Intrinsic Determinants of Neuronal Form and Function, pages 179–199
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Study of Dendritic Spines by Serial Electron Microscopy and Three-Dimensional Reconstructions

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INTRODUCTION

Dendritic spines are small protrusions studding the surfaces of many neurons. They were first described in Golgi preparations by Ramón y Cajal (1891) who recognized that the spines were likely to be sites of synaptic interactions between neurons. Gray (1959) later verified with electron microscopy that dendritic spines are a major postsynaptic target for excitatory afferent axons. Studies of Golgi-impregnated neurons have shown that spine density and morphology appear to be responsive to developmental, environmental, and disease-related influences (for reviews, see Schiebel and Schiebel, 1968; Greenough, 1985; or Coss and Perkel, 1985). Several electron microscopic studies suggest that dendritic spines might change shape following production of physiological plasticity in the hippocampus in the form of long-term potentiation (LTP) (Van Harreveld and Fifkova, 1975; Moshkov et al., 1977, Fifkova and Andersen, 1981; Lee et al., 1980; Desmond and Levy, 1983; Chang and Greenough, 1984; and see Lynch et. al., this volume). Many theoretical descriptions show that the specific shapes and dimensions of dendritic spines might influence the amount of current transferred from synapses on spine heads, through the spine necks, and into the parent dendrite (Chang, 1952; Diamond et al., 1970; Rall, 1974; Jack et al., 1975; Perkel, 1982–1983; Wilson, 1984; see also Rall and Segev this volume). These descriptions emphasize that small changes in spine dimensions could produce large changes in synaptic efficacy. Other studies suggest that spine location in the dendritic arbor might be important in regulating current transfer to the soma (Turner, 1984). All of these studies indicate the need for complete and accurate measures of anatomical features of different types of spiny dendrites and for empirical measures of their biophysical properties.

Understanding how features of dendritic spines are influenced by extrinsic or intrinsic factors requires measuring the spines, components of the

surrounding neuropil that interact with the spines, and organelles or other structures inside of the spines. The size of most of these structures occurs outside the limit of resolution of the light microscope, and therefore obtaining the desired measurements requires electron microscopy and three-dimensional reconstructions of the dendritic spines (Westrum and Blackstad, 1962; Feldman, 1975; Friere, 1978; Wilson et al., 1983; Spacek and Hartman, 1983). We discuss here how complete three-dimensional reconstructions of spiny dendrites were obtained from electron microscopy to begin evaluating how anatomical features of dendritic spines might modulate ionic events occurring in the spine following synaptic activation.

MATERIALS AND METHODS

Methods for collecting serial sections and for using a movie reconstruction system to obtain accurate microalignment have been described elsewhere (Stevens and Trogadis, 1984). Briefly, serial thin sections obtained from aldehyde-fixed rat hippocampus and cerebellum were collected on formvar-coated slot grids. Dendritic segments from pyramidal cells in area CA1 of the hippocampus and spiny branchlets of cerebellar Purkinje cells were photographed through serial sections in the electron microscope. The negatives were then rephotographed onto a continuous 35-mm filmstrip, referred to as a movie. The movie was mounted on a film transport system, which was controlled by a computer interface. Each spine was traced from its dendritic origin through dense neuropil to the synapse on its head. Portions of the spine that were sectioned from the dendrite were reconnected to portions that were attached to the dendrite. Accurate positioning of the sectioned portions of the spines was achieved by superimposing a stored photographic image of the previous section over the live photographic image of the section to be traced. All of the structures viewed in the local neuropil could then be used as feducials to align parts of the dendritic spines in sequential sections. These parts were then traced into the computer reconstruction system, and three-dimensional reconstructions were generated from the complete tracings.

DENDRITIC SPINE DENSITIES AND DISTRIBUTIONS

We chose to reconstruct every dendritic spine along segments of spiny dendrites instead of selecting individual spines scattered throughout the neuropil. In this way, unusual features of spines did not selectively attract our attention, and small spines were not missed for lack of enough distinguishing characteristics. This approach also provided a means to identify unambiguously and to label the origin of every spine emerging from the dendrite so as to obtain accurate measures of spine densities and distributions. These complete reconstructions were used to determine whether spine density and distribution are influenced by extrinsic features of the local neuropil.

