single mitochondrion usually lies in the center of the SER network (Fig. 1.7), and the SER is often found to surround individual mitochondria (see Fig. 1.9; Spacek and Lieberman, 1980) in a similar way to how it surrounds the nucleus. This intimate relationship between the SER and mitochondrial and nuclear membranes suggests inter-organelle communication, perhaps to regulate calcium during RNA synthesis, which occurs in both the mitochondria and the nucleus. In stratum radiatum of
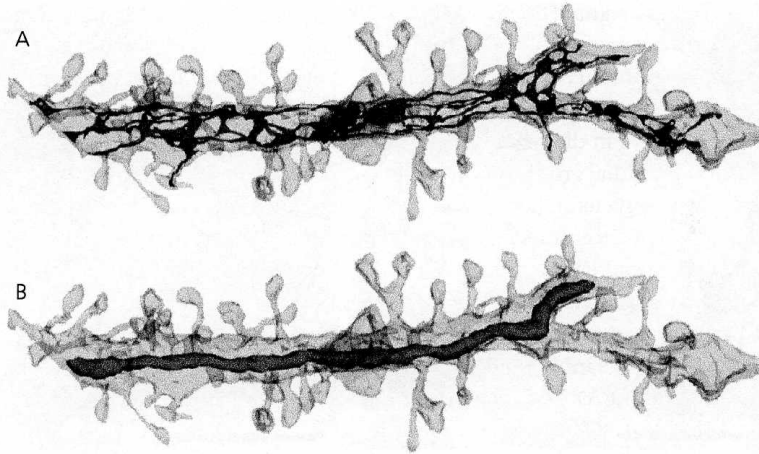
Fig. 1.7 Reconstruction of a segment of a lateral dendritic branch (12.5 μm long) from a CA1 pyramidal cell in the stratum radiatum of the hippocampus of an adult rat: (**A**) network of SER; (**B**) a mitochondrion runs down the center of the dendrite and is surrounded by the SER network.

area CA1, mitochondria comprise about 2% of the intracellular volume in the apical stem dendrites and fill 13% of the thinnest dendritic branches of the apical tuft (Nafstad and Blackstad, 1966). Mitochondria accumulate in dendrites at large synaptic specializations, for example in thalamic nuclei (Lieberman and Spacek, 1997) and in highly branched synaptic specializations of the CA3 pyramidal neurons known as thorny excrescences (Chicurel and Harris, 1989; Rollenhagen et al., 2007). As is known from time-lapse movies in cell culture (Overly et al., 1996), mitochondria are highly dynamic structures and can be redistributed to regions of synaptogenesis or enlarging synapses in response to metabolic demands (Li et al., 2004; Kraev et al., 2009).

The *cytoskeleton* of dendrites is composed of *microtubules, neurofilaments (intermediate filaments)*, and *actin filaments (microfilaments)*. Microtubules are long, thin structures, ~25 nm in diameter and ~90 μm long (Fiala et al., 2003), that are usually oriented parallel to the longitudinal axis of the dendrite, although they can be found to curve into dendritic branches from the parent dendrite. In regions of the dendrite that are relatively free of organelles, the microtubules are packed in a regular array at a density of 50–150 per μm², typically spaced at 80–200 nm (Fig. 1.6A). The number of microtubules in a dendritic cross section is proportional to the caliber of the dendrite (Fiala et al., 2003), which is also proportional to the number of spines per micron length of dendrite (Harris et al., 2007; Bourne and Harris, 2011). Microtubules are the "railroad tracks" of the cell, and they play an important role in the transport of mitochondria and other organelles (Overly et al., 1996; Ehlers, 2013). Microtubules transport SER which leads to local elaboration and redistribution of dendritic SER (Cui-Wang et al., 2012). In regions of a dendrite with a lot of SER, the microtubules are more widely dispersed (Figure 1.6B). The 63-kDa cytoskeleton-linking membrane protein (CLIMP63) is an integral membrane protein in the SER that when phosphorylated by protein kinase C (PKC) causes the SER to dissociate from microtubules and become more elaborate (Klopfenstein et al., 1998; Vedrenne et al., 2005; Cui-Wang et al., 2012). In contrast, where CLIMP63 is dephosphorylated, the SER is associated with microtubules and becomes straighter and more tubular (Cui-Wang et al., 2012). As already indicated, when the SER is more elaborate, cargo is slowed and can be offloaded to support synapses and

other structures in the neighborhood, whereas simplification of the SER enhances the movement of proteins and other cargo and slows offloading. Three-dimensional reconstructions reveal that the SER is a rather continuous network that often surrounds the mitochondrion in thin dendritic branches (Fig. 1.7).

Neurofilaments (10 nm in diameter) occur in dendrites but are more common in axons, where they are important for radial growth (Yuan et al., 2012). *Actin filaments* (7 nm in diameter) constitute the bulk of the cytoskeleton between microtubules and, as discussed later, the actin filaments tend to be more highly concentrated in dendritic synaptic specializations, especially filopodia and dendritic spine necks, where they provide a means of rapidly changing shape.

Organelles of the endosomal pathway are involved in membrane protein sorting and recycling and are commonly found in dendrites (Cooney et al., 2002; Park et al., 2006). *Coated pits* and *coated vesicles* represent the initial step in endocytosis and are frequently seen at the plasma membrane of dendrites (Fig. 1.8A). The cytoplasmic coat is composed of *clathrin*, giving it a distinctive periodic structure (Fig. 1.8C). Coated vesicles and coated pits occur more frequently in dendrites during development (Altman, 1971) and periods of synaptic remodeling (McWilliams and Lynch, 1981). *Recycling endosomes* appear as tubular compartments that can be distinguished from SER by their darker interior, more uniform diameter, smooth (as opposed to wavy) membrane, and the frequent occurrence of specialized coats at the ends of the tubule. These coated ends represent budding sites of recycling vesicles that are bound for the cell membrane. Thus, single *smooth vesicles* are generated when these vesicles lose their coats and are then recycled back to the plasma membrane. In addition, smooth vesicles of varying dimensions can be transported from the soma or other parts of the cell along microtubules. *Sorting endosomes* can be identified by the occurrence of similar tubular compartments connected to larger, spherical organelles with interior vesicles. These spherical compartments mature into *multivesicular bodies* that separate from the sorting endosome and eventually become late endosomes and lysosomes (see Fig. 1.5B, D). Thus, multivesicular bodies in dendrites occur alone or in conjunction with the sorting endosome compartments (Fig. 1.8B, D).
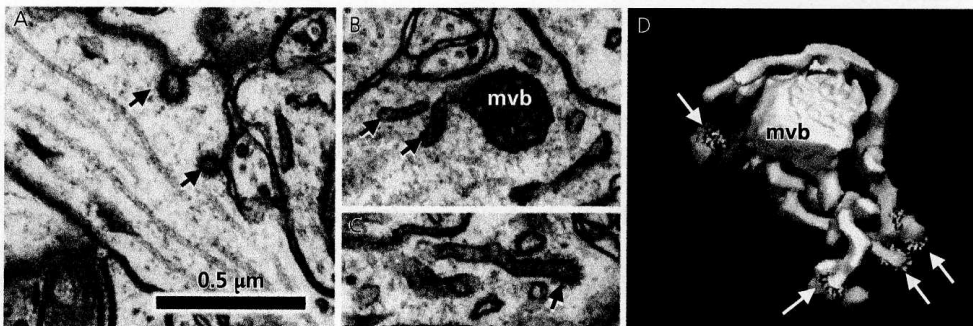
Fig. 1.8 Endosomes in lateral dendrites in stratum radiatum of area CA1 from the hippocampus of an adult rat. (**A**) Clathrin-coated pits (arrows) are the initial step in the endosomal pathway. (**B**) A multivesicular body (mvb) with its connected tubular compartments of a sorting endosome (arrows). (**C**) A tubular endosome associated with a sorting complex has a coated tip at one end (arrow). (**D**) Three-dimensional reconstruction of the sorting complex reveals a multivesicular body with many tubules. The top of the multivesicular body is removed to reveal the interior vesicles. Clathrin-coated tips are identified at the ends of a few tubules (arrows).

Autophagy is a normal process that occurs in dendrites, as evidenced by the presence of *macroautophagosomes, autophagosomes,* and *autophagosomal complexes* (Nixon, 2007, 2013; McBrayer and Nixon, 2013; Wolfe et al., 2013). Depending on the stage in the degradation process, these structures are characterized by a SER-like structure that forms a double-walled delimiting membrane and surrounds other organelles (e.g., see "vac" in Fig. 1.12), and ultimately sends them to the lysosome.

Dendrites contain other membranous subcellular structures. Amorphous vesicular clumps of membrane are common in dendritic growth cones and at sites of synaptogenesis along dendrites and in dendritic growth cones (Fiala et al., 1998). They also appear to be critical for the growth and formation of dendritic spines during development and synaptic plasticity (Park et al., 2006).

Structure of synaptic specializations of dendrites

The potential for connectivity is established primarily by the patterns of dendritic and axonal arborization and secondarily by the formation of a variety of synaptic specializations emerging from the dendrites (Table 1.3).

