Dendrites

SECOND EDITION

Edited by

Greg Stuart
Australian National University

Nelson Spruston
Northwestern University

Michael Häusser
University College London

OXFORD
UNIVERSITY PRESS

OXFORD
UNIVERSITY PRESS

Great Clarendon Street, Oxford OX2 6DP

Oxford University Press is a department of the University of Oxford.
It furthers the University's objective of excellence in research, scholarship,
and education by publishing worldwide in

Oxford New York

Auckland Cape Town Dar es Salaam Hong Kong Karachi
Kuala Lumpur Madrid Melbourne Mexico City Nairobi
New Delhi Shanghai Taipei Toronto

With offices in

Argentina Austria Brazil Chile Czech Republic France Greece
Guatemala Hungary Italy Japan Poland Portugal Singapore
South Korea Switzerland Thailand Turkey Ukraine Vietnam

Oxford is a registered trade mark of Oxford University Press
in the UK and in certain other countries

Published in the United States
by Oxford University Press Inc., New York

© Oxford University Press, 2008

The moral rights of the authors have been asserted
Database right Oxford University Press (maker)

First published 2008

All rights reserved. No part of this publication may be reproduced,
stored in a retrieval system, or transmitted, in any form or by any means,
without the prior permission in writing of Oxford University Press,
or as expressly permitted by law, or under terms agreed with the appropriate
reprographics rights organization. Enquiries concerning reproduction
outside the scope of the above should be sent to the Rights Department,
Oxford University Press, at the address above

You must not circulate this book in any other binding or cover
and you must impose the same condition on any acquirer

British Library Cataloguing in Publication Data

Data available

Library of Congress Cataloging in Publication Data

Data available

Typeset by Cepha Imaging Pvt. Ltd., Bangalore, India
Printed in Spain
on acid-free paper by
Cayfosa-Quebecor

ISBN 978-0-19-856656-4

10 9 8 7 6 5 4 3 2 1

Chapter 1

Dendrite structure

John C. Fiala, Josef Spacek, Kristen M. Harris

Summary

Dendrites are extensions of the cell body of the neuron specialized for receiving and processing synaptic inputs. Dendrites exhibit enormously diverse forms. In many cases the shape of the dendritic arbor can be related to the mode of connectivity between neurons, with dendrites often ramifying in characteristic spatial domains where they receive specific inputs. Synaptic inputs occur directly on the shaft of some dendrites, but other dendrites have specialized enlargements or protrusions to receive synapses. These synaptic specializations also occur in many different forms related to local connectivity requirements. The use of serial section electron microscopy to obtain detailed quantitative data on these structures has shown that synaptic specializations differ widely in dimensions, distribution, and intracellular composition. The shape and composition of dendrites are under the continual influence of the local environment, as demonstrated by various pathological conditions. Understanding the structural diversity of dendrites is essential for understanding the intricacies of dendritic function and the contribution dendrites make to mental processes.

Introduction

What is the purpose of dendrites? Why do they exhibit such an overwhelming variety and complexity of shapes? How are their shapes related to neuronal function? Ramón y Cajal posed and, to a remarkable degree, answered these questions 100 years ago in his numerous publications and compendium *Histology of the Nervous System* (recently translated into English: Ramón y Cajal 1995). Ramón y Cajal showed that the two types of neuronal processes, axons and dendrites, do not interconnect in anastomotic continuity. This established the *neuron doctrine* that neurons are independent entities. The many dendrites of a neuron receive electrical impulses from the axons of other neurons and conduct this activity to the neuron's own axon for transmission to other cells. This basic tenet is often referred to as *dynamic polarization.*

As Ramón y Cajal correctly surmised, dendrites are the receptive surfaces of the neuron. However, it is now known that dendrites can also be output devices. Dendrites containing synaptic vesicles make reciprocal synapses on other dendrites in the retina, olfactory bulb, lateral geniculate nucleus, and cerebral cortex (Price and Powell 1970; Lieberman 1973; Sloper and Powell 1978; Ellias and Stevens 1980). While such dendro-dendritic

communication pathways are relatively rare, in the retina and the olfactory bulb they play an important role in neuron-to-neuron communication.

Ramón y Cajal also saw that the complexity of dendrites reflects the number of connections that a neuron receives. Consider that a neuron without dendrites, having a roughly spherical cell body, has a very limited surface area for receiving inputs. By extending dendrites, the nerve cell can increase the surface area without excessively increasing cell volume. For example, 97% of the surface area of a motor neuron (excluding the axon) is dendritic (Ulfhake and Kellerth 1981). The dendrites occupy 300 000 μm^3 while providing 370 000 μm^2 of surface area for synaptic input. To provide an equivalent surface, a spherical cell body would have to be 340 μm in diameter and 20 000 000 μm^3 in volume. The fact that 80% of the surface area of proximal dendrites of motor neurons is covered with synapses (Kellerth *et al.* 1979) suggests that this increased surface area is indeed valuable for increasing the number of inputs to a neuron. 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 a larger variety of axons.

Dendrites make relatively local connections as compared with the axon. The axon, emerging either from the soma or a dendrite, may extend to distant targets a meter or more away from the cell body in some cases (e.g. motor neurons and corticospinal projection neurons). Dendrites are rarely longer than 1–2 μm, even in the largest neurons, and are often much smaller (Table 1.1). In many neurons the diameter of dendrites at their origin from the cell body is proportional to the diameter of the cell body (Ulfhake and Kellerth 1981; Chen and Wolpaw 1994). The proximal dendrites taper and ramify in proportion to their size, such that the total length and number of branches are correlated with proximal diameter. Thus, larger neurons typically have both larger cell bodies and more extensive dendritic fields (Figure 1.1).

Ramón y Cajal argued that phylogenetic differences in specific neuronal morphologies support the relationship between dendritic complexity and number of connections. The complexity of many types of vertebrate neurons, including cerebellar Purkinje cells, cortical pyramidal cells, and mitral cells of the olfactory bulb, increases with increasingly complex nervous systems. (See Chapter 2 for more phylogenetic differences.) These changes are driven both by the need to make more connections and by the need to make connections with additional cell types at specific locations. As expressed by Sholl (1956), it is the *mode of connectivity* between neurons that is the most critical property of their diverse morphologies. The primary contribution of dendrites to a neuron's mode of connectivity is through their characteristic branching and extension into specific spatial domains. Axons from particular sources ramify within these domains, such that particular portions of the dendritic arbor receive specific inputs.

Dendrite arbors

Classification of morphologies is difficult because of the large number of different dendritic arborization patterns in neurons and the enormous variation in individual shapes. Elementary expositions often use the simple scheme originated by Ramón y Cajal,

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

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	3200
CA1 pyramidal cell (rat)	21						11900
basal dendrites		5	1	30	0.5–1	130	5500
stratum radiatum		1	3	30	0.25–1	110	4100
stratum lacunosum-moleculare				15	0.25–1	500	2300
Cerebellar Purkinje cell (guinea pig)	25	1	3	440	0.8–2.2	200	9100
Principal cell of globus pallidus (human)	33	4	4	12	0.3–0.5	1000	7600
Meynert cell of visual cortex (macaque)	35						15400
basal dendrites		5	3	—	—	250	10200
apical dendrites		1	4	15	2–3	1800	5200
Spinal α-motoneuron (cat)	58	11	8	120	0.5–1.5	1100	52000

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

Sources: Ito (1984); Mariani (1990); Claiborne *et al.* (1990); Bannister and Larkman (1995a); Rapp *et al.* (1994); Palay (1978); Yelnik *et al.* (1984); Ulfhake and Kellerth (1981).

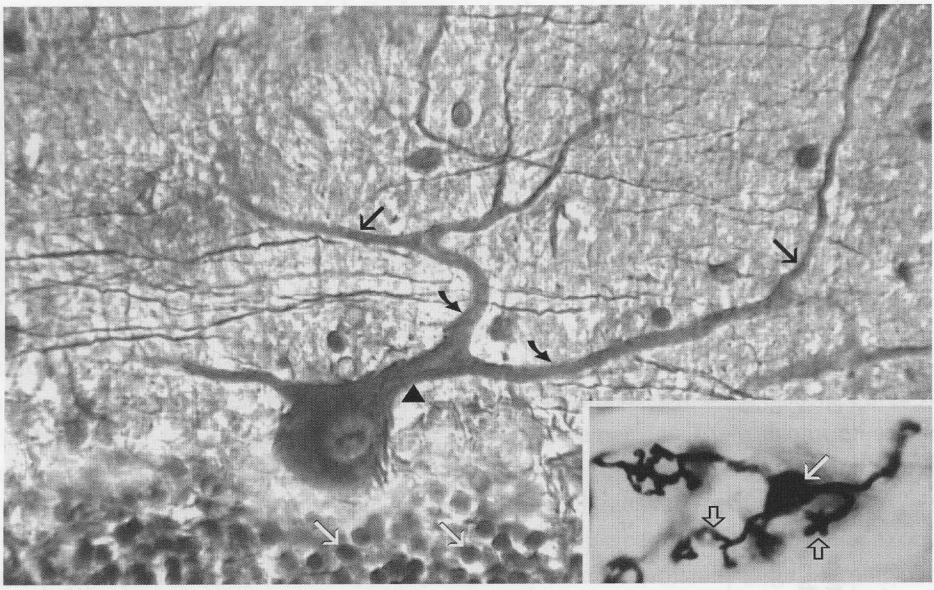


Fig. 1.1 Dendrites often look like tree branches. Thus, the name derives from the Greek word for tree: *dendron*. In this image of mouse cerebellar cortex silver-impregnated using the Bielschowski method, a thick primary dendrite (arrowhead) extends from the upper right of the cell body of a Purkinje neuron. The primary dendrite branches into secondary (curved arrows) and tertiary (straight arrows) dendrites within the plane of the section. The terminal dendritic branchlets are not visible with the Bielschowski method. Beneath the large Purkinje cell can be seen a layer of smaller granule cell bodies (white arrows). **Inset:** Higher magnification of a Golgi-impregnated granule cell (white arrow) reveals that this neuron possesses just a few, short dendrites with claw-like formations at the ends (open arrows).

in which neurons are classified as *unipolar*, *bipolar*, and *multipolar*, based on the number and orientation of processes emanating from the cell body. Ramón y Cajal intended this only as an introduction, however, and developed a detailed classification to differentiate the wide variety of multipolar neurons. A more geometrical classification of common mammalian dendritic arborizations will be useful for understanding the purposes of this diversity (Table 1.2).

Adendritic neurons have only a branched axon and no dendrites. Spinal dorsal root ganglia cells are examples of adendritic neurons. These neurons conduct sensory input to the central nervous system from the periphery but do not receive synaptic input from other neurons.

The slender neurons found throughout the brain may frequently be characterized as having a *spindle radiation*, with two sparsely branching dendrites emerging from opposite poles of the cell body. Other fusiform cells, such as the Lugaro cell of cerebellar cortex and the bipolar cells of cerebral cortex, may also be characterized as having this arborization pattern.

A common arborization pattern in the central nervous system is the *spherical* (or *stellate*) *radiation*. The principal neurons in nonlaminated nuclei, such as the inferior olive,

Table 1.2 Some characteristic dendritic arborization patterns

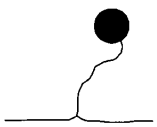
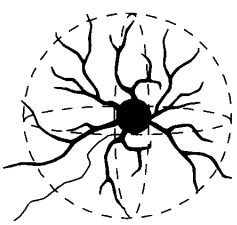
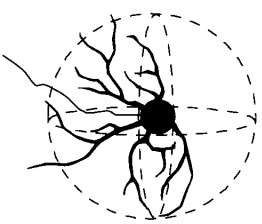
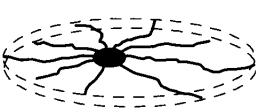
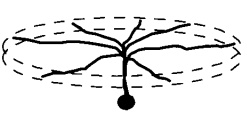
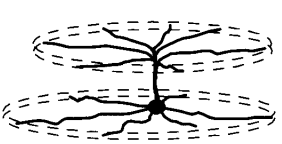
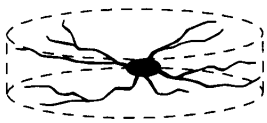
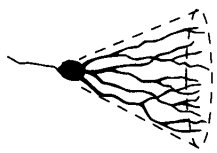
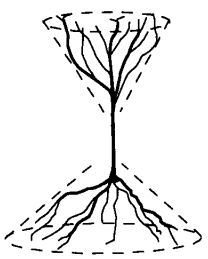
Pattern	Characteristics	Examples
Adendritic 	Cell body lacks dendrites	Dorsal root ganglion cells Sympathetic ganglion cells
Spindle radiation 	Two dendrites emerge from opposite poles of the cell body and have few branches	Lugaro cells Bipolar cells of cortex
Spherical radiation Stellate 	Dendrites 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

pontine nuclei, striatum, thalamus, etc., generally have spherical radiations of dendrites. Many interneurons, those neurons having axons that terminate locally, have spherical radiations as well. Although attempts have been made to describe the variety of stellate types in general terms (Ramón-Moliner 1968), classification often comes down to individual characteristics. For example, in the ventral cochlear nucleus there is a unique set of

descriptive morphologies: 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. In the cerebral cortex the primary distinguishing characteristic of the many stellate interneurons is not the dendritic arbor but rather the pattern of axon arborization (Jones 1975).

In some neurons, dendrites radiate in arbitrary directions from the cell body but are restricted to a planar region. This type of *laminar radiation* (see Table 1.2) is seen in horizontal cells of the retina (Kolb *et al.* 1994), and in some interneurons of cortex (Parra *et al.* 1988). Dendrites of retinal ganglion cells are laminar radiations offset by an apical stem. Nearly 20 kinds of retinal ganglion cells can be distinguished by their dendritic arborization patterns (Sterling 1990). Three basic types, alpha, beta, and gamma cells (Wingate *et al.* 1992), are easily distinguished physiologically (Fukuda *et al.* 1984). Apparently, the pattern of dendritic arborization of these neurons contributes to their physiological differences. The relationship between morphology and physiology is examined in more detail in other chapters.

In regions where there are distinct layers of cells and fibers, dendritic arbors are shaped to receive specific input sources in specific spatial domains. For example, the arbors of some retinal ganglion cells lie in the outer third of the inner plexiform layer (Sterling 1990). These receive contacts from bipolar cells that respond to light turning off. Other retinal ganglion cells have arbors in the inner two-thirds of the inner plexiform layer, receiving contacts from bipolar cells that respond to light turning on. The physiological differences between on and off ganglion cells arise in part from differences in their regions of dendritic arborization and sources of input.

Frequently, dendritic arbors ramify into more than one layer to access more than one type of afferent. Such is the case with the many multilaminar forms of retinal amacrine cells. At least 26 different types of amacrine cells can be identified based on their dendritic arborization and retinal tiling patterns (Mariani 1990; Kolb *et al.* 1992; MacNeil and Masland 1998). As with ganglion cells, the morphological differences in dendritic arbors almost certainly denote differences in the computational roles of these neurons.