Figures 1–4 illustrate a reconstruction of a segment of spiny branchlet from a cerebellar Purkinje cell. Triangles were used to mark the location of each spine origin on the three-dimensional reconstructions. This cerebellar dendritic segment was located in the middle of dense neuropil (star in Fig. 5), and the dendritic spines were distributed around the circumference of the dendrite. Clustering of spine origins can be seen in Figure 2 and 3. A region of dendrite above spine 39 was essentially free of spines. For comparison, a second cerebellar spiny branchlet located near to a blood vessel was reconstructed (see Fig. 18 below). The side near to the blood vessel was free of spine origins for the entire length of the dendritic segment (Harris et al., 1985). Similarly, five hippocampal dendritic segments were reconstructed, and spine origins were labeled. These also exhibited nonuniform clustering of dendritic spines along the length of the dendrites (Harris et al., 1985).

Spine densities were determined by measuring the length of each dendritic segment and computing the number of spines per micron. The accuracy of this measurement is dependent on the value assigned to section thickness. Usually this value is estimated by the interference color of the ultrathin sections (Meek, 1976). We tested our estimates for each cerebellar series by measuring the diameter of a Purkinje cell dendrite in a single section and across sections (Fig. 5). The first measurement does not require section thickness. The second measure of the diameter is obtained by counting the number of sections that the dendrite appeared in at the same location where the diameter was measured in a single section. Since Purkinje spiny branchlets are approximately circular at a single plane through them, these two measures of the diameter should be nearly equal, and the following equation should hold for section thickness:

$$\text{section thickness } (\mu\text{m/sect}) = \text{measured diameter } (\mu\text{m})/\text{No. of sections}$$

We obtained section thicknesses of 0.06 μm for one series and 0.07 μm for the other series; both values were in close agreement to the estimates obtained from the interference colors of silver-grey and silver, respectively.

Spine densities obtained by three different methods for Purkinje spiny branchlets (cereb) and hippocampal Cal spiny dendrites in stratum radiatum (hippo) are presented in Table 1. Historically, dendritic spine densities have been obtained from light microscopic observations by counting spines on Golgi-impregnated dendrites (Schiebel and Schiebel, 1968). The absolute values obtained from these counts however, are subject to the limits of light microscopic resolution of two adjacent spines (0.25 μm). Many spines can also be obscured by the dendritic shafts and by other spines (Feldman and Peters, 1979). Consequently, counts of dendritic spines in Golgi preparations are often underestimates of true spine densities.

Spine densities on Purkinje spiny branchlets have been estimated from Golgi preparations of adult rat cerebellum as .8–3 spines μm of dendritic

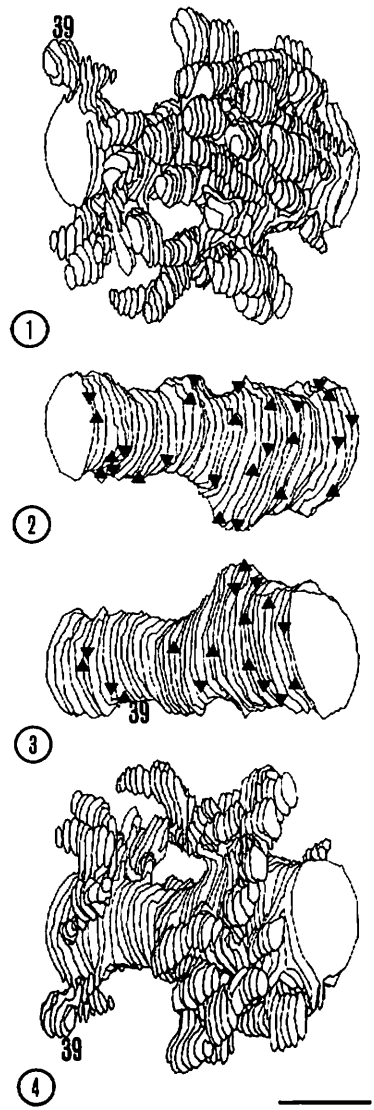


Fig. 1–4. Three-dimensional reconstructions of a segment of cerebellar spiny branchlet, which was located in the middle of dense neuropil in the molecular layer. Figures 1 and 4, and 2 and 3 are rotated 180 degrees from one another. Triangles indicate spine origins. Bar = 1.0 μm.