Shaft synapses reside directly on the surface of the dendrite without obvious changes in the dimensions of the dendrite at the synapses (e.g., Fig. 1.9). Both excitatory and inhibitory axons can form synapses directly on the dendritic shafts throughout the dendritic arbor. In the hippocampus and elsewhere, the interneuronal dendrites have most of their synapses directly on the dendritic shafts (~95%) with only a small fraction on dendritic specializations or protrusions (~5%) (Harris et al., 1985; Harris and Landis, 1986; Ascoli et al., 2008). In contrast, 95% of synapses onto the dendrites of CA1 pyramidal cells are located on dendritic spines, while shaft synapses make up only 5% (Harris et al. 1992; Kirov et al. 1999).

Varicosities in the dendrite are one type of synaptic specialization that can be found in certain neurons, such as amacrine cells of the retina, where dendritic varicosities both receive synapses from rod bipolar cells and make reciprocal synapses back onto the bipolar cells (Ellias and Stevens, 1980). Under normal, non-pathological conditions, many of the cortical, hippocampal, and cerebellar non-spiny interneurons also have dendritic varicosities containing subcellular components that support axo-dendritic synapses (DiFiglia and Carey, 1986; Harris and Landis, 1986; Ascoli et al., 2008).

Filopodia are transient synaptic specializations of dendrites. All neurons exhibit dendritic filopodia during development when extracellular space separates potential synaptic partners (Morest, 1969). Filopodia are identified in 3DEM by their length (often exceeding more than 2 μm) and a somewhat denser cytoplasm than mature spines; when visualized in culture, they are highly dynamic, extending and retracting within a few minutes (Dailey and Smith, 1996; Fischer et al., 1998), which could explain the diversity of lengths captured in 3DEM (Fiala et al., 1998). After the developmental period, filopodia diminish (Dunaevsky et al., 1999; Grutzendler et al., 2002) and then are usually distinguished from dendritic spines only by the absence of synapses (Bourne and Harris, 2011). Even during synaptogenesis most filopodia bear no synapses, but some filopodia make small synaptic contacts along their lengths with multiple presynaptic axons (Fiala et al., 1998). Multiple synapses with different axons can also be found surrounding the base of filopodia (Fiala et al., 1998), consistent with the rapid migration of synaptic proteins along filopodia in culture (Marrs et al., 2001). Long filopodia (>1 μm) are rarely seen on dendrites in the normal rat hippocampus beyond 2 weeks after birth, possibly because the more mature neuropil is densely packed with axonal boutons; hence, even a short dendritic outgrowth would encounter multiple potential synaptic partners. During maturation, filopodia are generally replaced with shaft synapses, stubby spines, or other types of synaptic specialization (Harris, 1999).

Table 1.3 Synaptic specializations of dendrites
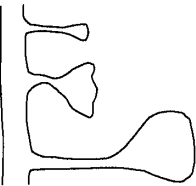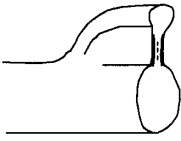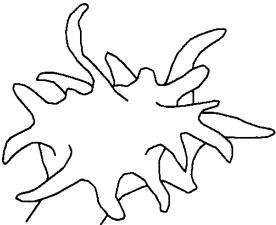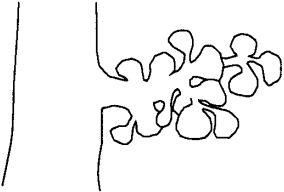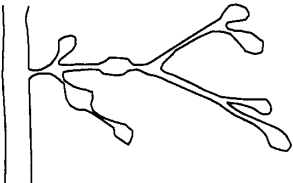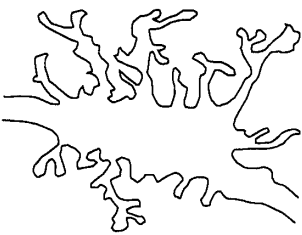
Pattern	Characteristics	Examples
Shaft		
	Synapses occur directly on the surface of the dendritic shaft without obvious swelling or specialization	Interneurons
Varicosity		
	An enlargement in a thinner dendrite associated with synaptic contacts	Retinal amacrine cells
Filopodium	A long, thin protrusion with a dense actin matrix and few internal organelles	All neurons during developmental synaptogenesis
Simple spine		
Sessile		
	Synaptic protrusions without a neck constriction	Cerebral pyramidal cells
		Cerebral pyramidal cells
	Sessile spine	Neurons of dentate nucleus
	Stubby spine	
	Crook thorn	
Pedunculated		
	Bulbous enlargement at tip	Cerebral pyramidal cells
	Thin spine	Cerebral pyramidal cells
	Mushroom spine	Olfactory bulb granule cell
	Gemmule	
Branched spine		
	Each branch has a unique presynaptic partner and each branch has the shape characteristics of a simple spine	CA1 pyramidal cells
		Granule cells of dentate gyrus
		Cerebellar Purkinje cells

Table 1.3 (continued) Synaptic specializations of dendrites

Pattern	Characteristics	Examples
Synaptic crest		
	Crest-like protrusion with a synapse on either side of a thin lamellar neck region	Cerebral pyramidal cells Neurons of habenula, subfornical organ, and interpeduncular nucleus
Claw ending		
	Synaptic protrusions at the tip of the dendrite associated with one or more glomeruli	Granule cells of cerebellar cortex and dorsal cochlear nucleus
Brush ending		
	Spray of complex dendritic protrusions at the end of the dendrite that extends into glomerulus and contains presynaptic elements	Unipolar brush cells of cerebellar cortex and dorsal cochlear nucleus
Thorny excrescence		
	Densely lobed dendritic protrusion into a glomerulus	Proximal dendrites of CA3 pyramidal cells and dentate gyrus mossy cells Proximal dendrites of thalamocortical relay cells
Racemose appendage		
	Twig-like branched dendritic appendages that contain synaptic varicosities and bulbous tips	Inferior olivary neurons Relay cells of lateral geniculate nucleus
Coralline excrescence		
	Dendritic varicosity extending numerous thin protrusions, velamentous expansions and tendrils	Neurons of dentate nucleus and lateral vestibular nucleus

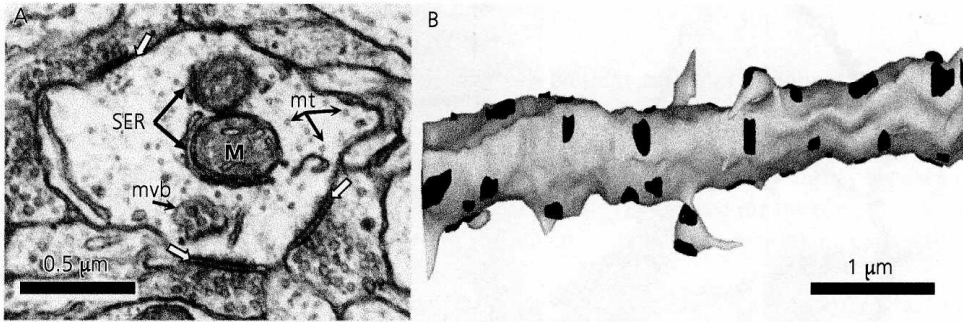**Fig. 1.9** An interneuronal dendrite from stratum radiatum of hippocampal area CA1 in a 21-day-old rat with mostly shaft synapses. (**A**) Electron micrograph illustrating a cross-sectional view where this dendrite hosts three excitatory synapses (white arrows) directly on the dendritic shaft. This section also illustrates that interneuronal dendrites have dispersed microtubules among a similar complement of subcellular components as the spiny dendrites including a close association between SER and mitochondria (black arrows, M), microtubules (mt), and a sorting endosome with a multivesicular body (mvb) illustrated on this section. (**B**) Three-dimensional reconstruction from serial section electron microscopy of a 4.6-μm segment of the dendrite reveals 45 excitatory synaptic contacts (black), 91% of which are located on the dendrite shaft and three on protrusions.

Simple dendritic spines are the most common synaptic specialization of dendrites (Fig. 1.10), being the site of more than 90% of excitatory synapses across brain regions. *Simple spines* have a *sessile* shape with no distinction between the diameter of the neck and head, or a *pedunculated* shape that has been described as thin or mushroom shaped (Fig. 1.10E). In the mature brain, 3DEM has revealed that simple spines vary greatly in size, with volumes ranging from less than 0.01 μm³ to more than 1.5 μm³ (Table 1.4). Simple spines of different sizes and shapes can be neighbors on the same parent dendrite (Harris and Stevens, 1988b, 1989) and occasionally form synapses with the same presynaptic bouton (Sorra and Harris, 1993; Sorra et al., 1998; Fiala et al., 2002a). Measuring spine neck dimensions is functionally relevant since the presence of a neck constriction can serve to isolate the spine head compartment from the dendrite (Holmes, 1990;

Table 1.4 Dimensions of simple spines on spiny neurons

Neuron	Neck diameter (μm)	Volume (μm³)	Surface area (μm²)	Synapse area (μm²)	Ratio of synapse area to surface area
Cerebellar Purkinje cell	0.1–0.3	0.06–0.2	0.7–2	0.04–0.4	0.17 ± 0.09
CA1 pyramidal cell	0.04–0.5	0.004–0.6	0.1–4	0.01–0.5	0.12 ± 0.06
Visual cortex pyramidal cell	0.07–0.5	0.02–0.8	0.5–5	0.02–0.7	0.10 ± 0.04
Neostriatal spiny neuron	0.1–0.3	0.04–0.3	0.6–3	0.02–0.3	0.125
Dentate gryus granule cell	0.05–0.5	0.003–0.2	0.1–3	0.003–0.2	—