In many brain regions there is an enormous variety of distinct neuronal shapes, as in the retina, but in other regions there appear to be relatively few. One example of the latter is the globus pallidus of primates, in which small interneurons appear to be infrequent. Principal components analysis reveals that the large pallidal neurons belong to a single population (Yelnik *et al.* 1984). The dendrites of these neurons fill cylindrical spatial domains approximately 1500×1000 μm in diameter and 250 μm thick (Tables 1.1 and 1.2). These dendritic disks are parallel to the boundaries of the globus pallidus and thus perpendicular to incoming striatal axons, such that each neuron receives a broad distribution of inputs.

Pyramidal cells often extend two distinct conical arbors, one from the apex and the other from 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. Pyramidal cells provide a good example of how arborization

patterns depend on the cell body location relative to preferred input sources. 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 usually do not have an apical stem at all, since one is not required to reach appropriate axons (Ramón y Cajal 1995).

Axonal inputs may pass near the apical stem dendrite of pyramidal cells, in which case additional dendritic branches emanate from the apical stem in a *cylindrical radiation*. This occurs, for example, in the large pyramidal cells of hippocampal area CA1 (Figure 1.2a). Here, the apical tuft arborizes in stratum lacunosum-moleculare to receive perforant path input from entorhinal cortex. The middle arbor in stratum radiatum receives the

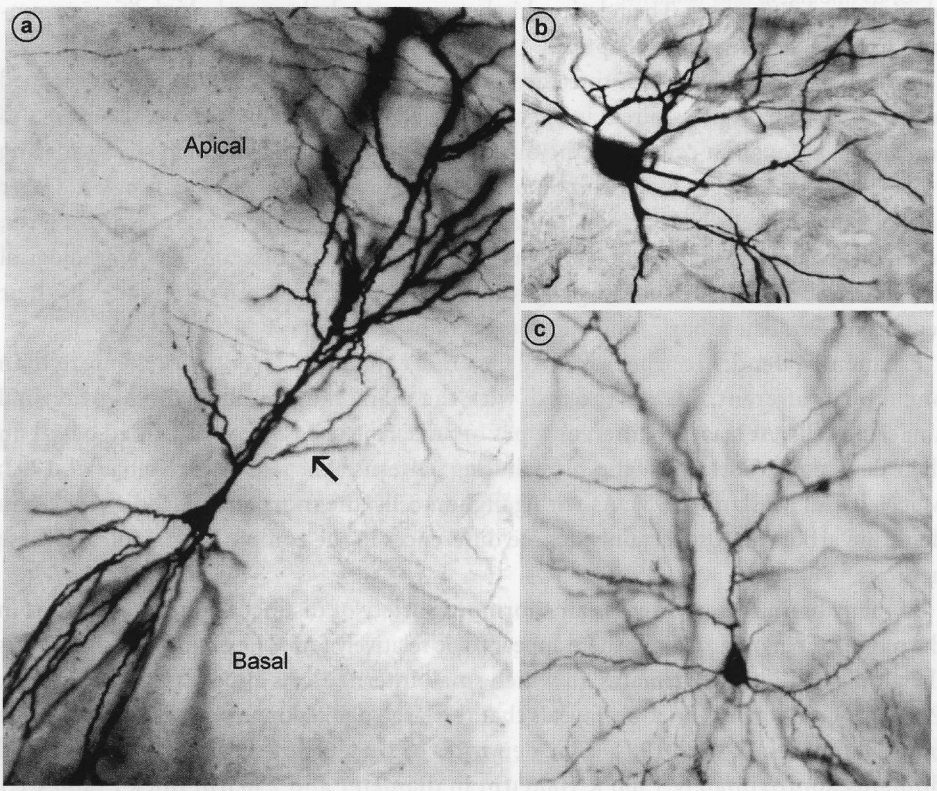


Fig. 1.2 Neurons silver-impregnated with the Golgi method. **(a)** A pyramidal neuron from hippocampal area CA1 in which basal and apical dendrites ramify in separate conical domains. Between these two denser domains, a few dendrites extend obliquely (arrow) from the apical stem in stratum radiatum (rat; Harris *et al.* 1980). **(b)** A thalamic projection neuron exhibits many primary dendrites extending from the cell body (rat; reprinted with permission from Spacek and Lieberman 1974). **(c)** A pyramidal neuron from the parietal area of the cerebral cortex has a few dendrites that extend almost horizontally from the base of the cell body, and a sparsely branching apical dendrite (mouse).

Schaffer axon 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 CA1 pyramidal cell may be characterized as having three different spatial domains of dendritic arborization, an apical cone, a basal cone, and a central cylinder. A similar pattern is frequently seen in neocortical pyramidal cells (Feldman and Peters 1978; Prieto and Winer 1999).

Table 1.2 summarizes some common arborization patterns, but it is far from exhaustive. Many other arbors can be characterized by elaborations on or combinations of the basic patterns of Table 1.2. Mitral cells of the olfactory bulb, for example, may exhibit a number of variations such as a 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 cones (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).

Not only is the geometry of the arborization important for connectivity, but so is the density of the arborization (Figure 1.3). At one extreme, a dendrite connects a single

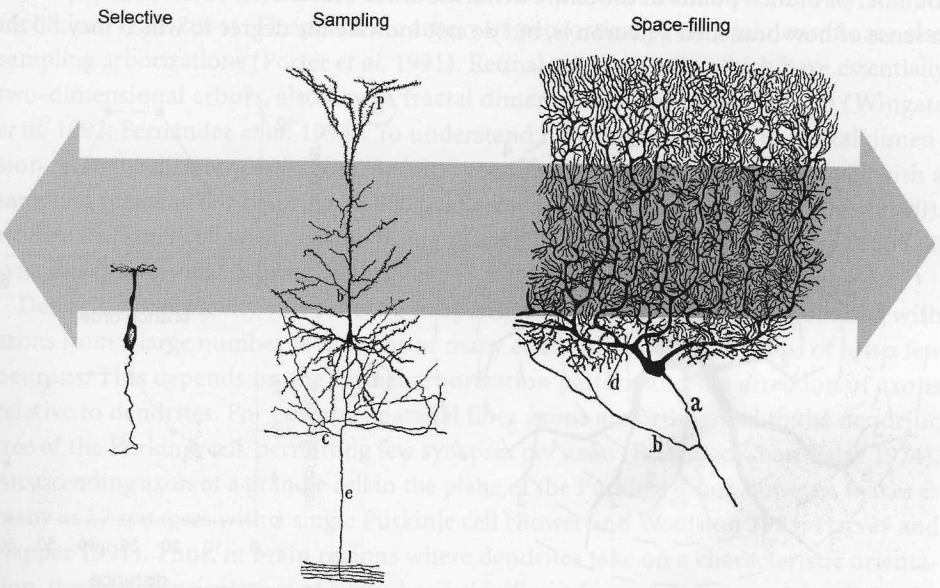


Fig. 1.3 The densities of dendritic arbors lie on a continuum of values. Differences in arbor density reflect differences in connectivity. At one extreme are selective arborizations in which each dendrite connects the cell body to a single remote target. An olfactory sensory cell is used to illustrate this. At the other extreme lie 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 (drawings of neurons from Ramón y Cajal 1995). By permission of Oxford University Press.

remote target to the rest of the neuron. This is a *selective* arborization. At the other extreme, dendritic branches occupy most of the domain of arborization in a *space-filling* arborization. An example of this is the cerebellar Purkinje cell arbor that synapses with at least half of the parallel fiber axons that pass through it (Palay and Chan-Palay 1974; Napper and Harvey 1991). Most dendritic arborizations lie between the selective and space-filling varieties and are therefore *sampling* arborizations. For example, a CA1 pyramidal cell arborizes in three different spatial domains and within each domain samples a tiny fraction of the available axons. Conical, cylindrical, and spherical radiations are usually sampling arborizations, with each dendritic branch receiving synapses along its length from a subset of the axons that pass nearby. Spherical radiations that are selective arborizations are also encountered, as in the dendrites of cerebellar granule cells, which make synapses only at their ends rather than along their entire length.

Many approaches are used to characterize the density of dendritic arborizations (Uylings and van Pelt 2002; Scorcioni *et al.* 2004). Branch ordering schemes are frequently used, such as the *centrifugal method* wherein the dendrites emerging from the cell soma are primary, their first branches are secondary, and so on, with increasing order until the tips are reached. The number of dendrite segments of each order characterizes the degree of branching of the arbor (Figure 1.4). A simpler scheme is to just count the number of branch points in the entire dendritic arbor (Table 1.1). Such schemes provide a sense of how branched a neuron is, but do not indicate the degree to which they fill the

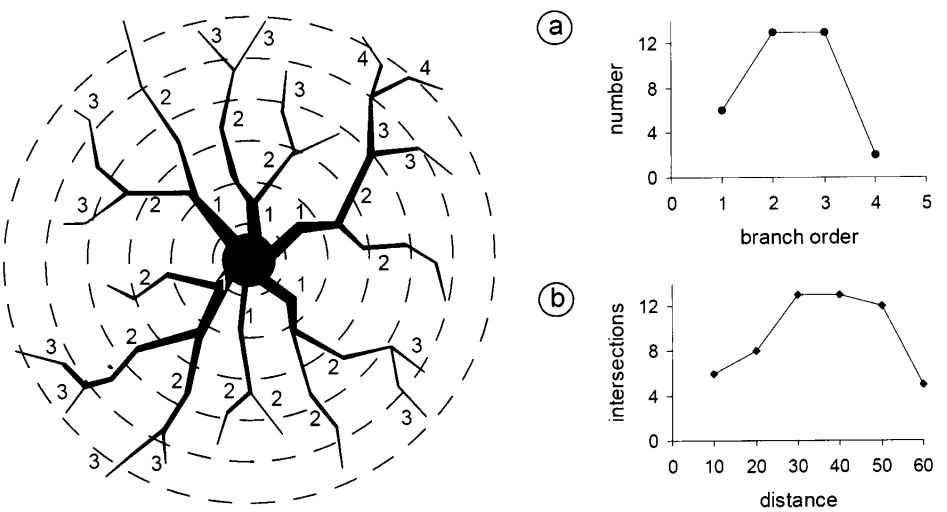


Fig. 1.4 Methods for characterizing dendritic branching. (a) A plot of the number of branches of each order using the centrifugal method of branch ordering. The *Strahler method* is similar but the dendritic tips are order 1 and branch numbers increase sequentially toward the soma. (b) A *Sholl plot* showing the number of intersections of the dendritic tree with circles of increasing radius from the center of the dendritic arbor. When three-dimensional data are available, concentric spheres are used rather than these circles centered on a two-dimensional projection of the neuron.

space of their arborizations, an important factor when considering their potential for connectivity.

Another measure, *fractal dimension*, can be used to quantify the degree to which an arborization fills its spatial domain. From basic geometry, linear objects have a dimension of 1; planar objects have a dimension of 2; and solid objects, such as a sphere, have a dimension of 3. In addition, there exist objects with non-integer dimensions, often called *fractal* objects, that 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 fractional dimension is useful for quantifying their space-filling tendencies (Smith *et al.* 1989; Panico and Sterling 1995; Fernández and Jelinek 2001). Selective arborizations have fractal dimensions close to unity, while space-filling arborizations have fractal dimensions close to the dimension of the geometrical region they occupy. For instance, the fractal dimension of the Purkinje cell dendritic arbor is about 1.8 in mammals, indicating that the Purkinje cell covers most of the two-dimensional area in which it ramifies. In agreement with Ramón y Cajal's assessment of the phylogenetic trend in complexity, the fractal dimension of Purkinje cells increases with phylogeny, from a value of 1.13 in lamprey up to a value of 1.86 in humans (Takeda *et al.* 1992).

Sampling arborizations have fractal dimensions greater than 1 but much less than the dimension of the spatial domain in which they arborize. Hippocampal pyramidal neurons arborize in a three-dimensional volume but have a fractal dimension of only 1.4–1.5 (Scorcioni *et al.* 2004). The dendritic arbors of neocortical pyramidal neurons are also sampling arborizations (Porter *et al.* 1991). Retinal ganglion cells, which have essentially two-dimensional arbors, also have a fractal dimension of approximately 1.5 (Wingate *et al.* 1992; Fernández *et al.* 1994). To understand how the differences in fractal dimension relate to differences in connectivity, consider that a retinal ganglion cell with a sampling planar arbor covering 25 000 μm^2 receives only 2000 synapses (Sterling 1990), while a Purkinje cell with a space-filling planar arbor of 50 000 μm^2 receives 160 000 synapses (Harvey and Napper 1991).

Dendritic complexity can reflect a propensity for a neuron to make contacts with axons from a large number of neurons or many connections with the axons of just a few neurons. This depends on the axonal arborization pattern and the direction of axons relative to dendrites. For example, parallel fiber axons are orthogonal to the dendritic tree of the Purkinje cell, permitting few synapses per axon (Palay and Chan-Palay 1974). An ascending axon of a granule cell in the plane of the Purkinje arbor, however, makes as many as 17 synapses with a single Purkinje cell (Bower and Woolston 1983; Harvey and Napper 1991). Thus, in brain regions where dendrites take on a characteristic orientation, the relative orientation of axons has a significant impact on connectivity. Since the direction of dendrites relative to that of afferent axons varies, pyramidal cells may receive many synapses from a single axon which runs parallel to a dendritic segment or few synapses from axons which traverse its dendrites perpendicularly (Sholl 1956; Sorra and Harris 1993). The ability of dendrites to connect with axons that pass nearby is also greatly influenced by dendritic protrusions, such as the dendritic spines which Ramón y Cajal first described more than a century ago.

Intracellular structure of dendrites

Electron microscopy reveals that the contents of large proximal dendrites are similar to those of the cell soma from which they arise (Figure 1.5). Somal organelles involved in protein synthesis, such as the *Golgi apparatus* and the *granular* (or *rough*) *endoplasmic reticulum*, extend into the proximal dendrites, reinforcing the view that dendrites are extensions of the cell body. These characteristically somal organelles diminish, however, with increasing distance from the cell body and decreasing dendrite diameter. Free ribosomes, those not attached to granular endoplasmic reticulum, occur throughout the cytoplasm of dendrites at a very low density compared to the soma. Frequently, they are closely clustered into groups of 3 to 30 called *polyribosomes* (Steward and Reeves 1988). Groups of polyribosomes often occur together in dendrites, especially at the branch points. In contrast to dendrites, axons are generally devoid of ribosomes except at the initial segment, suggesting that new proteins are transported from the cell body rather than manufactured within the axon.