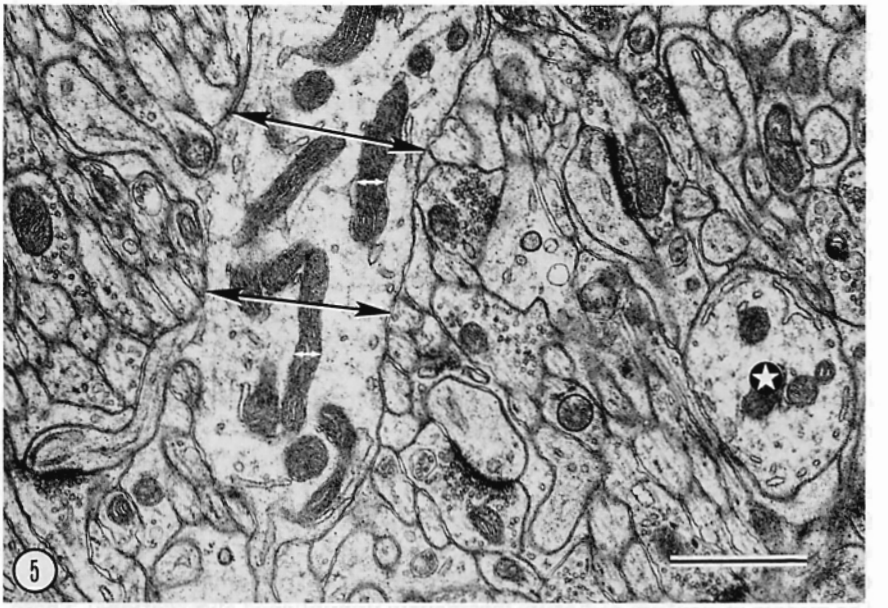


Fig. 5. Longitudinally and cross-sectioned spiny branchlets of cerebellar Purkinje cells located in the middle of dense neuropil. Dendrite 1 was used to test our estimates of section thickness. Large arrows on dendrite 1 indicate positions where dendrite diameter was first measured on this single section. This is also the location where the number of sections that this diameter appeared in were counted. Small arrows illustrate where mitochondria cut in longitudinal sections were also measured in a similar fashion to confirm section thicknesses. The star indicates the dendrite that was reconstructed and illustrated in Figures 1–4. Bar = 1 μm.

TABLE 1. Comparison of Three Methods to Obtain Spine Density Along Dendrites

	Spines/μm		
	Golgi	Acetate	Movie
Cereb	.8–3	7	11.6–14.3
Hippo	.4–5	1.3–3.6[a]	1.7–5.3

[a]Two dendritic spines were mistakenly included as being part of the dendritic segment when we first used this method on CAl spines.

length (Pysh and Weiss, 1979; Palay and Chan-Palay, 1974; Llinas and Hillman, 1969). Spine density along part of a cerebellar spiny branchlet was computed by Spacek and Hartman (1983) to be 7 spines/μm by tracing serial sections of the branchlet that were photographed in the electron microscope onto acetate sheets.

In our reconstructions of Purkinje spiny branchlets, we measured a spine density of 11.6 spines/μm for the dendritic segment adjacent to the blood vessel, which was 23% lower than the 14.3 spines/μm counted on the dendritic segment located in the middle of dense neuropil. These values from the movie reconstruction system were much greater than those obtained from the other two methods (Table 1).

Only two axons were observed between the dendritic segment and the blood capillary. This result suggests that the lower density of spines on this dendritic segment might occur from an extrinsic influence; namely the density of axons in the local neuropil.