Sources: Spacek and Hartman (1983), Wilson et al. (1983), Harris and Stevens (1988a), Harris and Stevens (1989), Trommald and Hullenburg (1997).
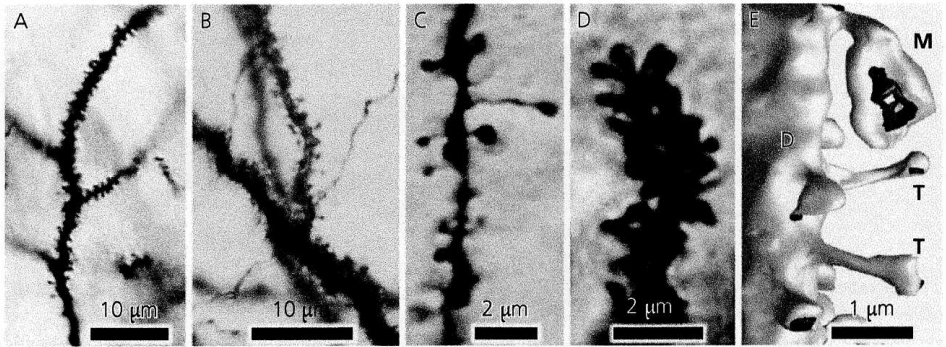
Fig. 1.10 Dendritic spines at increasing magnifications. (**A**) The apical dendrite of a neocortical pyramidal neuron has few spines near the soma (bottom of image) and many spines distally (Golgi; rat). (**B**) Spiny dendrites of a CA1 pyramidal hippocampal cell (Golgi; rat). (**C**) High magnification of a dendrite of a neocortical pyramidal neuron (Golgi; mouse). (**D**) Spiny branchlet of a cerebellar Purkinje cell (Golgi; mouse). (**E**) Three-dimensional reconstruction of a segment of CA1 pyramidal cell dendrite (D) showing typical shapes of thin (T) and mushroom (M) pedunculated spines. (serial section electron microscopy; rat).

Parts C and D reproduced from *Anatomy and Embryology*, 167(2), pp. 289–310, Three-dimensional analysis of dendritic spines, Josef Spacek and Miroslav Hartmann © 1983, Springer Science and Business Media. With permission from Springer Science and Business Media.

Koch and Zador, 1993; Svoboda et al., 1996; Tønnesen et al., 2014) and influence charge transfer and network properties (Yuste, 2013).

Additional types of simple spines are often found on specific neurons. One example is the bent sessile spines in the cerebellar dentate nucleus, called *crook thorns* (Chan-Palay, 1977). The granule cells of the olfactory bulb have particularly large pedunculated spines sometimes referred to as *gemmules*. These spines may be 5 μm long, with heads 1–2 μm in diameter (Cameron et al., 1991).

Branched spines are two or more simple spines that share a common stalk (Table 1.3). The individual branches exhibit the same range of morphologies as simple spines, and in a mature brain each branch synapses with a bouton from different axons (Harris and Stevens, 1988a, 1989; Trommald and Hulleberg, 1997; Sorra et al., 1998). Branching is a rare event because spine origins exhibit a non-random tendency to separate (Trommald et al., 1995; Ward et al., 1995); thus only about 2% of all dendritic protrusions on CA1 pyramidal cells (Harris et al., 1992) or dentate granule cells (Trommald and Hulleberg, 1997) are branched. Branched spines are slightly more frequent on dendrites with higher spine densities, such as Purkinje cell dendrites where approximately 6% of spines are branched (Harris and Stevens, 1988a). In addition, higher spine densities lead to larger numbers of branches per branched spine, with up to five branches having been found on the branched spine of a Purkinje cell (Harris and Stevens, 1988a), while branched spines of CA1 pyramidal cell rarely have more than two branches (Sorra et al., 1998). Exactly how simple spines become branched is unclear. Their rarity suggests an accidental proximity of synapses that emerge close enough to one another to have the same origin. Alternatively, one branch may emerge as a filopodium-like structure, a spine head protrusion, from an existing spine and make a synapse onto a different axon (Richards et al., 2005). The branched simple spines are distinguished from the thorny excrescences discussed later in that the different heads of a branched simple spine never

synapse with the same presynaptic axon; this also supports the hypothesis that they are not due to the splitting of a synapse (Fiala et al., 2002a).

Synaptic crests are specializations found occasionally on spiny neurons throughout the brain, but especially concentrated on the dendrites of the habenula, subfornical organ, and interpeduncular nucleus (Milhaud and Pappas, 1966; Akert et al., 1967; Lenn, 1976). Crest synapses are formed by two axons on either side of the thin lamellar neck of the crest. The synapses are closely apposed inside the crest and may exhibit characteristic subjunctional bodies connecting the two postsynaptic densities. In some instances a synaptic crest can contain multiple folds with many pairs of crest synapses (Lenn, 1976).

Synaptic complexes called glomeruli often occur when the terminals of large axons interact with particular dendrites. The dendrites extend multi-lobed protrusions into the glomeruli and make many synaptic contacts. A simple example is the *claw endings* of the dendrites of cerebellar granule cells (Fig. 1.1), which make several synapses with a single mossy cell axon terminal (Eccles et al., 1967). These cerebellar glomeruli also host *brush endings* of the unipolar brush cells, multi-lobed protrusions that are both presynaptic to the claw endings of the granule cells and postsynaptic to mossy fibers, which may account for their unusual appearance (Floris et al., 1994; Mugnaini et al., 1994; Mugnaini and Floris, 1994).

Thorny excrescences are another type of synaptic specialization associated with large axonal boutons. These specializations are exemplified by those found in the thalamus where, in a reconstructed glomerulus of the ventrobasal thalamic nucleus, 44 synapses were located on a single excrescence that invaginated a giant lemniscal bouton (Spacek and Lieberman, 1974). Mossy fiber axons from dentate granule cells synapsing onto CA3 pyramidal cells are another classic example (Chicurel and Harris, 1992; Hama et al., 1994). The complexity of the CA3 thorny excrescences varies, with some having as many as 16 lobes and others having just a few. Usually, all these lobes synapse with the same presynaptic bouton, but in some cases one or more lobes synapse with different axons.

Racemose appendages have a more sparsely lobed appearance and are common on neurons in the inferior olive (Ruigrok et al., 1990) and lateral reticular nucleus (Hrycyshyn and Flumerfelt, 1981a,b,c). This type of protrusion can also be found on spiny neurons such as neocortical pyramidal cells.

Coralline excrescences are found on dendrites of the small neurons of the cerebellar and vestibular nuclei. These complex dendritic varicosities exhibit numerous synaptic protrusions (Chan-Palay, 1977) and sometimes also have thin tendrils with a similar appearance to filopodia (Morest, 1969; Angaut and Sotelo, 1973; Sotelo and Angaut, 1973), leading to the suggestion that the coralline excrescences are growth processes on adult dendrites.

The dendritic synaptic specializations outlined in Table 1.3 do not describe all the specialized synaptic shapes found in dendrites. Furthermore, individual dendritic segments exhibit a wide variety of spine types as well as other synaptic specializations. Thus, the shape of dendritic synaptic specializations is not fixed by the postsynaptic neuron but appears to reflect the source and activation history of the presynaptic partner. Furthermore, synaptic specializations can be highly dynamic structures, capable of structural change throughout life. This structural plasticity of synapses blurs morphological classifications, and many related and intermediate forms are to be expected. For example, filopodia become dendritic spines, and small spines have been shown to grow or shrink over time and with experience, as discussed later. Furthermore, the distribution of the dimensions of dendritic spines and other synaptic specializations is broad, and essentially continuous. Super-resolution microscopy and 3DEM provide a means to obtain reasonably accurate measurements; hence, future measurements will enhance understanding of the functional

consequences of changes in the shapes of dendritic specializations during development and synaptic plasticity in the mature nervous system. As discussed in the next section, the subcellular composition of spines and other synaptic specializations further distinguishes them in their capacity to undergo calcium-mediated or protein-synthesis-dependent changes in structure and molecular composition.

Composition of synaptic specializations of dendrites

The diversity of shapes of synaptic specializations is accompanied by diversity in their intracellular composition. Like the dendritic shaft, different synaptic specializations in dendrites contain different intracellular components, including various synaptic and adhesive junctions, SER, the spine apparatus (a possible Golgi outpost), rough endoplasmic reticulum and polyribosomes, mitochondria, microtubules, smooth vesicles, and organelles of the endosomal and recycling pathways. The presence or absence of perisynaptic astroglial processes influences the structure and composition of the dendritic synaptic specializations. Furthermore, the size of a synaptic specialization correlates with the composition of subcellular structures. Thus, even within and surrounding the synaptic specializations, resources are present that can respond rapidly to local changes in synaptic efficacy.