The *cytoskeleton* of dendrites is composed of *microtubules*, *neurofilaments*, and *actin filaments*. 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). Microtubules are long, thin structures, approximately 24 nm in diameter, oriented to the longitudinal axis of the dendrite. Microtubules in the distal dendrites of CA1 pyramidal cells are about 90 μm in length (Fiala *et al.* 2003). In regions of the dendrite free of large organelles, microtubules are found in a regular array at a density of 50–150 per μm^2, typically regularly spaced at 80–200 μm (Figure 1.6). The number of microtubules in a dendritic cross-section is proportional to the dendrite diameter (Fiala *et al.* 2003). Axons also contain microtubules, usually with closer spacing than in dendrites or even very closely linked in the axon initial segment. However, the fine processes of glia do not contain microtubules ordered in a regular array.

Glial processes are highly irregular, extending protrusions into narrow crevices between axonal and dendritic processes (Figure 1.6). Axons tend to maintain a consistent, convex cross-section, except where they expand into varicosities containing mitochondria and/or synaptic vesicles. Small dendrites tend to be more irregular in cross-section than axons, while still maintaining convexity as compared with glia. The ultrastructure of glia also differs from that of axons and dendrites in that glia characteristically contain numerous glycogen granules and bundles of intermediate filaments (Figure 1.6). Axons and the dendrites of some types of interneurons may contain occasional glycogen granules, but the dendrites of principal neurons contain few such granules.

Smooth endoplasmic reticulum (SER) is an organelle found throughout dendrites, thought to be involved in the regulation of cytoplasmic calcium concentrations. SER in aldehyde-fixed brain tissue usually appears as a network of thin tubules and flattened cisternae having a clear interior bounded by a wavy membrane (Figure 1.6). SER is sometimes swollen into large vacuoles, depending on the state of the tissue. *Hypolemmal cisternae* are those parts of the SER network which lie just beneath the cell membrane. Often these cisternae form characteristic junctions (Figure 1.5) that could give access to the extracellular space (Henkart *et al.* 1976). In the three-dimensional view obtained by reconstruction from serial sections (Figure 1.7), the cisternae of SER form a continuous

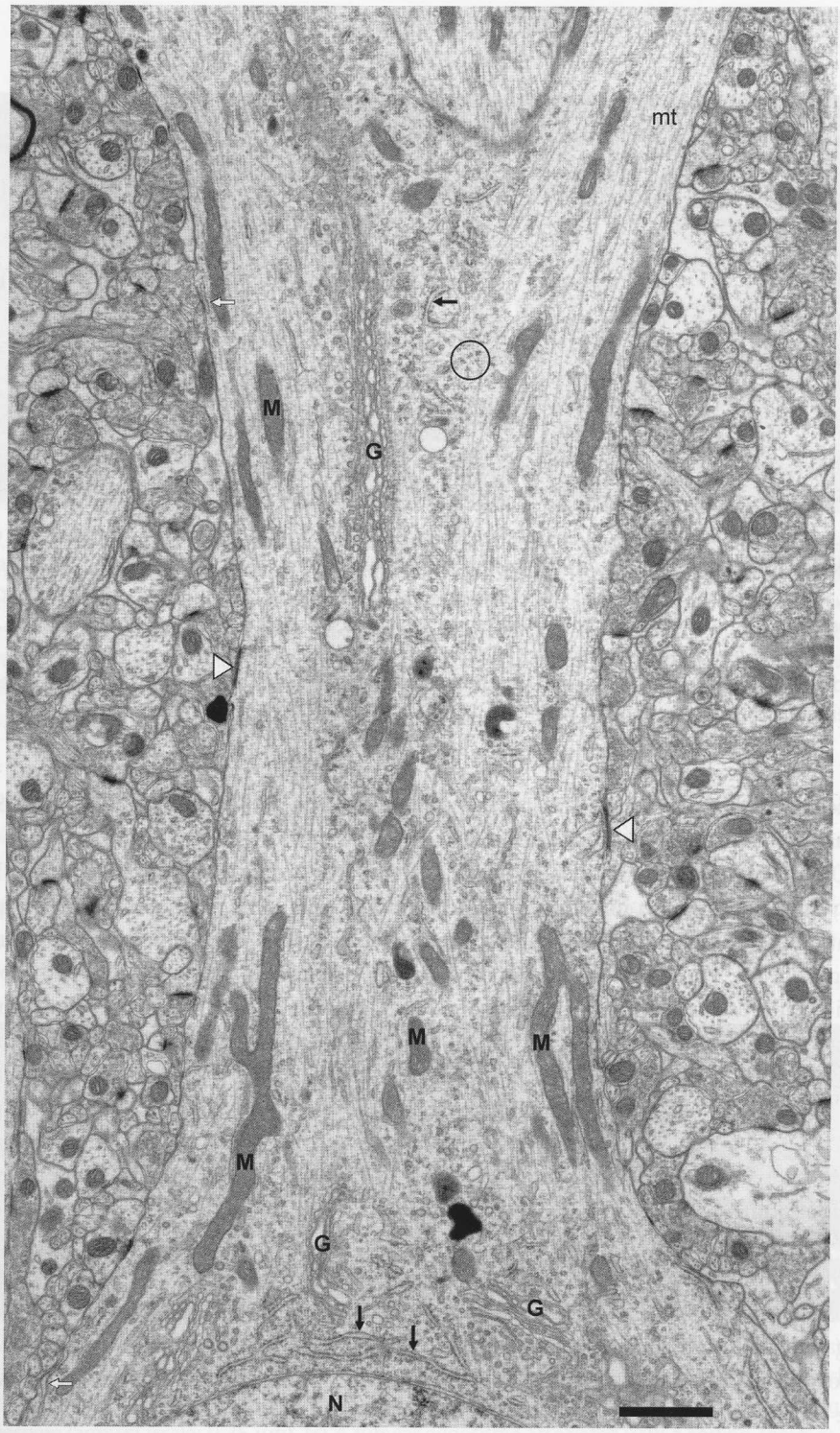


Fig.1.5 Somal organelles extend into the apical dendrite of a CA1 pyramidal cell. The secondary dendrite that branches off the apical stem (top-right) shows fewer of the characteristically somal organelles. Nucleus (N), granular endoplasmic reticulum (black arrows), Golgi apparatus (G), polyribosomes (circled), mitochondria (M), microtubules (mt), hypolemmal endoplasmic reticulum (white arrows), subsurface cisternal junctions of endoplasmic reticulum (white arrow heads) (rat; scale bar: 1 μm). Reproduced from *Dendrites, 1st edition* by Stuart, G. *et al.*, (1999). By permission of Oxford University Press.

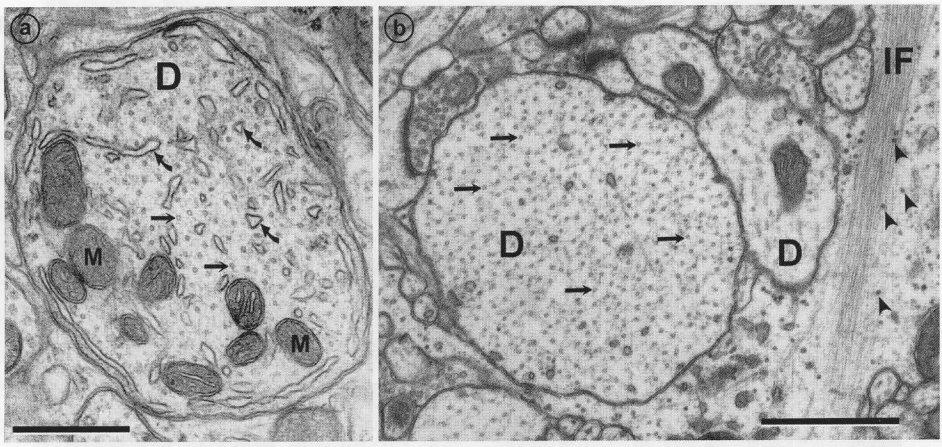


Fig. 1.6 Electron microscopy of large dendrites in cross-section. **(a)** A secondary dendrite (D) of a Purkinje cell contains an array of microtubules (horizontal arrows), cross-sectioned mitochondria (M), and large amounts of SER (curved arrows) (mouse; scale bar: 0.5 μm). **(b)** An apical stem dendrite (D) of a CA1 pyramidal cell displays a similar regular array of microtubules (arrows) but relatively few SER tubules and no mitochondria in this section. A nearby glial process is readily identified by the presence of numerous glycogen granules (arrowheads) and a bundle of intermediate filaments (IF) (rat; scale bar: 1 μm).

reticulum throughout the dendrites (Harris and Stevens 1988, 1989; Martone *et al.* 1993; Spacek and Harris 1997; Cooney *et al.* 2002).

Organelles of the early endosomal pathway involved in membrane protein sorting and recycling are common in dendrites (Cooney *et al.* 2002). *Coated pits* and *vesicles* representing the initial step in endocytosis are frequently seen at the membrane of dendrites (Figure 1.8a). The cytoplasmic coat is composed of *clathrin*, giving it a distinctive periodic structure recognizable as the same coat found on the tips of early endosomes (Figure 1.8c). Coated vesicles and coated pits occur more frequently in dendrites during development (Altman 1971) and during periods of synaptic remodeling (McWilliams and Lynch 1981). *Recycling endosomes* appear as tubular compartments that are not part of the endoplasmic reticulum. These can be distinguished from SER by their darker interior, more uniform diameter, and the frequent occurrence of specialized coats at the ends of the tubule. These coated ends represent sites of budding of recycling vesicles bound for the cell membrane. *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*, separate from the sorting endosome, and are transported to the cell body for processing in late endosomes and lysosomes. Thus, multivesicular bodies in dendrites occur alone or in conjunction with the sorting endosome compartments (Figure 1.8b and d).

Mitochondria in dendrites are typically rod-shaped organelles with the long axis parallel to the microtubules (Figure 1.5). They vary greatly in length, many less than 5 μm long but some more than 10 μm. In the apical dendrites of hippocampal pyramidal neurons, branched mitochondria can form a large network over 25 μm long (Popov *et al.* 2003).

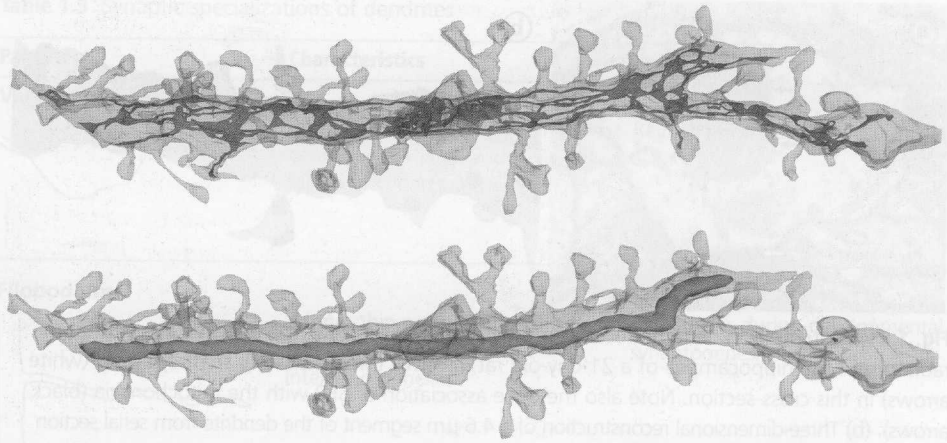


Fig. 1.7 Smooth endoplasmic reticulum is a single compartment in the dendrites. This reconstruction from serial section electron microscopy shows the SER network (top) in a 12.5-μm segment from the lateral dendrites of a CA1 pyramidal cell in stratum radiatum of the rat hippocampus. The SER network consists of thin tubules and wider cisternae coursing in the outer part of the dendrite. A mitochondrion (bottom) runs down the center of the SER network and has numerous contacts with it. The SER network continues into both branches on the right end of the segment, but only occasionally enters into the many dendritic spines that protrude from the dendrite shaft.

In the fine dendrites (<0.5 μm diameter), a single mitochondrion usually lies in the center of the SER network (Figure 1.7). Mitochondria often have a very intimate relationship with SER in dendrites, as shown in Figure 1.9; see also Spacek and Lieberman (1980). In stratum radiatum of area CA1, mitochondria comprise about 2% of intracellular space in the apical stem dendrites, while filling 13% of the thinnest branches in the apical tuft

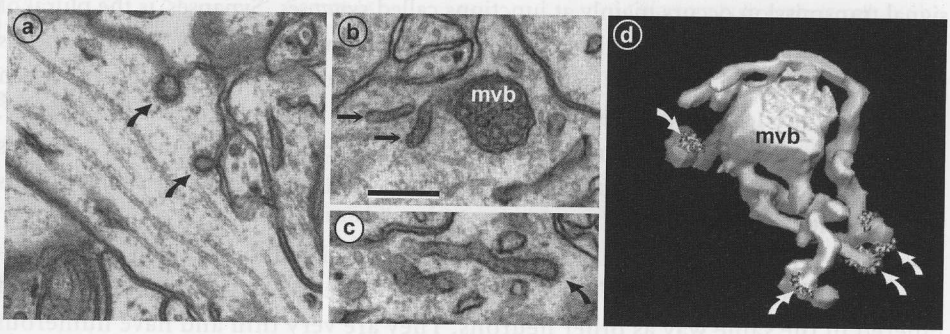


Fig. 1.8 Endosomes in rat hippocampal pyramidal cell dendrites. **(a)** Clathrin-coated pits (arrows) are the initial step in the endosomal pathway. These pits form intracellular vesicles destined for recycling endosomes. **(b)** A multivesicular body (mvb) with its connected tubular compartments of sorting endosome (arrows) (scale: 0.25 μm). **(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 multivesiccular 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). Adopted from *Dendrites, 1st edition* by Stuart, G. *et al.*, (1999). By permission of Oxford University Press.

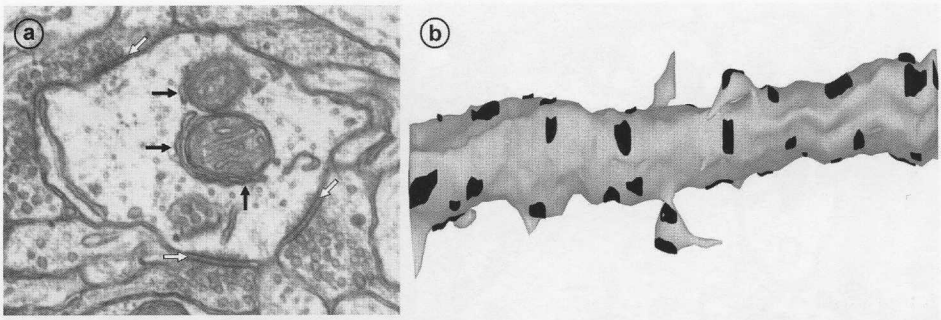


Fig. 1.9 An interneuron dendrite receives mostly shaft synapses. **(a)** The dendrite, from stratum radiatum of the hippocampus of a 21-day-old rat, receives three excitatory shaft synapses (white arrows) in this cross-section. Note also the close association of SER with the mitochondria (black arrows). **(b)** Three-dimensional reconstruction of a 4.6-μm segment of the dendrite from serial section electron microscopy reveals 45 excitatory synaptic contacts (black), 91% of which are located on the dendrite shaft. 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).