Postsynaptic densities

The synaptic specializations of dendrites receive chemical synapses consisting of apposed membranes separated by a gap called the synaptic cleft (Figs 1.11 and 1.12). Neurotransmitters released from synaptic vesicles on the presynaptic side of the cleft diffuse across the cleft to activate receptors in the postsynaptic membrane. The presynaptic element is usually a varicosity or end bulb of an axon, called a bouton. In aldehyde-fixed tissue, several different types of chemical synapses can be distinguished based on the size and shape of the presynaptic vesicles and perisynaptic structures (Colonnier, 1968; Peters and Palay, 1996). Two principal types are commonly referred
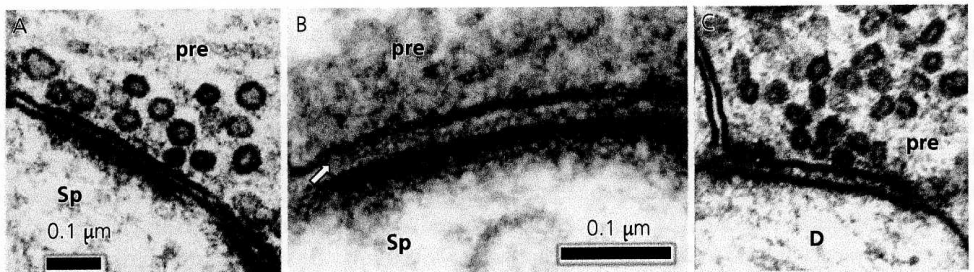
Fig. 1.11 Asymmetrical and symmetrical synapses on dendrites of spiny neurons. (**A**) An asymmetrical synapse located on a spine head (Sp) from the hippocampus of an adult rat illustrates the characteristic thickened postsynaptic density and round presynaptic (pre) vesicles. (**B**) High-magnification image of another asymmetrical synapse from mouse cerebellum shows dense material in the synaptic cleft (white arrow). (**C**) Symmetrical synapse on the shaft of a dendrite from rat hippocampus illustrates the thin and essentially equal densities in the presynaptic (pre) and postsynaptic (D) compartments and pleiomorphic presynaptic vesicles, which are smaller and more flattened than the vesicles of an asymmetric synapse (compare with those in A). The scale bar in part A is the same as for part C.
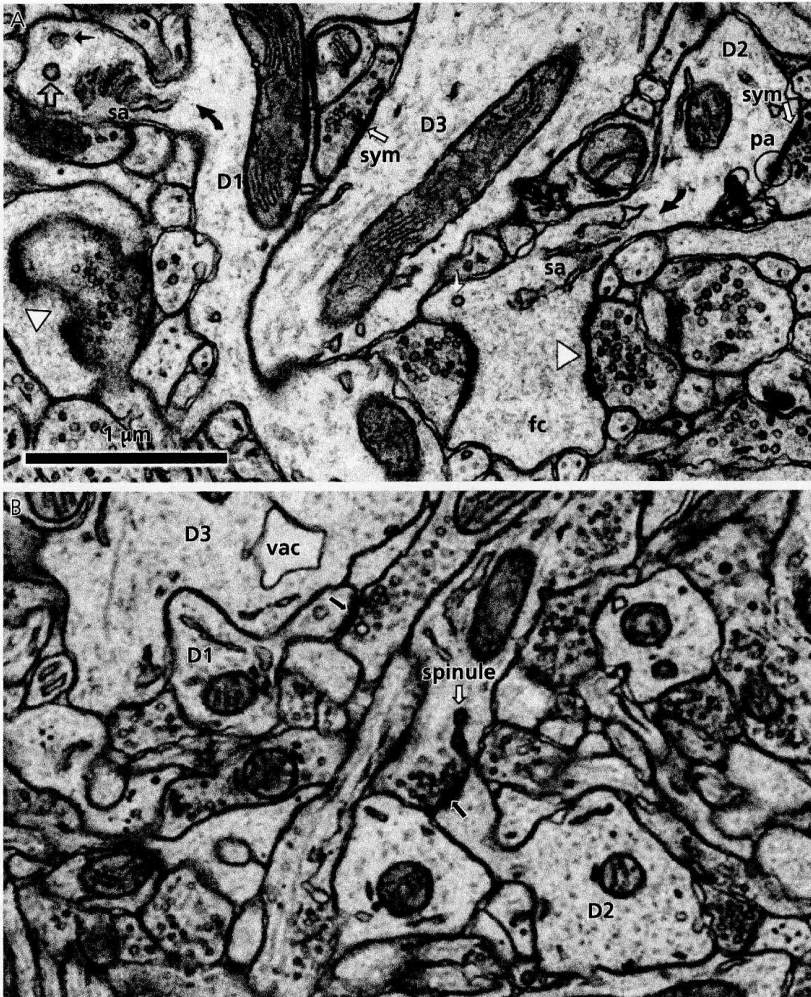
Fig. 1.12 Different dendritic spines contain unique subcellular compositions. (**A**) On this section, each of two dendrites (D1, D2) in rat CA1 stratum radiatum gives rise to large spines (curved arrows) that contain a spine apparatus (sa). The sa of the D1 spine is connected on adjacent sections to the tubule (small arrow), and this spine also contains a spherical vesicle (open arrow). The bouton presynaptic to the D2 spine wraps around the spine head so that the synapse appears on both sides in this section. The postsynaptic density is interrupted, or perforated, on one side (white arrowhead). In addition to the sa, the D2 spine contains a small clear profile (white arrow) found in series to be endosomal in nature. Both spines contain a floccular cytoplasm (fc) consistent with an actin matrix. Symmetric shaft synapses (sym, white arrows) are present on D2 and D3. Next to the D2 symmetric synapse is a punctum adhaerens junction (pa). (**B**) Two dendrites (D1, D2) give rise to small spines with macular synapses (black arrows). The spine of D2 has a spinule emerging from the edge of the synapse into the presynaptic axon. A third dendrite (D3) contains a vacuole (vac), which on serial section reconstruction is part of a macro-autophagosome. The scale bar in part A is for both A and B.

to as asymmetric and symmetric synapses. Asymmetric synapses are characterized by round pr-esynaptic vesicles about 30–50 nm in diameter and a prominent postsynaptic density (Figs 1.11A, B and 1.12A, B). The postsynaptic density is a densely stained structure that contains numerous re-ceptors, structural proteins, and signaling molecules that are important for synaptic transmission and plasticity (Kennedy, 2000; see also Chapter 6). The synaptic cleft also contains dense-staining material (Fig. 1.11B) probably containing the extracellular components of receptors and other signaling or adhesion molecules. Symmetric synapses have a much thinner postsynaptic density, matched by a nearly equal density on the presynaptic side, where many of the presynaptic vesicles appear flattened (Figs 1.11C and 1.12A). Asymmetric synapses are usually excitatory and use the neurotransmitter glutamate, whereas symmetric synapses use the inhibitory neurotransmitters gamma-aminobutyric acid (GABA) or glycine and the molecular composition of their receptors and downstream signaling molecules matches these structural and functional distinctions (Harris and Weinberg, 2012).

Asymmetric and symmetric synapses are differentially distributed along dendrites. By defini-tion, simple spines have an excitatory asymmetric synapse located on the spine head. Occasionally, simple spines have more than one synapse (Spacek and Hartmann, 1983; Fiala et al., 1998; Bourne and Harris, 2011). The second synapse can be either symmetric or asymmetric (Jones and Pow-ell, 1969). For example, in the visual cortex 84% of synapses are asymmetric and 16% symmetric (Beaulieu and Colonnier, 1985). Most of the asymmetric synapses (79%) occur on simple dendritic spines, while 21% occur on dendrite shafts and very few (0.1%) are found on cell bodies. The symmetric synapses are much less frequent and yet their positioning might effectively nullify the excitatory input because most (62%) occur directly on the dendrite shafts, 31% occur on a fraction of the dendritic spines, and 7% occur on cell bodies and axon initial segments. Symmetric synapses are only 7% of all dendritic spine synapses but 93% of all soma synapses in the visual cortex. In the neostriatum, as another example, approximately 8% of spines receive both an excitatory asymmet-ric and an inhibitory symmetric synapse (Wilson et al., 1983). Some striatal neurons make recip-rocal connections with the substantia nigra, and in this field 39% of their spines are contacted by a different type of symmetric synapse containing large, pleiomorphic vesicles which are probably dopaminergic (Freund et al., 1984). Glomeruli often contain both excitatory and inhibitory axon terminals. Thus, it is common for dendritic synaptic specializations that project into glomeruli to receive multiple types of synaptic contacts. For example, the racemose appendages of inferior olivary neurons receive both excitatory and inhibitory synapses (De Zeeuw et al., 1990).

Cell adhesion junctions (puncta adhaerentia) and nascent zones adjacent to synapses

Cell adhesion junctions, sometimes referred to as puncta adhaerentia (Figs 1.12A and 1.13), are char-acterized by pre- and postsynaptic thickenings but no presynaptic vesicles. They can be located at the edges of the postsynaptic densities of dendritic spines and other synaptic specializations (Uchida et al., 1996; Spacek and Harris, 1998; Benson and Huntley, 2012). Extensive adherent contacts form a meshwork in the glomeruli of thalamic relay nuclei (Lieberman and Spacek, 1997). Cell adhesion junctions contain a host of adhesion molecules (e.g., cadherins, neuroligins, and neurexins) with associated signaling cascades that differentiate them from the receptor-containing portions of the postsynaptic density (Fannon and Colman, 1996; Fields and Itoh, 1996; Benson and Huntley, 2012).

In addition to the puncta adhaerentia, we have identified the *nascent zone* (Fig. 1.13), an ultra-structurally distinct region at the edge of synapses in the intact mature hippocampus (Bell et al., 2014), which was previously described as a vesicle-free transition zone (Spacek and Harris, 1998).
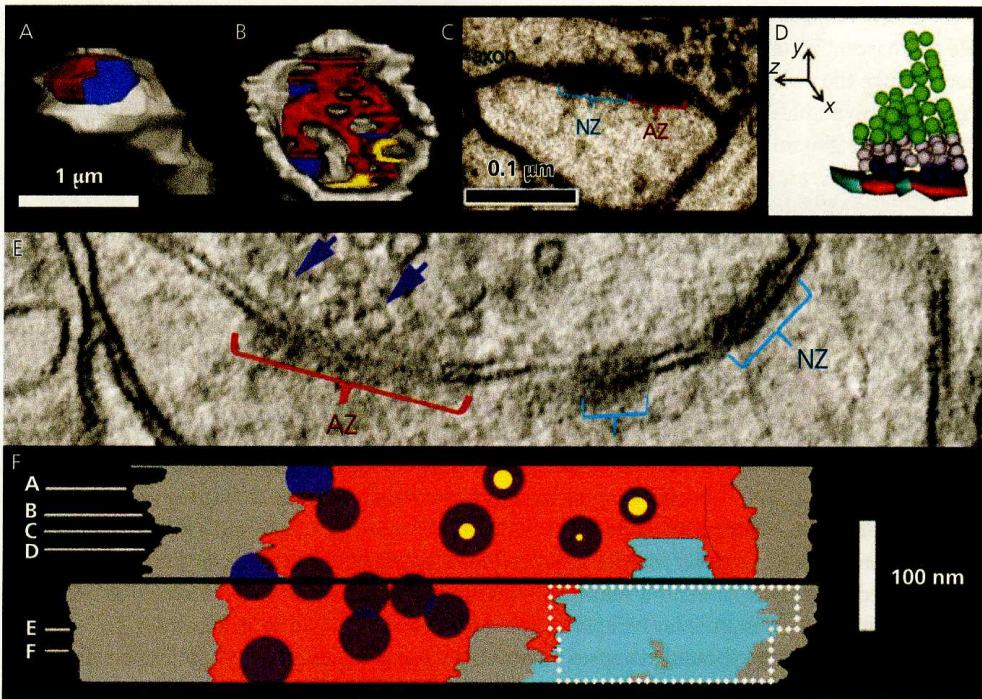
Fig. 1.13 Substructure of the postsynaptic density on dendritic spines. (**A**) 3DEM of a spine with a macular synapse on its head. The active zone (red) is adjacent to a large punctum adhaerens junction (blue). (**B**) A view of the synaptic face of a spine with a perforated postsynaptic density with a large active zone (red), three puncta adhaerens (blue), and two vesicle-free transition zones (yellow). (**C**) Nascent zones (NZ) is the revised name for vesicle-free transition zones. Nascent zones do not have any presynaptic vesicles, thus distinguishing them from the active zone (AZ). (**D**) 3DEM of the docked vesicles (dark blue) and the adjacent (light gray) and distant (green) reserve pool vesicles, viewed on edge to reveal the nascent zones (turquoise) and active zones (red). (**E**) Identification and measurement of nascent zones in virtual sections from tilt tomography revealed vesicles, with some pressed against the active zone (blue arrows), but no vesicles above the nascent zones. (**F**) Stacked projection of the axon–spine interface (gray), active zone (red), and nascent zone (teal) that were first traced through the 2–3-nm virtual sections and then displayed orthogonal to the virtual section planes with white lines illustrating the locations of the virtual sections and F equal to the virtual section in the micrograph E. Maximum diameters of docked vesicles are illustrated as dark blue circles with scaled pores (yellow circles) circumscribed in vesicles that had them. The scale bar in part F also applies to E.

Both nascent and active zones have a postsynaptic density, but, unlike the active zone, the presynaptic side of a nascent zone lacks the small clear synaptic vesicles that are required for the release of gluta-mate. Unlike the puncta adhaerentia, nascent zones have no presynaptic thickening. Synaptic edges are highly dynamic regions where alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs), which mediate fast excitatory transmission, diffuse laterally until they are stabilized by activity (Choquet and Triller, 2013; MacGillavry et al., 2013). How-ever, even if AMPARs were present in a nascent zone, the absence of presynaptic vesicles could render the nascent zone functionally silent because the diffusion path from release sites is too distant (Franks et al., 2002, 2003; Raghavachari and Lisman, 2004; Christie and Jahr, 2006; MacGillavry et al., 2013; Nair et al., 2013). Their distinct locations, composition, and morphology likely serve to modulate synaptic efficacy since their disassembly and reassembly may be needed for synaptic plasticity (Luthl et al., 1994; Muller et al., 1996; Tang et al., 1998); recent 3DEM results show that they disappear early and indeed are reassembled 2 hours after the induction of long-term potentiation (Bell et al., 2014).

The postsynaptic densities and associated adhesions exhibit size-dependent variations in mor-phology (Spacek and Hartmann, 1983; Harris and Stevens, 1988a, 1989; Humeau et al., 2005; Nichol-son and Geinisman, 2009). Most synapses have a continuous postsynaptic density when viewed in 3DEM, often called macular in shape (Figs 1.9, 1.10E (T spines), 1.12B, and 1.13A). Larger post-synaptic densities often exhibit interior regions devoid of pre- and postsynaptic density, which can be U-shaped or annular or exhibit multiple holes, and are often called perforated (Figs 1.12A and 1.13B), segmented, or multifocal synapses (Geinisman et al., 1987; Sorra et al., 1998).

Both macular and perforated synapses occur on a variety of dendritic synaptic specializations. When located on spines, the synaptic area occupies approximately 10% of the surface area of the spine head (Spacek and Hartmann, 1983; Harris and Stevens, 1988a, 1989). This relationship is consistent over different spine morphologies and neuron types (Table 1.4) and also appears to hold for more complicated synaptic specializations, such as the thorny excrescences of CA3 (Chicurel and Harris, 1992). Spine surface area, spine volume, bouton volume, and the number of synap-tic vesicles correlate with synapse size in most cases (Harris and Stevens, 1988a, 1989; Lisman and Harris, 1993; Schikorski and Stevens, 2001). Thus, smaller thin spines have smaller synapses, which tend to be macular, and larger mushroom spines have larger synapses, which can be perfo-rated (Sorra et al., 1998; Bourne and Harris, 2007). Larger synapses contain more receptors and other signaling molecules, and therefore represent more effective connections (Nusser, 2000; Mat-suzaki et al., 2001; Tanaka et al., 2005; Holderith et al., 2012). Differences in synaptic efficacy have important implications for both long-term information storage and short-term neurotransmis-sion. For example, excitatory synapses in the most distal apical dendrites of CA1 pyramidal cells are more often perforated and larger than those synapses more proximal to the cell body (Megias et al., 2001; Nicholson et al., 2006). This property may help compensate for distance-dependent attenuation of postsynaptic potentials (see Chapter 12).

Smooth endoplasmic reticulum

Some synaptic specializations also contain elements of the dendritic SER network (Figs 1.12 and 1.14). In pyramidal neurons of the cerebral cortex and hippocampus, only about 15% of dendritic spines contain SER, and this organelle is mostly absent from thin spines (Spacek and Harris, 1997). All dendritic spines of cerebellar Purkinje cells contain SER (Spacek, 1985a; Harris and Stevens, 1988a). In claw endings of cerebellar granule cells, each mitochondrion is surrounded by a single cistern of SER. Organelles derived from SER also appear subjacent to the puncta adhaerentia on both pre- and postsynaptic sides.
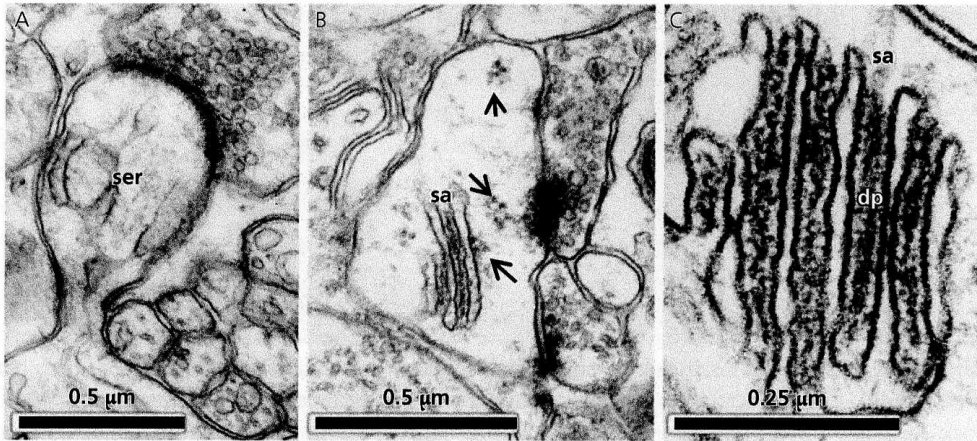
Fig. 1.14 Smooth endoplasmic reticulum in dendritic spines. (**A**) A cerebellar Purkinje cell spine showing the network of SER that extends into the spine from the dendrite. (**B**) The head of a neocortical mushroom spine containing a spine apparatus (sa) and polyribosomes (arrows). The lowest cluster of polyribosomes is located immediately adjacent to the sa, suggesting it may be rough endoplasmic reticulum. (**C**) High-magnification image of the spine apparatus (sa) in a mushroom spine of the mouse visual cortex shows the inner dense plates (dp) between cisternae of SER.