(Nafstad and Blackstad 1966). Mitochondria may be distributed based on metabolic demands related to synaptic inputs (Li *et al.* 2004). Dendrites which receive a high density of inputs, such as the fine branchlets of cerebellar Purkinje neurons, appear to have a high density of mitochondria (Peters *et al.* 1991). A remarkable accumulation of mitochondria was observed in regions of dendritic cytoplasm adjacent to giant presynaptic axons in thalamic nuclei (Lieberman and Spacek 1997).

Synaptic specializations of dendrites

Although a dendrite is adjacent to many axons and other dendrites throughout its length, signal transmission occurs mainly at junctions called *synapses*. 'Synapses' is the plural of 'synapsis', a term first employed by Foster and Sherrington (Foster 1897). Synapsis means 'connection' (Murray *et al.* 1919), apropos for the site of functional connectivity between neurons. In modern usage, the singular of 'synapses' is usually expressed as *synapse*. Synapses can reside directly on the surface of the dendrite, so-called *shaft* synapses (Figure 1.9), or on specialized enlargements or protrusions of the dendrite (Table 1.3), as with dendritic spine synapses.

Varicosities in the dendrite are one type of synaptic specialization found in certain neurons, such as amacrine cells of the retina. The dendrites of AI amacrine cells are not tapered in the same way as other neurons. They are very thin and have numerous swellings at contacts with rod bipolar cells (Ellias and Stevens 1980). The varicosities both receive synapses from rod bipolar cells and make reciprocal synapses on the bipolar cells. Dendritic varicosities are also frequently described in interneurons (e.g. DiFigilia and Carey 1987), but the purpose of these varicosities is much less clear since they are neither presynaptic nor exclusive sites of synaptic input.

Table 1.3 Synaptic specializations of dendrites

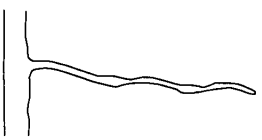
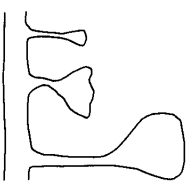
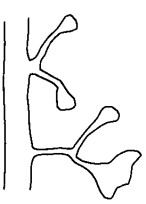
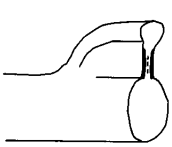
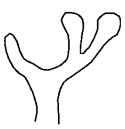
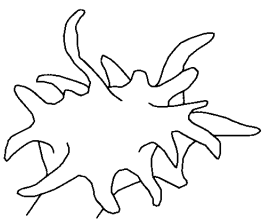
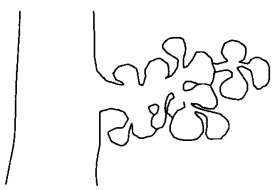
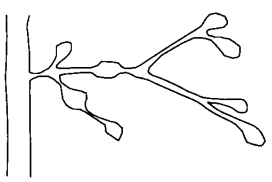
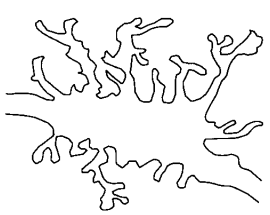
Pattern	Characteristics	Examples
Varicosity 	An enlargement in a thinner dendrite associated with synaptic contacts	Retinal amacrine cells
Filopodium 	A long, thin protrusion with a dense actin matrix and few internal organelles	All neurons during developmental synaptogenesis
Simple spine		
Sessile 	Synaptic protrusions without a neck constriction	
	Sessile spine	Cerebral pyramidal cells
	Stubby spine	Cerebral pyramidal cells
	Crook thorn	Neurons of dentate nucleus
Pedunculated 	Bulbous enlargement at tip	
	Thin spine	Cerebral pyramidal cells
	Mushroom spine	Cerebral pyramidal cells
	Gemmule	Olfactory bulb granule cell
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
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

Table 1.3 (continued) Synaptic specializations of dendrites

Pattern	Characteristics	Examples
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 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

Filopodia are another synaptic specialization of dendrites. All neurons exhibit dendritic filopodia transiently during development (Morest 1969). These filopodia are highly dynamic, extending and retracting within a few minutes (Dailey and Smith 1996; Fischer *et al.* 1998). After the developmental period, however, filopodia diminish (Dunaevsky *et al.* 1999; Grutzendler *et al.* 2002). Filopodia appear to play a role in synaptogenesis, often making nascent synaptic contacts (Fiala *et al.* 1998), although many filopodia are synapseless. Long filopodia are rarely seen on dendrites in normal adult brain, perhaps because such long protrusions are not required for establishing new contacts. Adult neuropil is densely backed with axonal boutons, such that only a short dendritic outgrowth is required for a dendrite to encounter a new presynaptic partner. Filopodia are generally replaced with shaft synapses or other types of synaptic specializations during maturation.

Dendritic spines are the most common synaptic specializations of dendrites. *Simple spines* are protrusions from the dendrite of usually no more than 2 μm, often ending in a bulbous head attached to the dendrite by a narrow stalk or neck (Figure 1.10). Simple spines are frequent on the dendrites of many types of neurons, including cerebral pyramidal cells, striatal neurons, granule cells of the dentate gyrus, cartwheel cells of the dorsal cochlear nucleus, and cerebellar Purkinje cells. Most interneurons have few, if any, dendritic spines (Figure 1.9). More generally, neurons are classified as *spiny*, *sparsely spiny*, and *nonspiny* (or *smooth*) according to the density of simple spines on their dendrites (Feldman and Peters 1978). Such a classification is complicated by the fact that different dendrites of a given neuron may exhibit widely different spine densities. Even along the length of a dendritic segment, spine densities can vary widely. Nominally nonspiny dendrites often exhibit a few simple spines.

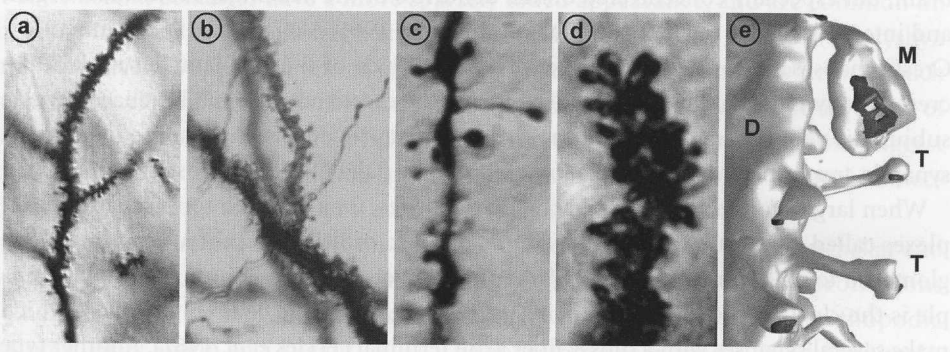


Fig. 1.10 Dendritic spines at increasing magnifications. **(a)** Apical dendrite of a neocortical pyramidal neuron has few spines near the soma (bottom of image) and many spines distally (Golgi; rat). **(b)** Spiny dendrites of a CA1 pyramidal cell of hippocampus (Golgi; rat). **(c)** High magnification of a neocortical pyramidal neuron dendrite (Golgi; mouse). **(d)** Spiny branchlet of a cerebellar Purkinje cell (Golgi; mouse). Images c and d reprinted with permission from Spacek and Hartmann (1983). **(e)** Three-dimensional reconstruction of a segment of CA1 pyramidal cell dendrite (D) showing thin (T) and mushroom (M) pedunculated spines (serial section electron microscopy; rat).

CA1 pyramidal neurons are very spiny, averaging 2.5 spines per μm over the total dendritic length of about 12 μm. By comparison, pyramidal cells of visual cortex are much less spiny averaging only about 1.5 spines per μm (Larkman 1991). Higher spine densities can be found on a few other neuron types, such as certain neostriatal neurons that have 7.2 spines per μm (Graveland *et al.* 1985). The spiniest neuron may be the cerebellar Purkinje cell, with spine densities reaching 15 spines per μm on the spiny branchlets (Harris and Stevens 1988). A Purkinje neuron in the rat may have over 160 000 spines (Napper and Harvey 1988). There are so many spines on many spiny neurons that they contribute substantially to the cell's surface area. In the case of the CA1 pyramidal cell, spines contribute nearly half of the dendritic surface area (Bannister and Larkman 1995b).

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 synapse on different boutons (Harris and Stevens 1988; Trommald and Hulleberg 1997; Sorra *et al.* 1998). Branched spines occur infrequently on spiny neurons, being only 2% of all dendritic protrusions on CA1 pyramidal cells (Harris *et al.* 1992) or dentate granule cells (Trommald and Hulleberg 1997). Apparently, branched spines are formed by accidental proximity of spine origins. This is a rare event because spine origins exhibit a nonrandom tendency to separate (Trommald *et al.* 1995; Ward *et al.* 1995). Branched spines are more frequent on dendrites with higher spine densities, such as Purkinje cell dendrites, where approximately 6% of spines are branched (Harris and Stevens 1988). Likewise, higher spine densities lead to larger numbers of branches per branched spine. Up to five branches have been found on Purkinje cell branched spines (Harris and Stevens, 1988), while CA1 pyramidal cell branched spines rarely have more than two branches (Sorra *et al.* 1998).

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).

When large axon terminals interact with dendrites, they often do so in synaptic complexes called glomeruli. Dendrites extend complex, multilobed protrusions into these glomeruli to make large numbers of synaptic contacts with the bouton. A simple example is the *claw endings* of the dendrites of cerebellar granule cells (Figure 1.1), which make several synapses with a mossy fiber axon terminal (Eccles *et al.* 1967). Another type of specialized dendritic ending associated with some cerebellar glomeruli is the *brush endings* of unipolar brush cells. These multilobed protrusions have a unique appearance which may be related to the fact that they are presynaptic to the claw endings of cerebellar granule cells as well as postsynaptic to mossy fibers (Mugnaini *et al.* 1994).

Thorny excrescences are another type of synaptic specialization associated with large axonal boutons (Figure 1.11). CA3 pyramidal cells have large numbers of simple spines, but 90% of the dendritic protrusions on the proximal apical dendrite are thorny excrescences

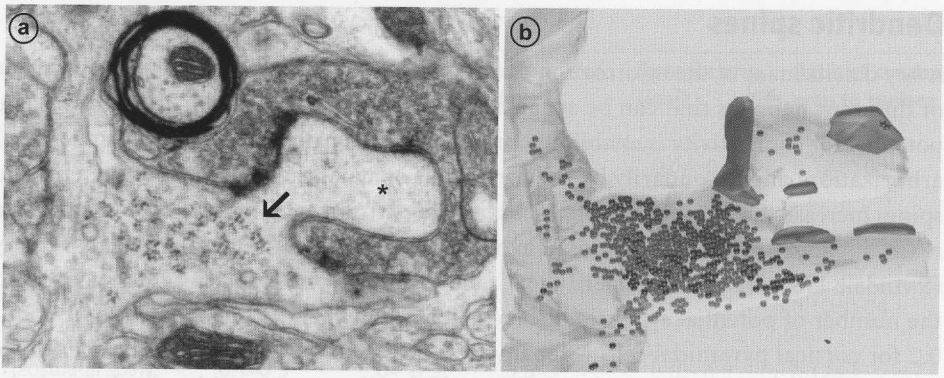


Fig. 1.11 A small thorny excrescence of a thalamocortical relay neuron of the ventrobasal nucleus. **(a)** Electron micrograph of section through the excrescence. Beneath the synaptic lobe (asterisk) is a region (arrow) with a large number of polyribosomes. **(b)** Reconstruction of the complete excrescence from serial sections reveals the intense concentration of polyribosomes. Synaptic areas are gray regions (rat; adapted from http://synapse-web.org/).

synapsing with the large boutons of mossy fiber axons from dentate granule cells (Chicurel and Harris 1992). The complexity of these thorny excrescences varies, with some having many lobes and others having just a few. Three-dimensional reconstructions have been obtained of excrescences with as many as 16 lobes, all of which synapse with a single giant mossy fiber bouton. In a reconstructed glomerulus of the ventrobasal thalamic nucleus, 44 synapses were located on an excrescence invaginating a giant lemniscal bouton (Spacek and Lieberman 1974). The density of lobes in thorny excrescences often gives them the appearance of a bunch of grapes (Hama *et al.* 1994).

Racemose appendages have a more sparsely lobed appearance, and are common on neurons in inferior olive (Ruigrok *et al.* 1990) and lateral reticular nucleus (Hrycyshyn and Flumerfelt 1981). This type of protrusion can also be found on spiny neurons such as neocortical pyramidal cells. In fact, single pyramidal neurons can exhibit a variety of spine types and other appendages (Prieto and Winer 1999). Such observations suggest that the shape of dendritic synaptic specializations is determined largely by local connectivity requirements, rather than by neuron or presynaptic partner type.

Coralline excrescences are found on dendrites of the small neurons of the cerebellar and vestibular nuclei. These complex varicosities exhibit numerous synaptic protrusions (Chan-Palay 1977), and sometimes thin tendrils similar in appearance to filopodia (Morest 1969; Sotelo and Angaut 1973). The appearance of filopodia, in adult animals, suggests that these coralline excrescences represent growth processes of dendrites. All synaptic specializations of dendrites may be relatively dynamic structures, capable of some degree of structural change throughout life. This structural plasticity blurs morphological classifications, since synaptic specializations may evolve into different forms, such as from filopodia to spines, over time. Furthermore, the classification of Table 1.3 by no means exhausts the shapes of synaptic specializations of dendrites, as perhaps many related and intermediate forms are to be expected.

Dendritic spines

A key determinant of the information storage capacity of neural networks is the number of different neurons that can be synaptically connected (Chklovskii *et al.* 2004). The potential for connectivity is established primarily by the patterns of dendritic and axonal arborization, and secondarily by the formation of synaptic specializations. 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 increasing brain volume only slightly (Swindale 1981; Harris and Kater 1994; Chklovskii 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). This suggests that dendritic spines play a significant role in determining which axons synapse with a dendrite.

A CA1 pyramidal cell in the rat has 30 000 spines, with 55% of them located in stratum radiatum and 40% in stratum oriens (Megias *et al.* 2001). Consequently, different regions of the arbor have different spine densities. Spine densities on the lateral branches of the apical stem in stratum radiatum average about 3 per μm of dendrite as measured by both serial section electron microscopy (Harris *et al.* 1992; Megias *et al.* 2001) and by light microscopy with correction (Bannister and Larkman 1995b; Trommald *et al.* 1995). The distal dendrites of the basal cone have a similar spine density, while spine densities are much lower in the apical tuft. The apical stem itself in stratum radiatum has the highest spine density of 7 spines per μm (Megias *et al.* 2001). The most proximal dendrites of the pyramidal neuron receive inhibitory inputs in the form of shaft synapses, and so these regions, like the cell body, are devoid of spines.