Reproduced from Anatomy and Embryology, 171(2), pp. 235–243, Three-dimensional analysis of dendritic spines, Josef Spacek, © 1985, Springer Science and Business Media. With permission from Springer Science and Business Media.

The spine apparatus (possible Golgi outpost), rough endoplasmic reticulum, and ribosomes

Large spines often possess a large, perforated synapse and contain a spine apparatus (Fig. 1.12A). As mentioned above, the spine apparatus has structural and molecular features similar to the Golgi apparatus (Gray, 1959; Spacek and Harris, 1997; Pierce et al., 2000; 2001; Deller et al., 2007). The largest mushroom spines and gemmules often contain the spine apparatus, as do the thorny excrescences of hippocampal area CA3. The spine apparatus can also be found in association with polyribosomes (Fig. 1.14B). High concentrations of polyribosomes have been found in the lobes of thalamic thorny excrescences (Fig 1.15) and those of the CA3 pyramidal cells (Chicurel and Harris, 1989). Under quiescent conditions, only about 12% of simple dendritic spines contain *polyribosomes* (Steward et al., 1996; Ostroff et al., 2002). During tetanus-induced long-term potentiation, polyribosomes increase dramatically in spines (Ostroff et al., 2002; Bourne et al., 2007), contrasting with theta-burst induction of long-term potentiation where polyribosome frequency increases transiently in several spines, but then concentrates by 2 hours in those spines with the most enlarged synapses (Chirillo et al., 2015). The spine apparatus is often found to have a structural association with puncta adhaerentia and nascent zones, indicating a possible role in synthesizing or maintaining them (Spacek and Harris, 1998; Bell et al., 2014). In addition, this location could enhance signaling via metabotropic glutamate receptors, which are also concentrated at the edges of excitatory synapses (Baude et al., 1993; Kennedy, 2000).
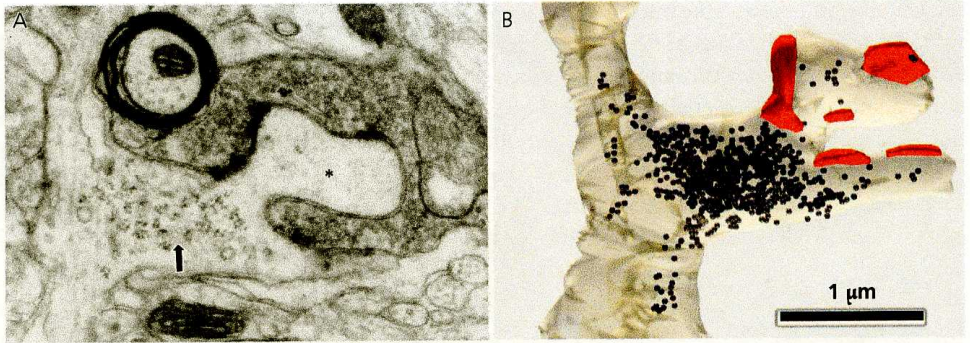
Fig. 1.15 A small thorny excrescence emerging from a thalamocortical relay neuron of the ventrobasal nucleus. (**A**) Electron micrograph of a section through the excrescence. Beneath the synaptic lobe (rectangle with cross) is a region (arrow) with a large number of polyribosomes. (**B**) 3DEM of the excrescence (gray) reveals the intense concentration of polyribosomes (black spheres) beneath the synapses (red).

Mitochondria

Small synaptic specializations such as filopodia and simple dendritic spines rarely contain mitochondria as most are too small, but larger dendritic synaptic specializations often contain mitochondria. Gemmules of olfactory bulb granule cells often have mitochondria in their head, and since these spines make reciprocal synapses on mitral cell dendrites it has been suggested that the presence of these mitochondria may be related to their presynaptic function (Cameron et al., 1991). Other specializations with both postsynaptic and presynaptic functions, such as the varicosities of AI amacrine cells and the brush endings of unipolar brush cells, likewise contain numerous mitochondria. Although the large thorny excrescences and claw endings do not have synaptic vesicles, they may contain a mitochondrion, suggesting a role beyond vesicular release. The larger synaptic specializations of the relay neurons of the lateral geniculate nucleus also contain many mitochondria but have no presynaptic function (Wilson et al., 1984). During development, mitochondria appear to visit the base of dynamic dendritic protrusions in culture (Li et al., 2004). Hence, mitochondria may provide differential support in the forms of calcium regulation, local protein synthesis, and production of ATP, depending on the function, size, and developmental status of a synaptic specialization.

Microtubules

Small dendritic protrusions such as simple spines and filopodia have cytoskeletons based primarily on actin rather than microtubules, although tubulin is ubiquitous in subcellular fractions of the postsynaptic density. Actin-based cytoskeletons are thought to facilitate rapid, calcium-induced changes in shape (Fifkova, 1985; Fischer et al., 1998; Halpain et al., 1998). Microtubules are not readily observed through serial section electron microscopy in mature hippocampal, cortical, or cerebellar dendritic spines under normal conditions, although they do occur normally in the extra large CA3 thorny excrescences (Ebner and Colonnier, 1975; Chicurel and Harris, 1992) and in hippocampal and cortical dendritic spines during development, apparently emanating from the spine apparatus (Westrum and Gray, 1977; Westrum et al., 1980). During the short period of synaptic quiescence after making a hippocampal slice, microtubules protrude into mature dendritic

spines; however, these spine microtubules can no longer be detected 30 minutes after the slice has recovered in vitro (Fiala et al., 2003). Recent work in hippocampal cultures suggests that microtubules are highly dynamic, and rapidly enter and retract from dendritic spines (Gu et al., 2008; Hu et al., 2008; Jaworski et al., 2009; Dent et al., 2011) such that at any one moment less than 1% of spines may contain a microtubule. This transience, along with their sensitivity to calcium, may explain why microtubules are rarely detected in a polymerized state in mature dendritic spines of aldehyde-fixed brain in vivo.

Organelles of the endosomal and recycling pathways

Smooth and coated vesicles and elements of the endosomal pathway also occur in synaptic specializations. The discrete tubules and vesicles of the endosomal compartments are distinguished from the essentially continuous network of SER through 3DEM. In addition, recycling endosomes engulf gold particles conjugated with bovine serum albumin (BSA-gold) that had been delivered in the extracellular space of a hippocampal slice (Cooney et al., 2002). About 50% of normal hippocampal dendritic spines contain no membrane-bound organelles, whereas some spines contain endosomes and others contain SER. Only rarely does a single spine contain both organelles. An endosomal sorting complex occurs about once every 10 μm along the length of a dendrite and hence serves about 10–20 hippocampal dendritic spines (Cooney et al., 2002). Interestingly, the endocytosis-related proteins clathrin, AP-2, dynamin (Racz et al., 2004; Lu et al., 2007), and an isoform of the exocytosis-related protein syntaxin (Kennedy et al., 2010) are all localized to a putative endocytic zone at the edge of postsynaptic densities. Endosomal compartments are dynamically regulated during synaptic plasticity and may provide a local source of new membrane for spinal enlargement (Park et al., 2006). Some endosomal compartments in dendrites may also be involved in autophagocytosis, a process in which proteins and other structures are engulfed by a membrane-bound organelle and incorporated into the lysosomal pathway for subsequent degradation (Bingol and Sheng, 2011). However, the complement of organelles in each instance is unique, suggesting local regulation of subcellular functions, possibly in response to different levels of neuronal activity. Extensive tubular bodies also appear in the dendritic cytoplasm subjacent to puncta adhaerentia (Spacek and Harris, 1998).