The fact that different regions of a dendritic arbor have different characteristic spine densities reflects differences in connectivity to different excitatory inputs (Scheibel and Scheibel 1970; Feldman 1984). Another example of this is the giant pyramidal cells of Meynert in visual cortex (Chan-Palay *et al.* 1974). The basilar dendrites and the apical dendrite in layer 5 are densely covered with spines, but as the apical dendrite enters layer 4, spine density rapidly decreases. Spines only reappear when the dendritic arbor reaches layer 2. Apparently, Meynert cells avoid connecting with excitatory axons distributing in layers 3 and 4, as reflected in significantly reduced spine density.

The diversity of structure in synaptic specializations of dendrites is most effectively studied with serial section electron microscopy (e.g. Figures 1.10 and 1.11). This methodology has been used extensively to study simple spines on spiny neurons. In the mature brain, simple spines vary greatly in size, with volumes ranging from less than 0.01 μm^3 to more than 1.5 μm^3 (Table 1.4). Simple spines of different sizes and shapes can be neighbors on the same parent dendrite (Harris and Stevens 1988; 1989), and occasionally form synapses with the same presynaptic bouton (Sorra and Harris 1993; Fiala *et al.* 2002a).

In pyramidal cells, two main types of simple spines can be distinguished: *sessile* and *pedunculated* (Jones and Powell 1969). Sessile spines do not exhibit a substantial neck constriction (Table 1.3). Sessile spines are often called *stubby* spines (Peters and Kaiserman-Abramof 1970), especially when the length of the spine is less than or equal to its width (Harris *et al.* 1992). The bulbous head of pedunculated spines attaches to the

Table 1.4 Dimensions of simple spines on spiny neurons

Neuron	Neck diameter (μm)	Volume (μm^3)	Surface area (μm^2)	Synapse area (μm^2)	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 Hartmann (1983); Wilson *et al.* (1983); Harris and Stevens (1988; 1989); Trommald and Hulleberg (1997).

dendrite through a thin stalk or neck. Among pedunculated spines, two varieties are commonly distinguished. *Thin* spines are those with a small head, while large-headed spines are called *mushroom* spines. On the dendrites of CA1 pyramidal neurons in stratum radiatum, the thin shape is most common (60%) while mushroom shapes comprise about 20%, and stubby and sessile shapes less than 10% of all spines (Harris *et al.* 1992).

Additional types of simple spines are often found on specific neurons. One example is the bent sessile spines in 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).

Not all spiny neurons show the same distribution of spine shapes as pyramidal cells (Table 1.4). For example, spines on the tertiary dendrites of cerebellar Purkinje cells constitute a single morphological class with head diameters similar to thin spines on pyramidal cells and neck diameters similar to those of mushroom spines (Spacek and Hartmann 1983). In other spiny neurons there is a continuum of spine shapes, with obvious stubby, thin, and mushroom varieties but many intermediate shapes. For instance, some mushroom spines have very long necks, while some thin spines have rather large heads. This makes distinct shape classes often difficult to distinguish (Wilson *et al.* 1983; Trommald and Hulleberg 1997). Still, the majority of simple spines have a thin shape (Peters and Kaiserman-Abramof 1970; Graveland *et al.* 1985; Harris *et al.* 1992).

The separation of simple spines into thin-necked and thick-necked varieties may be functionally relevant since the presence of a neck constriction can serve to isolate the spine head compartment from the dendrite (Holmes 1990; Koch and Zador 1993; Svoboda *et al.* 1996). However, the distribution of spine neck diameters in many

brain regions is not bimodal, despite the fact that it covers a very broad range (Wilson *et al.* 1983; Trommald and Hulleberg 1997). The fact that cerebellar spines have spine neck dimensions similar to thick-necked spines on pyramidal cells (Spacek and Hartmann 1983) suggests a functionally distinct, thick-necked spine class might be associated with spine endoplasmic reticulum.

Fine structure of synaptic specializations

The diversity of shapes of synaptic specializations is accompanied by a diversity in intracellular composition, and the size of a synaptic specialization seems to be a factor in this composition. Filopodia and simple spines rarely contain mitochondria — most are far too small. An exception is gemmules of olfactory bulb granule cells that often have mitochondria in the head. Since these spines make reciprocal synapses on mitral cell dendrites, it has been suggested that the presence of mitochondria may be related to 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. Thorny excrescences and claw endings do not have synaptic vesicles, yet they also may contain a mitochondrion in the head of a protrusion. The larger synaptic specializations of the relay neurons of lateral geniculate nucleus also contain many mitochondria but have no presynaptic function (Wilson *et al.* 1984).

Some synaptic specializations also contain elements of the dendritic SER network (Figures 1.12 and 1.13). In CA1 pyramidal neurons of the hippocampus, only about 15% of dendritic spines contain SER, this organelle being mostly absent from thin spines (Cooney *et al.* 2000). All dendritic spines of cerebellar Purkinje cells contain SER (Spacek 1985a; Harris and Stevens 1988). In claw endings of cerebellar granule cells, each mitochondrion is surrounded by a single cisterna of SER. In the cerebral cortex and hippocampus, large spines possessing large, perforated synapses contain more SER, which is often flattened and laminated into a characteristic appearance (Figure 1.13), called the *spine apparatus* (Gray 1959; Spacek 1985a). Mushroom spines and gemmules frequently contain spine apparati, as do the thorny excrescences of hippocampal area CA3. An organelle morphologically identical to the spine apparatus has been frequently observed in the dendrite shaft and axon initial segment. In these extraspinous locations, the term *cisternal organelle* is usually applied.

Smooth and coated vesicles, endosomes, and polyribosomes also occur in synaptic specializations. 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. High concentrations of polyribosomes have been found in the lobes of thorny excrescences of CA3 pyramidal cells (Chicurel and Harris 1992), and can be observed in thalamic thorny excrescences as well (Figure 1.11). In quiescent conditions, only about 12% of simple dendritic spines contain any polyribosomes (Steward *et al.* 1996; Ostroff *et al.* 2002). With intense activation, however, polyribosomes increase dramatically in spines (Ostroff *et al.* 2002). The presence of polyribosomes in the

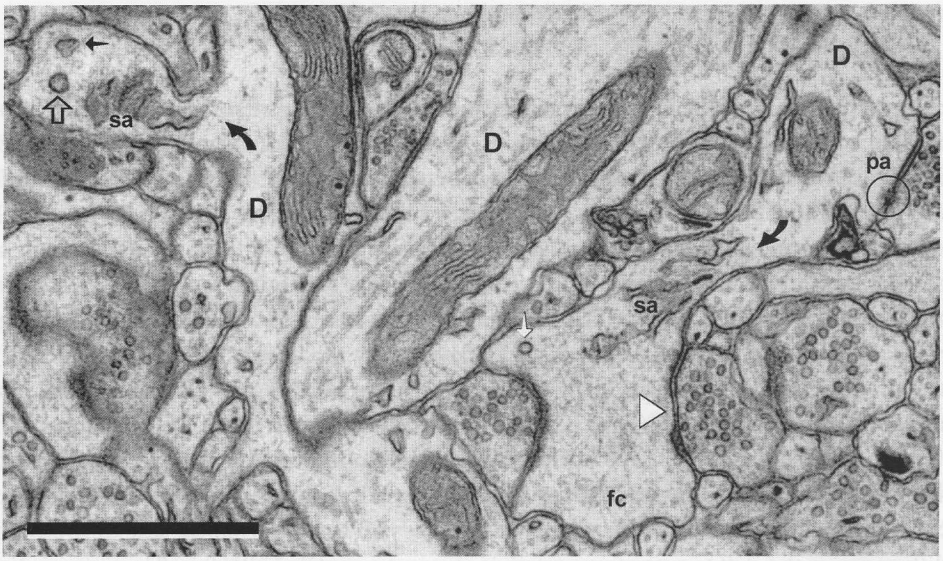


Fig. 1.12 Simple spines contain a unique complement of organelles. Dendrites (D) from stratum radiatum give rise to large spines (curved arrows). The spines contain mainly a darker, floccular cytoplasm (fc) consistent with a denser actin matrix. The spines contain a specialization of SER called the spine apparatus (sa). A further extension of SER can be seen in one spine (small arrow). This spine also contains a spherical vesicle (clear arrow). The other large spine contains a smaller clear profile (white arrow) that can be seen to be a tubule, perhaps endosomal, in cross-section by examining adjacent serial sections. The bouton presynaptic to this 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). Also visible is a punctum adhaerens junction (pa) next to a symmetric synapse on the dendrite shaft (rat; scale bar: 1 μm). Reproduced from *Dendrites 1/e* by Stuart, G. *et al.*, (1999). By permission of Oxford University Press.

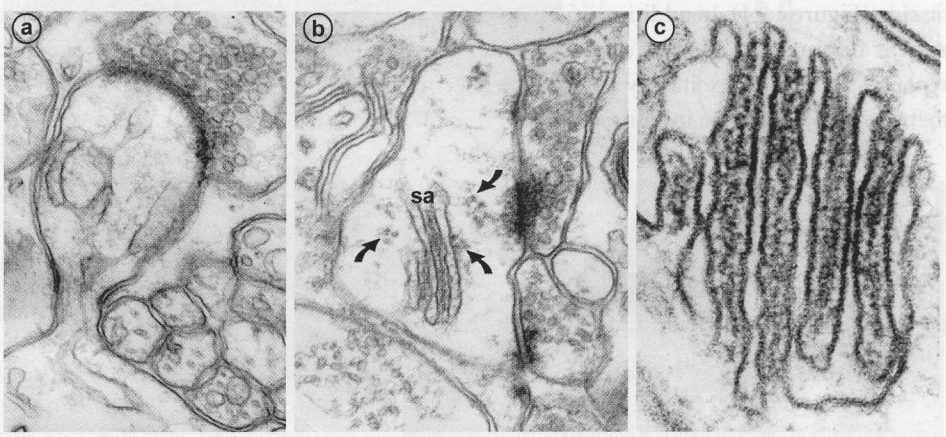


Fig. 1.13 Smooth endoplasmic reticulum in dendritic spines. **(a)** A cerebellar Purkinje cell spine showing the network of SER that extends into the spine from the dendrite. **(b)** The head of a neocortical mushroom spine containing a spine apparatus (sa) and closely associated polyribosomes (arrows). **(c)** High-magnification image of the spine apparatus in a mushroom spine of the visual cortex shows the inner dense plates between cisternae of SER. The electron-dense material reveals periodically organized granulofilamentous arrays when sectioned tangentially (mouse; reprinted with permission from Spacek 1985a).

postsynaptic region, especially in large, perforated, and perhaps the most active synapses may, in conjunction with the spine apparatus, represent proteosynthetic machinery engaged in synaptic plasticity and formation of short-term memory (Steward and Reeves 1988).

Small dendritic protrusions, such as simple spines and filopodia, have cytoskeletons based on actin rather than microtubules. Only after dendritic injury can microtubules be observed in dendritic spines (Fiala *et al.* 2003). Actin-based cytoskeletons may facilitate rapid, calcium-induced changes in shape (Fifkova 1985; Fischer *et al.* 1998; Halpain *et al.* 1998). Microtubules are occasionally found in the stems of larger synaptic specializations such as thorny excrescences (Ebner and Colonnier 1975; Chicurel and Harris 1992).

The synaptic specializations of dendrites receive chemical synapses, consisting of apposed membranes separated by a gap called the *synaptic cleft* (Figure 1.14). 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 the form of the perisynaptic structures (Colonnier 1968; Peters and Palay 1996). Two principal types are commonly referred to as *asymmetric* and *symmetric* synapses. Asymmetric synapses are characterized by round presynaptic vesicles ~30–50 nm in diameter and a prominent *postsynaptic density* (Figure 1.14). The postsynaptic density is a densely stained structure that contains numerous receptors, structural proteins, and signaling molecules that are important for synaptic transmission and plasticity (Kennedy 2000). Symmetric synapses have a much thinner postsynaptic density, matched by an equal density on the presynaptic side (Figure 1.14). In addition, many of the presynaptic vesicles at symmetric synapses appear flattened due to the effects of aldehyde fixation (Bodian 1970). Asymmetric synapses are usually excitatory and use the neurotransmitter glutamate. In contrast, symmetric synapses use the inhibitory neurotransmitters GABA or glycine.

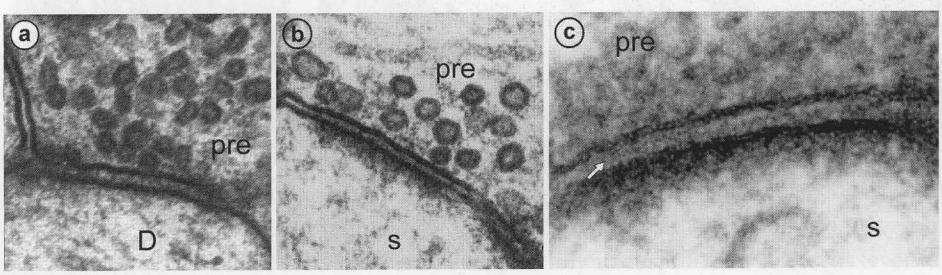


Fig. 1.14 Symmetrical and asymmetrical synapses on dendrites of spiny neurons. **(a)** Symmetrical synapse with equal densities in the presynaptic (pre) and postsynaptic (D) compartments, and flattened vesicles. Symmetrical synapses are usually found on the shaft of the dendrite (D) (rat hippocampus). **(b)** An asymmetrical synapse, with a thick postsynaptic density and round vesicles presynaptically. Asymmetrical synapses are found primarily on the heads of spines (s) (rat hippocampus). **(c)** Higher magnification image of an asymmetrical synapse shows, in addition to the postsynatic density, the dense material of the synaptic cleft (arrow) (mouse cerebellum).

Simple spines typically have a single asymmetric synapse located on the spine head. Occasionally, spines have a second synapse, usually on the spine neck (Spacek and Hartmann 1983). The second synapse can be either symmetric or asymmetric (Jones and Powell 1969). In neostriatum, approximately 8% of spines receive a second symmetric synapse (Wilson *et al.* 1983). The striatal neurons that make reciprocal connections with substantia nigra have an additional 39% of their spines contacted by a different (probably dopaminergic) type of symmetric synapse containing large, pleomorphic vesicles (Freund *et al.* 1984). In visual cortex, 84% of synapses are asymmetric, while 16% are symmetric (Beaulieu and Colonnier 1985). Most of the asymmetric synapses (79%) occur on dendritic spines, while 21% occur on dendrite shafts, and very few (0.1%) are found on cell bodies. The inhibitory synapses show a different pattern. Most are found on dendrite shafts (62%), while 31% occur on dendritic spines, and 7% occur on cell bodies and axon initial segments. Thus, symmetric synapses are only 7% of all dendritic spine synapses, but 93% of all soma synapses in this brain region.

Glomeruli often contain inhibitory axons in addition to the primary excitatory terminals. Thus, it is common for 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).

When viewed in three dimensions, synapses can be seen to exhibit size-dependent variations in morphology (Spacek and Hartmann 1983). Small synapses, like those on the heads of thin spines, are typically *macular*, consisting of a single round region without holes (Figure 1.15). Larger synaptic junctions often exhibit interior regions devoid of

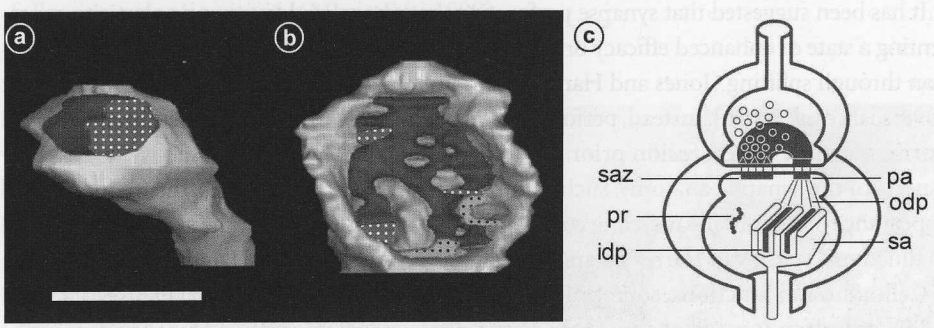


Fig. 1.15 Structure of excitatory synapses onto dendritic spines. **(a)** Three-dimensional reconstruction of a thin spine with a macular synapse (dark region) on the head. The synaptic area is adjacent to a large punctum adhaerens junction (gray region with white dots). **(b)** A view of the synaptic face of a mushroom spine with a perforated synapse. The perforations are places in the interior of the synaptic region which are nonsynaptic, seen as islands of gray spine surface within the dark synaptic region. Adjacent to the synaptic area are three puncta (with white dots) and two synaptic, vesicle-free junctions (with black dots) (scale: 1 μm). **(c)** Schematic of a large cortical dendritic spine containing a polyribosome (pr) and a spine apparatus (sa) with its inner dense plates (idp). A bundle of microfilaments forming an outer dense plate (odp) often extends from the inner dense plates to a punctum adhaerens (pa) that is structurally connected to the region of transmitter release: the synaptic active zone (saz) (rat; Spacek and Harris 1998). A and B reproduced from *Dendrites 1e* by Stuart, G. *et al.*, (1999). By permission of Oxford University Press.

pre- and postsynaptic density. These synapses can be U-shaped or annular, or exhibit multiple holes, and are often called *perforated* synapses. In some cases, a contact between a single bouton and a dendrite is composed of two or more disjoint synaptic regions. This type of perforated synapse is sometimes referred to as a *segmented*, or multifocal, synapse (Geinisman *et al.* 1987).

Macular and perforated synapses can both be found on simple spines as well as on the shafts of dendrites. When located on spines, the synaptic area occupies approximately 10% of the surface area of the spine head (Spacek and Hartmann 1983). This relationship between synapse and spine area is consistent over different spine morphologies and neuron types (Table 1.4). This relationship appears to hold for more complicated synaptic specializations as well, such as the thorny excrescences of CA3 (Chicurel and Harris 1992). Spine surface area, spine volume, bouton volume, and number of synaptic vesicles correlate with synapse size in most cases (Harris and Stevens 1988; 1989; Harris and Sultan 1995; Schikorski and Stevens 1999). Thus, smaller, thin spines have smaller synapses, which tend to be macular. Larger mushroom spines have larger synapses, which can be perforated (Harris and Stevens 1989; Harris *et al.* 1992).

Larger synapses contain more receptors and other signaling molecules, and therefore represent more efficacious connections (Tanaka *et al.* 2005). Differences in synaptic efficacy have important implications for both long-term information storage and short-term neurotransmission. For example, excitatory synapses in the most distal apical dendrites of CA1 pyramidal cells are more often larger, perforated synapses than those synapses more proximal to the cell body (Megias *et al.* 2001). This property may help compensate for distance-dependent attenuation of postsynaptic potentials (see Chapter 8).

It has been suggested that synapse perforations may be related to synaptic plasticity, representing a state of enhanced efficacy or an intermediate stage in a process of synapse proliferation through splitting (Jones and Harris 1995). But dendritic spines do not split (Sorra *et al.* 1998; Fiala *et al.* 2002a). Instead, perforations may be related to the excess membrane inserted during synaptic vesicle fusion prior to bulk endocytosis (Spacek and Harris 2004). Many aspects of the synaptic anatomy, such as the number of synaptic vesicles, the frequency and appearance of membrane recycling components, and the curvature of the synapse, are related to functional state (Van Harreveld and Trubatch 1975; Applegate and Landfield 1988).

Cell-adhesion junctions, sometimes referred to as *puncta adhaerentia* (Figures 1.13 and 1.15), are often located at the edges of the postsynaptic densities of dendritic spines (Spacek and Harris 1998). These junctions contain a different set of structural and signaling molecules from those in the postsynaptic density (Fannon and Colman 1996; Fields and Ito 1997). Puncta may also modulate synaptic efficacy, since their disassembly and reassembly may be needed for synaptic plasticity (Lüthi *et al.* 1994; Muller *et al.* 1996; Tang *et al.* 1998). The spine apparatus is often found to have a topical and structural relationship to puncta, indicating a possible role in synthesizing or maintaining them (Spacek and Harris 1998). Puncta adhaerentia may be progenitors of chemical synapses, as the first axon–dendrite contacts during synaptogenesis resemble puncta (Fiala *et al.* 1998). In some brain regions, such as thalamic relay nuclei, there are extensive adherent contacts between neurons, consisting of a meshwork of multiple puncta in

which synapses also intimately participate (Lieberman and Spacek 1997). Extensive *multitubular bodies*, unusual organelles derived from SER, and extensive SER characteristically appear in the dendritic cytoplasm subjacent to these contacts.

Dendritic spines occasionally have even smaller protrusions 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). These *spinules* are surrounded by invaginations of apposed membrane often with a clathrin-like coat visible on the cytoplasmic side at the tip of the invagination. Spinules in the hippocampus originate from all parts of the dendrite surface, often at the edges of synapses or from within perforations (Figure 1.16). The function of spinules is not known; however, it may involve 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).

Finally, synapses differ in the degree to which they are surrounded by glial processes (Figure 1.16). In cerebellar cortex, nearly all spine synapses are completely ensheathed by the Bergmann astroglial processes (Spacek 1985b). In contrast, only 58% of hippocampal synapses have even partial astroglial ensheathment (Ventura and Harris 1998), which is comparable to cortical spine synapses (Spacek 1985b). Thus, many, but certainly not all synapses have astrocytic processes at their perimeter, whereby neurotransmitter spillover between neighboring synapses could be detected and limited (Bergles *et al.* 1997). Another important aspect of astrocytes at excitatory synapses is energy metabolism. Perisynaptic astrocytes contain rich stores of glycogen that provide local energy replacement following synaptic activity (Magistretti 1999).

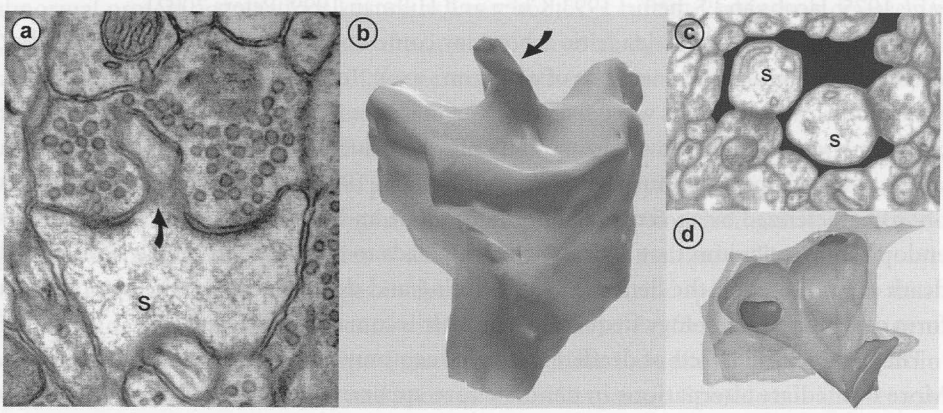


Fig. 1.16 Perisynaptic structure of dendritic spines. **(a)** A spinule (arrow) extends into the presynaptic bouton from the middle of a synapse on a large dendritic spine (s) of a hippocampal pyramidal cell (rat; Harris and Stevens 1989). **(b)** Full three-dimensional structure of this spine and spinule (arrow). **(c)** An astrocytic process (black) contacts the edges of synapses onto two dendritic spines (s) in cerebellar cortex (mouse). **(d)** Three-dimensional reconstruction reveals that the two spines are completely enveloped by the astrocyte (translucent gray).

Structural pathology

The characteristic dendritic arbors of neurons are created through a combination of intrinsic developmental programs and environmental influences, as discussed further in subsequent chapters. The cellular environment continues throughout life to have an influence on dendrites, and a number of structural pathologies arise from various adverse conditions. Pathologies of dendrite structure often accompany neurological diseases (Chapter 20).

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 proper dendrite development and maintenance. 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 1986). Consider another example from the hippocampus, in which lesion of entorhinal cortex atrophies the dendritic arbors of granule cells in the dentate gyrus (Caceres and Steward 1983). Total dendritic length is reduced to less than 2000 ∝m (cf. Table 1.1) 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 1983), possibly due to neuron loss and the associated deafferentation. Even aging seems to produce a degree of dendritic atrophy (Scheibel *et al.* 1975; Jacobs and Scheibel 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). The 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 stroke. Hypoxia causes a loss of energy that retards the ability of dendrites to maintain ionic polarity at the cell membrane. Disruption of ion homeostasis results in swelling of dendrites within minutes, usually producing a number of varicosities that give a dendrite the appearance of beads on a string (Figure 1.17). Dendritic varicosities of this type are also seen in brain tissue damaged by convulsions (Scheibel and Scheibel 1977), extreme cold (Kirov *et al.* 2004), and tumor (Spacek 1987). Dendritic swelling affects the

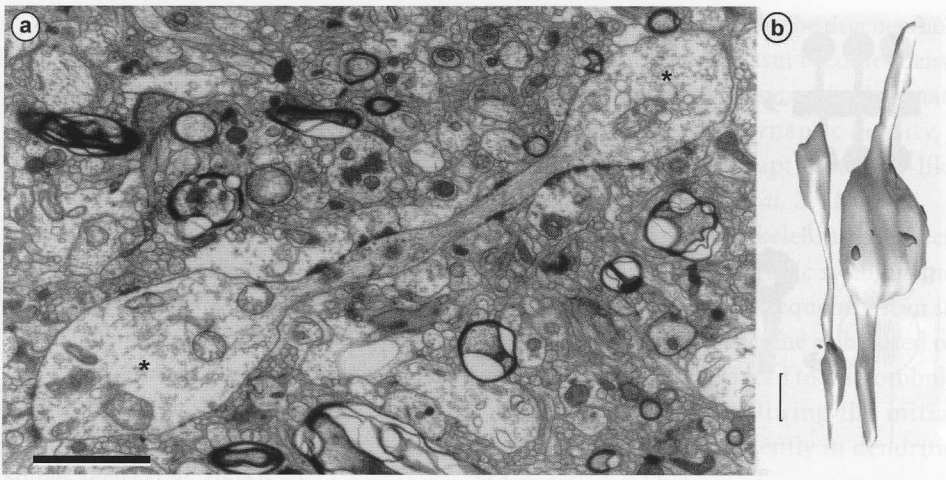


Fig. 1.17 Dendrites beaded by hydropic swelling. **(a)** Electron micrograph showing a segment of dendrite in human peritumorous neocortex in which two varicosities (asterisks) with watery cytoplasm are separated by a narrower region of denser cytoplasm (scale: 2 μm). **(b)** Three-dimensional reconstruction from serial section electron microscopy of two beaded dendritic segments that receive synaptic contacts (dark gray areas) (scale: 0.5 μm).

cytoplasmic organelles as well. 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.

The synaptic specializations of dendrites are also prone to structural distortions by a variety of insults and diseases. The pathologies of dendritic spines have been particularly well studied, with two general categories of spine pathology being distinguished, pathologies of distribution and pathologies of ultrastructure, as summarized in Figure 1.18.

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 toxin or alcohol exposure. Spine loss is also seen in epilepsy, prionoses, 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

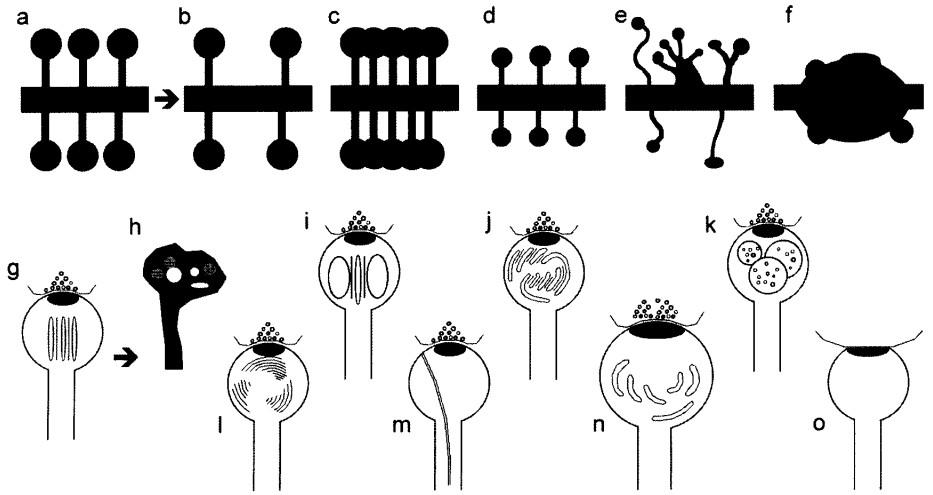


Fig. 1.18 Schematic of observed dendritic spine pathologies (Fiala *et al.* 2002b). **(a-f)** Pathologies of distribution as seen, for example, by light microscopy, involve differences from normal spines (a) by decreased density (b), increased density (c), reduction in spine size (d), distortions of normal spine shapes (e), and abnormally varicose dendrites that absorb spines (f). **(g-o)** Observed pathologies from normal spine ultrastructure (g) include shrunken spines with dense cytoplasm (h), altered endoplasmic reticulum (i), hypertrophied spine apparatus (j), hypertrophied multivesicular bodies (k), hypertrophied cytoskeleton (l), spine microtubules (m), giant spines (n), and axonless spines with an axon-free postsynaptic density (o).

spines may represent a failure of normal developmental synapse elimination, as has been suggested for phenylketonuria and fragile-X syndrome.