Dendritic spines occasionally have even smaller protrusions, *spinules*, that extend from them into the interior of surrounding structures such as boutons or glia (Westrum and Blackstad, 1962; Tarrant and Routtenberg, 1977; Sorra et al., 1998; Spacek and Harris, 2004; Richards et al., 2005). These spinules are surrounded by invaginations of apposed membrane, often with a clathrin-like coat visible on the cytoplasmic side of the engulfing membrane at the tip of the invagination. Spinules in the hippocampus originate from all parts of the dendrite surface, often at the edges of synapses (Fig. 1.12B) and especially from within perforations of the postsynaptic density (Fig. 1.16). The function of spinules is not known; however, they could be involved in bulk membrane recycling or signaling by way of transendocytosis (Spacek and Harris, 2004). Similar structures are found on other types of synaptic specializations, such as on the claw endings of cerebellar granule cells (Eccles et al., 1967) and on the lobes of thorny excrescences (Chicurel and Harris, 1992). It has been suggested that perforations in the postsynaptic density may be related to synaptic plasticity and represent an intermediate stage in a process of proliferation of synapses through splitting (Jones and Harris, 1995; Luscher et al., 2000). However, dendritic spines do not split (Sorra et al., 1998; Fiala et al., 2002a). Indeed, perforations in the postsynaptic density are rather transient structures during plasticity (Applegate et al., 1987; Applegate and Landfield, 1988; Geinisman et al., 1996). Thus, the perforations in the postsynaptic density may arise as a consequence of presynaptic vesicle membrane fusion that pushes the cell adhesion molecules of the pre- and postsynaptic densities
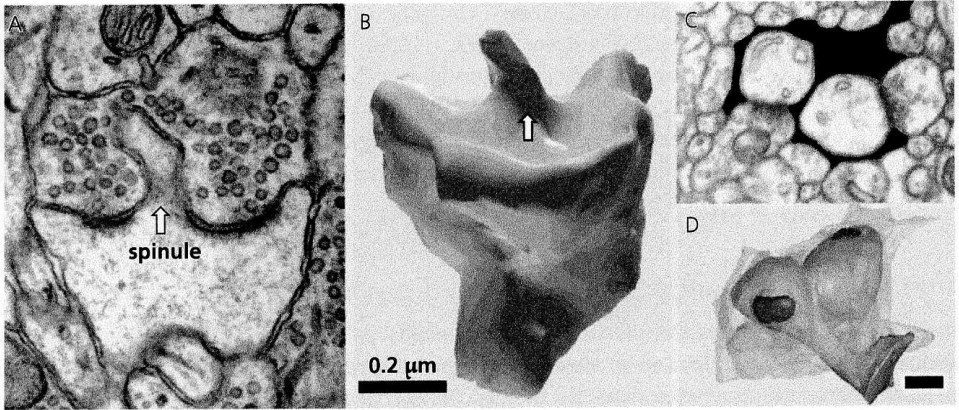
Fig. 1.16 Perisynaptic structure of dendritic spines. (**A**) A spinule (arrow) extends into the presynaptic bouton from the middle of a perforated postsynaptic density on a dendritic spine in adult rat CA1. (**B**) 3DEM of spine with the same spinule (arrow). (**C**) An astrocytic process (black) contacts the edges of synapses onto two dendritic spines in mouse cerebellar cortex. (**D**) 3DEM reveals that these two spines are completely enveloped by the astrocyte (translucent gray).

Part A reproduced from KM Harris and JK Stevens, Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics, *The Journal of Neuroscience*, 9(8), pp. 2982–2997, © 1989, The Society for Neuroscience.

sideways and opens a perforation; the postsynaptic density is then knitted back together as bulk endocytosis later ingests the membrane of the postsynaptic spinule (Applegate et al., 1987; Applegate and Landfield, 1988; Geinisman et al., 1996; Spacek and Harris, 2004; Bourne and Harris, 2011, 2012).

Perisynaptic astroglia

Finally, synapses differ in the degree to which they are surrounded by glial processes. In the cerebellar cortex, nearly all spine synapses are completely ensheathed by Bergmann astroglial processes (Fig. 1.16C, D) (Spacek, 1985b). In contrast, only 58% of hippocampal synapses have even partial astroglial ensheathment (Ventura and Harris, 1999), which is comparable to cortical spine synapses (Spacek, 1985b). Thus, many but certainly not all synapses have astrocytic processes at their perimeter, whereby spillover of neurotransmitter between neighboring synapses could be detected and limited (Bergles and Jahr, 1997). Another important aspect of perisynaptic astroglia at excitatory synapses is energy metabolism. Perisynaptic astrocytes contain rich stores of glycogen that provide local energy replacement following synaptic activity (Magistretti et al., 1999). Perisynaptic astroglial processes provide crucial factors, both contact-mediated and secreted factors, for the formation and maturation as well as the maintenance and elimination of synapses (Clarke and Barres, 2013). Interestingly, localization of perisynaptic astroglia in the human hippocampus is also highly susceptible to pathology such as epilepsy (Witcher et al., 2010).

Diversity in spine and synapse density across dendritic arbors

Neurons have been generally classified as *spiny, sparsely spiny*, and *non-spiny* (or *smooth*) according to the density of simple dendritic spines throughout the dendritic arbors (Feldman and Peters,

1978). Dendritic spines increase the number of potential synaptic partners for a neuron by extending the reach of dendrites to a larger pool of axons while only slightly increasing brain volume (Swindale, 1981; Harris and Kater, 1994; Chklovskii et al., 2004). The ratio of actual synapses to the number of potential synapses, the so-called *filling fraction*, is estimated to be about 0.2 for cortical pyramidal cells (Stepanyants et al., 2002). Indeed, dense 3DEM reconstructions from hippocampal CA1 neuropil reveal that only 20% of axons touching a dendrite actually made a synapse with that dendrite (Mishchenko et al., 2010).

Classification of neurons by spine density is complicated by the fact that different dendrites of a given neuron may exhibit widely different spine densities. Spine densities can vary ten-fold along the length of a single dendritic segment, and nominally non-spiny dendrites often exhibit a few spines. For example, a CA1 pyramidal cell in a rat has about 30,000 spines, with 55% of them located in the apical dendritic arbor and 40% in the basilar dendritic arbor (Megias et al., 2001). The spine density correlates with dendrite caliber, ranging from less than one spine per micron at the tapered dendritic tips, to two or three spines per micron on oblique dendritic branches and ten or more spines per micron along the thick apical dendrites of hippocampal CA1 pyramidal neurons as they pass through the middle of the stratum radiatum (Harris et al., 1992; Trommald et al., 1995; Bannister and Larkman, 1995b; Megias et al., 2001). High spine densities are found on other neuron types, such as certain neostriatal neurons that have seven spines per micron (Wilson et al., 1983; Graveland et al., 1985). By comparison, pyramidal cells of visual cortex are less spiny, averaging only about one and a half spines per micron (Larkman, 1991).

The spiniest dendrites may be the spiny branchlets of cerebellar Purkinje cells, with 3DEM revealing spine densities reaching 15 spines per micron (Harris and Stevens, 1988a). Thus, a single Purkinje neuron in an adult rat may have over 160,000 spines (Napper and Harvey, 1988a,b). The giant pyramidal cells of Meynert in the visual cortex have basilar dendrites and an apical dendrite in layer 5 that are densely covered with spines, few spines along the apical dendrite in layers 3–4, with a high density of spines occurring again as the dendritic arbor reaches layer 2 (Chan-Palay et al., 1974). For most spiny neurons, the dendrites emerging from the soma receive inputs at shaft synapses; hence, like the soma, proximal dendrites are usually devoid of spines. Thus, differences in characteristic spine densities across the dendritic arbor may reflect differences in connectivity to various inputs.

Dendritic pathology

The characteristic dendritic arbors of neurons are created through a combination of intrinsic developmental programs and environmental influences, as discussed further in later chapters. The cellular environment continues to influence dendrites throughout life, and a number of structural pathologies arise from various adverse conditions (Fiala et al., 2002b; Kuwajima et al., 2013). These pathologies must be distinguished from the normal changes in dendritic and synaptic structure that accompany development, learning, memory, and a host of experiences (Bourne and Harris, 2008, 2012).

One strong influence on dendrite structure is excitatory synaptic input, as studied for many years by lesion-induced degeneration in Golgi-impregnated brain tissue (Globus, 1975). Axonal inputs are necessary for the proper development and maintenance of dendrites. For instance, when granule cells are eliminated from the developing cerebellar cortex, Purkinje cells exhibit profoundly reduced, deformed, and misoriented dendritic arbors (Altman and Bayer, 1997). Dendrite structure continues to be dependent on the preservation of axonal afferents throughout adulthood, since deafferentation is followed by atrophy of the dendritic arbor (e.g., Anderson and Flumerfelt,

1986a,b). The hippocampus offers another example, in which a lesion of the entorhinal cortex atrophies the dendritic arbors of granule cells in the dentate gyrus (Caceres and Steward, 1983): the total dendritic length is reduced to less than 2,000 μm (cf. Table 1) 10 days after the lesion. Most of this reduction occurs in the distal dendrites that normally receive the entorhinal input. Remarkably, the dendritic arbors of deafferented neurons often recover (at least partially) within a few months through reafferentation from sprouting axon collaterals.

Reductions in dendritic arborization are seen in many pathological conditions, such as mental retardation (Kaufmann and Moser, 2000), prionosis (Beck et al., 1975), and Alzheimer's disease (Scheibel, 1982), possibly due to neuronal loss and the associated deafferentation (see also Chapter 24). Even aging seems to produce a degree of dendritic atrophy (Scheibel et al., 1975; Jacobs et al., 1993; Chen and Hillman, 1999; Peters, 2002). Trophic support for the dendritic arbor may come not only from synaptic inputs but also from the synaptic connections of a neuron's axon. In the peripheral nervous system, the dendritic arbor shrinks when a neuron's axon is transected (Purves et al., 1988). In the central nervous system, however, axotomy often produces a retrograde degeneration in which the axotomized neuron dies (Ramón y Cajal, 1991). This neurodegeneration is often characterized by an accumulation in the soma and proximal dendrites of granular endoplasmic reticulum that disintegrates and leads to chromatolysis. The signs of cell death are reflected in the dendrites as shrinking and densification of the cytoplasm, a form of dendritic pathology frequently seen after traumatic injury.

The pathological effects of deafferentation or axotomy may require days to materialize. More immediate alternations in dendrites are apparent with hypoxia or ischemia, as during a stroke. Hypoxia causes a loss of energy that retards the ability of dendrites to maintain ionic polarity at the cell membrane. Abnormal dendritic varicosities are seen in brain tissue damaged by tumors (Fig. 1.17; Spacek, 1987), convulsions (Scheibel and Scheibel, 1977), and cold temperatures.