Absence of normal levels of presynaptic activity without destruction of the input axons, can result in a reduction in synapse and spine size, as reported in 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 dendritic spine shape is seen in mental retardation and other conditions. The unusually long and tortuous spines having 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 the 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. Although they are observed occasionally in the normal brain, occurrences of axonless spines increase in many pathological conditions, such as developmental agenesis of cerebellar granule cells (Altman and Bayer 1997).

Alterations in spine organelles result from diverse causes. One cause may be degeneration of the postsynaptic cell, as is often the case when the dendritic cytoplasm becomes dense and dark. Another cause may be excitotoxic injury from excessive presynaptic glutamate release, 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, such as after traumatic injury. Hydropic swelling and vacuolization of the endoplasmic reticulum of dendritic spines are 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 microtubule depolymerization in the dendrite shaft, as mentioned above. During the initial repolymerization on recovery, microtubules may reassemble transiently in dendritic spines (Fiala *et al.* 2003).

Concluding remarks

A hundred years after Ramón y Cajal, 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 appears to contribute to dendritic computation, particularly when the dendrite is endowed with active mechanisms (Chapter 14). The synaptic specializations extended by dendrites also contribute significantly to connectivity. They allow thin dendrites to reach multiple axons such that larger numbers of synapses interdigitate in a relatively small brain volume. However, they presumably have additional functions related to neuronal computation and learning (Chapter 18). The enormous diversity in the structure, composition, and plasticity of dendrites and their synaptic specializations suggests that the functional contributions of these structures to mind and brain are enormously diverse.

Acknowledgments

For kind permission to reprint figures as indicated in the captions, the authors thank the original publishers Blackwell Publishing, Oxford; and Springer-Verlag, Berlin. Thanks to Marcia Feinberg for assistance with some of the serial section electron microscopy. This work was supported, in part, by National Institutes of Health grants NS21184 and NS33574, and Human Brain Project grant EB002170 to KMH.

References

Akert K, Pfenninger K and Sandri C (1967). Crest synapses with subjunctional bodies in the subfornical organ. *Brain Research*, **6**, 118–21.

Altman J (1971). Coated vesicles and synaptogenesis. A developmental study in the cerebellar cortex of the rat. *Brain Research*, **30**, 311–22.

Altman J and Bayer S (1997). *Development of the cerebellar system in relation to its evolution, structure, and functions*. CRC Press, Boca Raton, FL.

Amaral DG and Witter MP (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience*, **31**, 571–91.

Anderson WA and Flumerfelt BA (1986). Long-term effects of parallel fiber loss in the cerebellar cortex of the adult and weanling rat. *Brain Research*, **383**, 245–61.

Applegate MD and Landfield PW (1988). Synaptic vesicle redistribution during hippocampal frequency potentiation and depression in young and aged rats. *Journal of Neuroscience*, **8**, 1096–1111.

Bannister NJ and Larkman AU (1995a). Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus. I. Branching patterns. *Journal of Comparative Neurology*, **360**, 150–60.

Bannister NJ and Larkman AU (1995b). Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus. II. Spine distributions. *Journal of Comparative Neurology*, **360**, 161–71.

Banno T and Kohno K (1996). Conformational changes of smooth endoplasmic reticulum induced by brief anoxia in rat Purkinje cells. *Journal of Comparative Neurology*, **369**, 462–71.

Beaulieu C and Colonnier M (1985). A laminar analysis of the number of round-asymmetrical and flat-symmetrical synapses on spines, dendritic trunks, and cell bodies in area 17 of the cat. *Journal of Comparative Neurology*, **231**, 180–9.

Beck E, Bak IJ, Christ JF, Gajdusek DC, Gibbs CJ Jr and Hassler R (1975). Experimental kuru in the spider monkey. Histopathological and ultrastructural studies of the brain during early stages of incubation. *Brain*, **98**, 595–612.

Bergles DE and Jahr CE (1997). Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron*, **19**, 1297–1308.

Bodian D (1970). An electron microscopic characterization of classes of synaptic vesicles by means of controlled aldehyde fixation. *Journal of Cell Biology*, **44**, 115–24.

Bower JM and Woolston DC (1983). Congruence of spatial organization of tactile projections to granule cell and Purkinje cell layers of cerebellar hemispheres of the albino rat: vertical organization of cerebellar cortex. *Journal of Neurophysiology*, **99**, 745–66.

Caceres A and Steward O (1983). Dendritic reorganization in the denervated dentate gyrus of the rat following entorhinal cortical lesions: a Golgi and electron microscopic analysis. *Journal of Comparative Neurology*, **214**, 387–403.

Cameron HA, Kaliszewski CK and Greer CA (1991). Organization of mitochondria in olfactory bulb granule cell dendritic spines. *Synapse*, **8**, 107–18.

Chan-Palay V (1977). *Cerebellar dentate nucleus: organization, cytology, and transmitters.* Springer-Verlag, Berlin.

Chan-Palay V, Palay SL and Billings-Gagliardi SM (1974). Meynert cells in the primate visual cortex. *Journal of Neurocytology*, **3**, 631–58.

Chen S and Hillman DE (1999). Dying-back of Purkinje cell dendrites with synapse loss in aging rats. *Journal of Neurocytology*, **28**, 187–96.

Chen XY and Wolpaw JR (1994). Triceps surae motoneuron morphology in the rat: a quantitative light microscopic study. *Journal of Comparative Neurology*, **343**, 143–157.

Chicurel ME and Harris KM (1992). Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. *Journal of Comparative Neurology*, **325**, 169–82.

Chklovskii DB (2004). Synaptic connectivity and neuronal morphology: two sides of the same coin. *Neuron*, **43**, 609–17.

Chklovskii DB, Mel BW and Svoboda K (2004). Cortical rewiring and information storage. *Nature*, **431**, 782–8.

Claiborne BJ, Amaral DG and Cowan WM (1990). Quantitative, three-dimensional analysis of granule cell dendrites in the rat dentate gyrus. *Journal of Comparative Neurology*, **302**, 206–19.

Colonnier M (1968). Synaptic pattern on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Research*, **9**, 268–87.

Cooney JR, Hurlburt JL, Selig DK, Harris KM and Fiala JC (2002). Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *Journal of Neuroscience*, **22**, 2215–24.

Dailey ME and Smith SJ (1996). The dynamics of dendritic structure in developing hippocampal slices. *Journal of Neuroscience*, **16**, 2983–94.

De Zeeuw CI, Ruigrok TJ, Holstege JC, Jansen HG and Voogd J (1990). Intracellular labeling of neurons in the medial accessory olive of the cat. II. Ultrastructure of dendritic spines and their GABAergic innervation. *Journal of Comparative Neurology*, **300**, 478–94.

DiFiglia M and Carey J (1986). Large neurons in the primate neostriatum examined with the combined Golgi-electron microscopic method. *Journal of Comparative Neurology*, **244**, 36–52.

Dunaevsky A, Tashiro A, Majewska A, Mason C and Yuste R (1999). Developmental regulation of spine motility in the mammalian central nervous system. *Proceedings of the National Academy of Sciences of the U S A* **96**, 13438–43.

Ebner FF and Colonnier M (1975). Synaptic patterns in the visual cortex of turtle: an electron microscopic study. *Journal of Comparative Neurology*, **160**, 51–80.

Eccles JC, Ito M and Szentagothai J (1967). *The cerebellum as a neuronal machine*. Springer-Verlag, New York.

Ellias SA and Stevens JK (1980). The dendritic varicosity: a mechanism for electrically isolating the dendrites of cat retinal amacrine cells? *Brain Research*, **196**, 365–72.

Fannon AM and Colman DR (1996). A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins. *Neuron*, **17**, 423–34.

Feldman ML (1984). Morphology of the neocortical pyramidal neuron. In A Peters and EG Jones, eds. *Cerebral cortex*, vol. 1, pp. 123–200. Plenum Press, New York.

Feldman ML and Peters A (1978). The forms of non-pyramidal neurons in the visual cortex of the rat. *Journal of Comparative Neurology*, **179**, 761–94.

Fernández E, Eldred WD, Ammermuller J, Block A, Von Bloh W and Kolb H (1994). Complexity and scaling properties of amacrine, ganglion, horizontal, and bipolar cells in the turtle retina. *Journal of Comparative Neurology*, **347**, 397–408.

Fernández E and Jelinek HF (2001). Use of fractal theory in neuroscience: methods, advantages, and potential problems. *Methods*, **24**, 309–21.

Fiala JC, Allwardt B and Harris KM (2002a). Dendritic spines do not split during hippocampal LTP or maturation. *Nature Neuroscience*, **5**, 297–8.

Fiala JC, Feinberg M, Popov V and Harris KM (1998). Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *Journal of Neuroscience*, **18**, 8900–11.

Fiala JC, Kirov SA, Feinberg MD, Petrak LJ, George P, Goddard CA and Harris KM (2003). Timing of neuronal and glial ultrastructure disruption during brain slice preparation and recovery in vitro. *Journal of Comparative Neurology*, **465**, 90–103.

Fiala JC, Spacek J and Harris KM (2002b). Dendritic spine pathology: cause or consequence of neurological disorders? *Brain Research Reviews*, **39**, 29–54.

Fields RD and Ito K (1997). Neural cell adhesion molecule in activity-dependent development and synaptic plasticity. *Trends in Neurosciences*, **19**, 473–80.

Fifkova E (1985). Actin in the nervous system. *Brain Research*, **9**, 187–215.

Fischer M, Kaech S, Knutti D and Matus A (1998). Rapid actin-based plasticity in dendritic spines. *Neuron*, **20**, 847–54.

Foster M (1897). *A textbook of physiology* (17th edn), p. 929. Macmillan, New York.

Freund TF, Powell JF and Smith AD (1984). Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. *Neuroscience*, **13**, 1189–1215.

Fukuda Y, Hsiao CF, Watanabe M and Ito H (1984). Morphological correlates of physiologically identified Y-, X-, and W-cells in cat retina. *Journal of Neurophysiology*, **52**, 999–1013.

Geinisman Y, Morrell F and De Toledo-Morrell L (1987). Axospinous synapses with segmented postsynaptic densities: a morphologically distinct synaptic subtype contributing to the number of profiles of 'perforated' synapses visualized in random sections. *Brain Research*, **423**, 179–88.

Globus A (1975). Brain morphology as a function of presynaptic morphology and activity. In H Reisen, ed. *Developmental neuropsychology of sensory deprivation*, pp. 9–91. Academic Press, New York.

Graveland GA, Williams RS and Difiglia M (1985). A Golgi study of the human neostriatum: neurons and afferent fibers. *Journal of Comparative Neurology*, **234**, 317–33.

Gray EG (1959). Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *Journal of Anatomy (London)*, **93**, 420–33.

Grutzendler J, Kasthuri N and Gan WB (2002). Long-term dendritic spine stability in the adult cortex. *Nature*, **420**, 812–16.

Hama K, Arii T and Kosaka T (1994). Three-dimensional organization of neuronal and glial processes: high voltage electron microscopy. *Microscopy Research and Technique*, **29**, 357–67.

Harris KM, Cruce WL, Greenough WT and Teyler TJ (1980). A Golgi impregnation technique for thin brain slices maintained in vitro. *Journal of Neuroscience Methods*, **2**, 363–71.

Harris KM, Jensen FE and Tsao B (1992). Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *Journal of Neuroscience*, **12**, 2685–2705.

Harris KM and Kater SB (1994). Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annual Review of Neuroscience*, **17**, 341–71.

Harris KM and Stevens JK (1988). Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience*, **8**, 4455–69.

Harris KM and Stevens JK (1989). Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience*, **9**, 2982–97.

Harris KM and Sultan P (1995). Variation in the number, location, and size of synaptic vesicles provides an anatomical basis for the nonuniform probability of release at hippocampal CA1 synapses. *Neuropharmacology*, **34**, 1387–95.

Harvey RJ and Napper RMA (1991). Quantitative studies on the mammalian cerebellum. *Progress in Neurobiology*, **36**, 437–63.

Henkart M, Landis DMD and Reese TS (1976). Similarity of junctions between plasma membranes and endoplasmic reticulum in muscle and neurons. *Journal of Cell Biology*, **70**, 338–47.

Hirano A (1981). *A guide to neuropathology.* Igaku-Shoin, New York and Tokyo.

Holmes WR (1990). Is the function of dendritic spines to concentrate calcium? *Brain Research*, **519**, 338–42.

Hrycyshyn AW and Flumerfelt BA (1981). Cytology and synaptology of the lateral reticular nucleus of the cat. *Journal of Comparative Neurology*, **197**, 459–75.

Ito M (1984). *The cerebellum and neural control.* Raven Press, New York.

Jacobs B and Scheibel AB (1993). A quantitative dendritic analysis of Wernicke's area in humans. I. Lifespan changes. *Journal of Comparative Neurology*, **327**, 83–96.

Jones DG and Harris RJ (1995). An analysis of contemporary morphological concepts of synaptic remodeling in the CNS: perforated synapses revisited. *Reviews in the Neurosciences*, **6**, 177–219.

Jones EG (1975). Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. *Journal of Comparative Neurology*, **160**, 205–68.

Jones EG and Powell TPS (1969). Morphological variations in the dendritic spines of the neocortex. *Journal of Cell Science*, **5**, 509–29.

Kaufmann WE and Moser HW (2000). Dendritic anomalies in disorders associated with mental retardation. *Cerebral Cortex*, **10**, 981–91.

Kellerth JO, Berthold CH and Conradi S (1979). Electron microscopic studies of serially sectioned cat spinal alpha-motoneurons. III. Motoneurons innervating fast-twitch (type FR) units of the gastrocnemius muscle. *Journal of Comparative Neurology*, **184**, 755–67.

Kennedy MB (2000). Signal-processing machines at the postsynaptic density. *Science*, **290**, 750–4.

Kirov SA, Petrak LJ, Fiala JC and Harris KM (2004). Dendritic spines disappear with chilling but proliferate excessively upon rewarming of mature hippocampus. *Neuroscience*, **127**, 69–80.

Kirov SA, Sorra KE and Harris KM (1999). Slices have more synapses than perfusion-fixed hippocampus from both young and mature rats. *Journal of Neuroscience*, **19**, 2876–86.

Kishi K, Mori K, and Tazawa Y (1982). Three-dimensional analysis of dendritic trees of mitral cells in the rabbit olfactory bulb. *Neuroscience Letters*, **28**, 127–32.

Koch C and Zador A (1993). The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience*, **13**, 413–22.

Kolb H, Fernández E, Schouten J, Ahnelt P, Linberg KA and Fisher SK (1994). Are there three types of horizontal cell in the human retina? *Journal of Comparative Neurology*, **343**, 370–86.

Kolb H, Linberg KA and Fisher SK (1992). Neurons of the human retina: a Golgi study. *Journal of Comparative Neurology*, **318**, 147–87.

Larkman AU (1991). Dendritic morphology of pyramidal neurones of the visual cortex of the rat. II. Spine distributions. *Journal of Comparative Neurology*, **306**, 332–43.

Lenn NJ (1976). Synapses in the interpeduncular nucleus: electron microscopy of normal and habenula lesioned rats. *Journal of Comparative Neurology*, **166**, 73–100.

Li Z, Okamoto K, Hayashi Y and Sheng M (2004). The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell*, **119**, 873–78.

Lieberman AR (1973). Neurons with presynaptic perikarya and presynaptic dendrites in the rat lateral geniculate nucleus. *Brain Research*, **59**, 35–59.

Lieberman AR and Spacek J (1997). Filamentous contacts: the ultrastructure and three-dimensional organization of specialized non-synaptic interneuronal appositions in thalamic relay nuclei. *Cell Tissue Research*, **288**, 43–57.

Lüthi A, Laurent JP, Figurov A, Muller D and Schachner M (1994). Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. *Nature*, **372**, 777–9.

MacNeil MA and Masland RH (1998). Extreme diversity among amacrine cells: implications for function. *Neuron*, **20**, 971–82.

Magistretti PJ (1999). Brain energy metabolism. In MJ Zigmond, FE Bloom, SC Landis, JL Roberts and LR Squire, eds. *Fundamental neuroscience*, pp. 389–413. Academic Press, New York.

Mariani AP (1990). Amacrine cells of the rhesus monkey retina. *Journal of Comparative Neurology*, **301**, 382–400.

Martone ME, Zhang Y, Simpliciano VM, Carragher BO and Ellisman MH (1993). Three-dimensional visualization of the smooth endoplasmic reticulum in Purkinje cell dendrites. *Journal of Neuroscience*, **13**, 4636–46.

McWilliams JR and Lynch G (1981). Sprouting in the hippocampus is accompanied by an increase in coated vesicles. *Brain Research*, **211**, 158–64.

Megias M, Emri Z, Freund TF and Gulyas AI (2001). Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*, **102**, 527–40.

Milhaud M and Pappas GD (1966). The fine structure of neurons and synapses of the habenula of the cat with special reference to subjunctional bodies. *Brain Research*, **3**, 158–73.

Morest DK (1969). The growth of dendrites in the mammalian brain. *Zeitschrift für Anatomie und Entwicklungsgeschichte*, **128**, 290–317.

Mugnaini E, Floris A and Wright-Goss M (1994). Extraordinary synapses of the unipolar brush cell: an electron microscopic study in the rat cerebellum. *Synapse*, **16**, 284–311.

Muller D, Wang C, Skibo G, Toni N, Cremer H, Calaora V, Rougon G and Kiss JZ (1996). PSA-NCAM is required for activity-induced synaptic plasticity. *Neuron*, **17**, 413–22.

Murray JAH, Bradley H, Craigie WA and Onions CT (eds.) (1919). *A New English Dictionary on Historical Principles*. Clarendon Press, Oxford.

Nafstad PHJ and Blackstad TW (1966). Distribution of mitochondria in pyramidal cells and boutons in hippocampal cortex. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **73**, 234–45.

Napper RM and Harvey RJ (1988). Number of parallel fiber synapses on an individual Purkinje cell in the cerebellum of the rat. *Journal of Comparative Neurology*, **274**, 168–77.

Ostapoff EM, Feng JJ and Morest DK (1994). A physiological and structural study of neuron types in the cochlear nucleus. II. Neuron types and their structural correlation with response properties. *Journal of Comparative Neurology*, **346**, 19–42.

Ostroff LE, Fiala JC, Allwardt B and Harris KM (2002). Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron*, **35**, 535–45.

Overly CC, Rieff HI and Hollenbeck PJ (1996). Organelle motility and metabolism in axons vs. dendrites of cultured hippocampal neurons. *Journal of Cell Science*, **109**, 971–80.

Palay SL (1978). The Meynert cell, an unusual cortical pyramidal cell. In MAB Brazier and H Petsch, eds. *Architectonics of the cerebral cortex*, pp. 31–42. Raven Press, New York.

Palay SL and Chan-Palay V (1974). *Cerebellar cortex: cytology and organization*. Springer-Verlag, New York.

Panico J and Sterling P (1995). Retinal neurons and vessels are not fractal but space-filling. *Journal of Comparative Neurology*, **361**, 479–90.

Parra P, Gulyas AI and Miles R (1998). How many subtypes of inhibitory cells in the hippocampus? *Neuron*, **20**, 983–93.

Peters A (2002). Structural changes that occur during normal aging of primate cerebral hemispheres. *Neuroscience and Biobehavioral Reviews*, **26**, 733–41.

Peters A and Kaiserman-Abramof IR (1970). The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *American Journal of Anatomy*, **127**, 321–56.

Peters A and Palay SL (1996). The morphology of synapses. *Journal of Neurocytology*, **25**, 687–700.

Peters A, Palay SL and Webster H De F (1991). *The fine structure of the nervous system*. Oxford University Press, New York.

Popov VI, Medvedev NI, Rogachevskii VV, Ignat'ev DA, Stewart MG and Fesenko EE (2003). Three-dimensional organization of synapses and astroglia in the hippocampus of rats and ground squirrels: new structural and functional paradigms of the synapse function. *Biofizika*, **48**, 289–308.

Porter R, Ghosh S, Lange GD and Smith TG Jr (1991). A fractal analysis of pyramidal neurons in mammalian motor cortex. *Neuroscience Letters*, **130**, 112–16.

Price JL and Powell TPS (1970). The synaptology of the granule cells of the olfactory bulb. *Journal of Cell Science*, **7**, 125–55.

Prieto JJ and Winer JA (1999). Layer VI in cat primary auditory cortex: Golgi study and sublaminar origins of projection neurons. *Journal of Comparative Neurology*, **404**, 332–58.

Purves D, Snider WD and Voyvodic JT (1988). Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. *Nature*, **336**, 123–8.

Ramón y Cajal S (1991). *Degeneration and regeneration of the nervous system*. J Defelipe and EG Jones, eds; trans. RM May. Oxford University Press, New York.

Ramón y Cajal S (1995). *Histology of the nervous system of man and vertebrates* (trans. N Swanson and LW Swanson). Oxford University Press, New York (originally published: *Histologie du système nerveux de l'homme et des vertèbrès* (trans. L Azoulay), Paris, 1909–11).

Ramón-Moliner E (1968). The morphology of dendrites. In GH Bourne, ed. *The structure and function of nervous tissue,* vol. 1, pp. 205–67. Academic Press, New York.

Rapp M, Segev I and Yarom Y (1994). Physiology, morphology and detailed passive models of guinea-pig cerebellar Purkinje cells. *Journal of Physiology*, **474**, 101–18.

Ruigrok TJH, De Zeeuw CI, Van Der Burg J and Voogd J (1990). Intracellular labeling of neurons in the medial accessory olive of the cat. I. Physiology and light microscopy. *Journal of Comparative Neurology*, **300**, 462–77.

Scheibel AB (1983). Dendritic changes. In B Reisberg, ed. *Alzheimer's disease*, pp. 69–73. Free Press, New York.

Scheibel ME, Lindsay RD, Tomiyasu U and Scheibel AE (1975). Progressive dendritic changes in aging human cortex. *Experimental Neurology*, **47**, 392–403.

Scheibel ME and Scheibel AM (1970). Of pattern and place in dendrites. *International Review of Neurobiology*, **13**, 1–26.

Scheibel ME and Scheibel AM (1977). Specific postnatal threats to brain development: dendritic changes. In SR Berenberg, ed. *Brain: fetal and infant: current research on normal and aAbnormal development*, pp. 302–15. Martinus Nijhoff Medical Division, The Hague.

Schikorski T and Stevens CF (1999). Quantitative fine-structural analysis of olfactory cortical synapses. *Proceedings of the National Academy of Sciences of the U S A*, **96**, 4107–12.

Scorcioni R, Lazarewicz MT and Ascoli GA (2004). Quantitative morphometry of hippocampal pyramidal cells: differences between anatomical classes and reconstructing laboratories. *Journal of Comparative Neurology*, **473**, 177–93.

Sholl DA (1956). *The organization of the cerebral cortex.* Wiley, New York.

Sloper JJ and Powell TP (1978). Dendro-dendritic and reciprocal synapses in the primate motor cortex. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **203**, 23–38.

Smith TG, Marks WB, Lange GD, Sheriff WH and Neale EA (1989). A fractal analysis of cell images. *Journal of Neuroscience Methods*, **27**, 173–80.

Sorra KE, Fiala JC and Harris KM (1998). Critical assessment of the involvement of perforations, spinules, and spine branching in hippocampal synapse formation. *Journal of Comparative Neurology*, **398**, 225–40.

Sorra KE and Harris KM (1993). Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA1. *Journal of Neuroscience*, **13**, 3736–48.

Sotelo C and Angaut P (1973). The fine structure of the cerebellar central nuclei in the cat. I. Neurons and neuroglial cells. *Experimental Brain Research*, **16**, 410–30.

Spacek J (1985a). Three-dimensional analysis of dendritic spines. II. Spine apparatus and other cytoplasmic components. *Anatomy and Embryology*, **171**, 235–43.

Spacek J (1985b). Three-dimensional analysis of dendritic spines. III. Glial sheath. *Anatomy and Embryology*, **171**, 245–52.

Spacek J (1987). Ultrastructural pathology of dendritic spines in epitumorous human cerebral cortex. *Acta Neuropathologica (Berlin)*, **73**, 77–85.

Spacek J and Harris KM (1997). Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *Journal of Neuroscience*, **17**, 190–203.

Spacek J and Harris KM (1998). Three-dimensional organization of cell adhesion junctions at synapses and dendritic spines in area CA1 of the rat hippocampus. *Journal of Comparative Neurology*, **393**, 58–68.

Spacek J and Harris KM (2004). Trans-endocytosis via spinules in adult rat hippocampus. *Journal of Neuroscience*, **24**, 4233–41.

Spacek J and Hartmann M (1983). Three-dimensional analysis of dendritic spines. I. Quantitative observations related to dendritic spine and synaptic morphology in cerebral and cerebellar cortices. *Anatomy and Embryology*, **167**, 289–310.

Spacek J and Lieberman AR (1974). Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. *Journal of Anatomy (London)*, **117**, 487–515.

Spacek J and Lieberman AR (1980). Relationships between mitochondrial outer membranes and agranular reticulum in nervous tissue: ultrastructural observations and a new interpretation. *Journal of Cell Science*, **46**, 129–47.

Sterling P (1990). Retina. In GM Shepherd, ed. *The synaptic organization of the brain* (3rd edn), pp. 170–215. Oxford University Press, New York.

Stepanyants A, Hof PR and Chklovskii DB (2002). Geometry and structural plasticity. *Neuron*, **34**, 275–88.

Steward O, Falk PM and Torre ER (1996). Ultrastructural basis for gene expression at the synapse: synapse-associated polyribosome complexes. *Journal of Neurocytology*, **25**, 717–34.

Steward O and Reeves TM (1988). Protein-synthetic machinery beneath postsynaptic sites on CNS neurons: association between polyribosomes and other organelles at the synaptic site. *Journal of Neuroscience*, **8**, 176–84.

Svoboda K, Tank DW and Denk W (1996). Direct measurement of coupling between dendritic spines and shafts. *Science*, **272**, 716–719.

Swindale NV (1981). Dendritic spines only connect. *Trends in Neuroscience*, **4**, 240–41.

Takeda T, Ishikawa A, Ohtomo K, Kobayashi Y and Matsuoka T (1992). Fractal dimension of dendritic tree of cerebellar Purkinje cell during onto- and phylogenetic development. *Neuroscience Research*, **13**, 19–31.

Tanaka J, Masanori M, Tarusawa E *et al.* (2005). Number and density of AMPA receptors in single synapses in immature cerebellum. *Journal of Neuroscience*, **25**, 799–809.

Tang L, Hung CP and Schuman EM (1998). A role for the cadherin family of cell adhesion molecules in hippocampal long-term potentiation. *Neuron*, **20**, 1165–75.

Tao-Cheng JH, Vinade L, Smith C *et al.* (2001). Sustained elevation of calcium induces Ca(2+)/calmodulin-dependent protein kinase II clusters in hippocampal neurons. *Neuroscience*, **106**, 69–78.

Tarrant SB and Routtenberg A (1977). The synaptic spinule in the dendritic spine: electron microscopic study of the hippocampal dentate gyrus. *Tissue and Cell*, **9**, 461–73.

Trommald M and Hulleberg G (1997). Dimensions and density of dendritic spines from rat dentate granule cells based on reconstructions from serial electron micrographs. *Journal of Comparative Neurology*, **377**, 15–28.

Trommald M, Jensen V and Andersen P (1995). Analysis of dendritic spines in rat CA1 pyramidal cells intracellularly filled with a fluorescent dye. *Journal of Comparative Neurology*, **353**, 260–74.

Ulfhake B and Kellerth JO (1981). A quantitative light microscopic study of the dendrites of cat spinal alpha-motoneurons after intracellular staining with horseradish peroxidase. *Journal of Comparative Neurology*, **202**, 571–83.

Uylings HBM and van Pelt J (2002). Measures for quantifying dendritic arborizations. *Network: Computation in Neural Systems*, **13**, 397–414.

Van Harreveld A and Trubatch J (1975). Synaptic changes in frog brain after stimulation with potassium chloride. *Journal of Neurocytology*, **4**, 33–46.

Ventura R and Harris KM (1999). Three-dimensional relationships between hippocampal synapses and astrocytes. *Journal of Neuroscience*, **19**, 6897–6906.

Ward R, Moreau B, Marchand M-J and Garenc C (1995). A note on the distribution of dendritic spines. *Journal für Hirnforschung*, **36**, 519–22.

Westrum LE and Blackstad T (1962). An electron microscope study of the stratum radiatum of the rat hippocampus (regio superior, CA1) with particular emphasis on synaptology. *Journal of Comparative Neurology*, **119**, 281–309.

Wilson CJ, Groves PM, Kitai ST and Linder JC (1983). Three-dimensional structure of dendritic spines in the rat neostriatum. *Journal of Neuroscience*, **3**, 383–98.

Wilson JR, Friedlander MJ and Sherman SM (1984). Fine structural morphology of identified X- and Y-cells in the cat's lateral geniculate nucleus. *Proceedings of the Royal Society of London, Series B*, **221**, 411–46.

Wingate RJ, Fitzgibbon T and Thompson ID (1992). Lucifer yellow, retrograde tracers, and fractal analysis characterize adult ferret retinal ganglion cells. *Journal of Comparative Neurology*, **323**, 449–74.

Yelnik J, Percheron G and Francois C (1984). A Golgi analysis of the primate globus pallidus. II. Quantitative morphology and spatial orientation of dendritic arborizations. *Journal of Comparative Neurology*, **227**, 200–21.