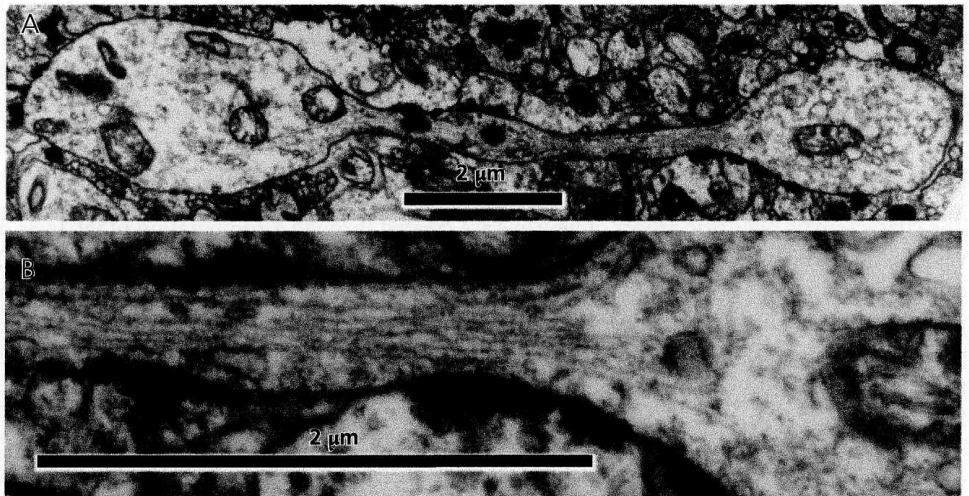**Fig. 1.17** Dendrites beaded by hydropic swelling. (**A**) Electron micrograph showing a segment of dendrite in human peritumorous neocortex in which two varicosities with watery cytoplasm are separated by a narrower region of denser cytoplasm. (**B**) Higher magnification of the intervaricosity region shows that it contains some vesicular components and microtubules and filaments that do not span the varicosities.

Dendritic swelling also affects the cytoplasmic organelles. The SER can become dilated or even swollen into large vacuoles. In Purkinje cells the dendritic SER first forms into lamellar arrays of cisternae (Banno and Kohno, 1996). Mitochondria can also be swollen in dendrites following hypoxia, and microtubules can be completely depolymerized.

A number of progressive neurodegenerative disorders are associated with other forms of dendrite pathology (Hirano, 1981). A different type of dendritic varicosity containing abnormal protein aggregations known as Lewy bodies occurs in Parkinson's disease. In Creuzfeldt–Jakob disease, a form of prionosis, vacuolar dystrophy within dendrites causes a spongiform appearance of the neuropil. Other inclusions of various metabolites may fill the dendritic cytoplasm in different enzymopathies.

Pathology of synaptic specializations of dendrites

The synaptic specializations of dendrites are also prone to structural distortions by a variety of insults and diseases (see Chapter 24). The pathologies of dendritic spines have been particularly well-studied, as summarized in Fig. 1.18 and reviewed in Fiala et al. (2002b) and Kuwajima et al. (2013). Two general categories of spine pathology are distinguished as pathologies of distribution and pathologies of ultrastructure.
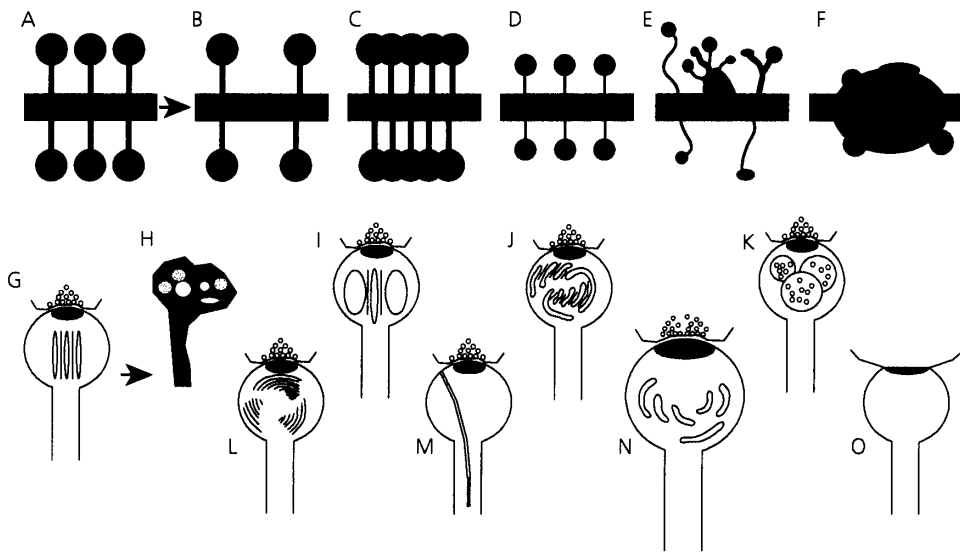
Fig. 1.18 Schematic of dendritic spine pathologies. (A–F) Pathologies of distribution as seen, for example, by light microscopy, involve differences from normal spines (A) by: (B) decreased density, (C) increased density, (D) reduction in spine size, (E) distortions of normal spine shapes, and (F) abnormally varicose dendrites that absorb spines. (G–O) Observed pathologies from normal spine ultrastructure (G) include: (H) shrunken spines with dense cytoplasm, (I) altered endoplasmic reticulum, (J) hypertrophied spine apparatus, (K) hypertrophied multivesicular bodies, (L) hypertrophied cytoskeleton, (M) spine microtubules, (N) giant spines, and (O) axonless spines with an axon-free postsynaptic density.

Many conditions lead to changes in the number of dendritic spines along spiny dendrites. Spine loss commonly occurs within a few days of deafferentation. Permanent spine loss is evident in most forms of mental retardation, including those resulting from prenatal infection, malnutrition, and exposure to toxins or alcohol. Spine loss is also seen in epilepsy, prionosis, and various neurodegenerative disorders. Increased spine density is seen paradoxically in some types of deafferentation, such as when Purkinje cells are deprived of their climbing fiber input. Increased spine numbers have also been reported following chronic use of stimulatory drugs. In some cases, an overabundance of dendritic spines may represent a failure of normal elimination of synapses during development, as has been suggested for fragile-X syndrome (Cruz-Martin et al., 2010; Portera-Cailliau, 2012).

The absence of normal levels of presynaptic activity without the destruction of the input axons can result in a reduction in synapse and spine size, as reported in the visual cortex following visual deprivation from birth (Globus, 1975). In contrast, deafferentation often results in excessive enlargement of the remaining spines and synapses as an apparent compensatory mechanism for the decreased synaptic input. Deafferentation can also lead to lengthening of dendritic spines, and a similar distortion of the shape of dendritic spines is seen in mental retardation and other conditions. The unusually long and tortuous spines, which have no head enlargement or multiple swellings along their length, resemble in many respects the filopodia seen during developmental synaptogenesis. This resemblance suggests that synaptogenic mechanisms are active in pathological conditions and long, tortuous spines are an additional compensatory response to loss of afferents (Fiala et al., 2002b).

Another response to deafferenting conditions is the formation of axonless spines. Axonless spines exhibit an intracellular structure that looks like a postsynaptic density, but this structure does not form part of a synapse with an axon. Rather, axonless spines contact glia or the dendrites of other neurons (Spacek, 1982). Although they are observed occasionally in the normal brain, the occurrence of axonless spines increases in many pathological conditions, for example developmental agenesis of cerebellar granule cells (Altman and Bayer, 1997).

Alterations in spine organelles can occur for several reasons. One may be degeneration of the postsynaptic cell, as is often the case when the dendritic cytoplasm becomes dense and dark. Another reason may be excitotoxic injury due to excessive presynaptic release of glutamate, which characteristically leads to thickening of the postsynaptic density, a condition often seen in ischemia. Ischemia also induces postsynaptic density-like structures that lie free in the cytoplasm of the dendrite (Tao-Cheng et al., 2001).

Changes in spine endosomes, endoplasmic reticulum, and cytoskeleton have been occasionally observed in edema, for example after traumatic injury. Hydropic swelling and vacuolization of the endoplasmic reticulum of dendritic spines is frequently seen in edematous tissue. In addition to swelling, the spine apparatus can become elaborated or atrophied. Loss of ionic regulation at the plasma membrane can also lead to depolymerization of microtubules in the dendrite shaft, as already mentioned.

Concluding remarks

A hundred years after Ramón y Cajal's pioneering work, the intricacies of the relationship between structure and function in neurons are still being discovered. The pattern of dendritic arborization is clearly related to connectivity but also contributes to dendritic computation, particularly when the dendrite is endowed with active mechanisms (see Chapter 12). The synaptic specializations extended by dendrites also make a significant contribution to connectivity,

allowing thin dendrites to interdigitate in a relatively small brain volume. Their diverse structures presumably have diverse functions related to neuronal computation and learning (see Chapter 18). What these changes in role or differences in dendritic and synaptic structure confer during normal development, learning, and memory remains an area of active investigation. Assuming that form predicts function, the diversity in dendritic and synaptic structure and composition discussed here suggests that many mechanisms are available for adjusting the strength of connectivity across the brain.

Acknowledgments

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