The CA1 pyramidal cells are less spiny than the cerebellar Purkinje cells, and consequently it is easier to obtain accurate estimates of spine density from Golgi-impregnated dendrites of these cells (Table 1). As part of two earlier studies (Harris et al., 1980; Harris and Teyler, 1984), we counted dendritic spines on 28 lateral dendrites from CA1 pyramidal cells located in the middle of stratum radiatum (previously unpublished observations). These lateral dendrites tapered from 2.0 μm at the intersection with the apical dendrite to 0.3 μm at the dendritic terminals (a value at the limit of resolution of the light microscope, indicating that they might taper even more). Dendritic spines were counted along segments of these branches ranging in length from 10 to 40 μm. Dendritic spine density ranged from 1.33 to 5.00 spines/μm on these dendritic branches. Other studies of Golgi-impregnated CA1 pyramidal cells have computed spine densities of 0.4–1.9 spines/μm for the lateral branches in stratum radiatum (Wenzel et al., 1973; Minkwitz, 1976; Frotscher et al., 1978; Haschke et al., 1980). These spine density ranges overlap the ranges we obtained from tracing CA1 dendrites onto acetate sheets, or through serial reconstructions by the movie system (Table 1).

Do Dendritic Spines Increase Receptive Surface Area for Synapses?

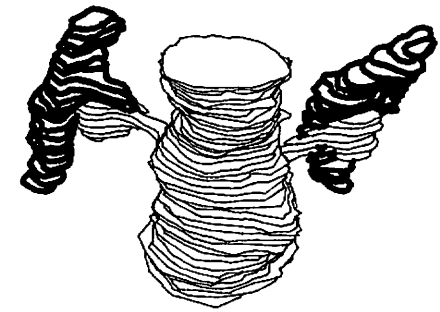
When dendritic spines were first discovered, it was suggested that they provided additional surface area on the dendrite where synapses could form (Berkley, 1896; Swindale, 1981; Coss and Perkel, 1985). This hypothesis has been largely dismissed, because electron microscopic views of spiny dendrites showed that dendritic membrane between the spines often lacks synapses (Gray, 1959, 1982). It has not yet been shown however, that the synapses found on the dendritic spines would indeed fit onto the dendritic shaft if the spines were removed.

To test whether the synapses would fit, we graphically removed all the spines from our three-dimensional reconstructions of the Purkinje and CA1 dendrites by severing them from their parent dendrites at their origins. Then we computed the remaining dendritic surface area that would be available for synapse formation. For the two Purkinje spiny branchlets this remaining area totaled 13–18.8 μm^2. The total synaptic area on these spines was 3.83–8.58 μm^2. Therefore, 29–45% of the remaining dendritic surface would have been covered with the synapses if the spines were removed. For the five CA1 pyramidal cell dendrites that were reconstructed, 5–9% of the remaining dendritic surface area would have been covered by the spine synapses. Together, these results show that the synapses formed on the dendritic spines would easily fit on the parent dendrite if the spines were removed and further suggest that spines do not simply increase dendritic surface area available for synapse formation. It does not tell us, however, if the presynaptic axons would have to change their course through the neuropil to make these contacts on the dendrite (Peters and Kaiserman-Abramhof, 1970; Swindale, 1981). The presynaptic axonal varicosities might also be too large to crowd next to one another along the dendrite if the spines were removed.

DENDRITIC SPINES AND THEIR PRESYNAPTIC AXONS

Our anatomical observations show that spine distribution is not uniform, even along short dendritic segments. Could this nonuniform clustering of spine origins influence activity of neighboring spines (see also Rall and Segev, this volume)? Does nonuniform clustering of dendritic spines reflect features of the organization of the afferent input to the dendritic spines? To begin understanding more about the anatomical interactions of the dendritic spines and their presynaptic axons, we reconstructed each presynaptic varicosity that was connected to the dendritic spines.

Most dendritic spines of Purkinje spiny branchlets and CA1 pyramidal cell dendrites form the only synapse found on their presynaptic varicosity (Fig. 6). However, many of the varicosities forming synapses with the Purkinje spiny branchlets had two or more synapses on them (Table 2). Most sharing of presynaptic varicosities occurred between two spines of the same dendritic segment (Fig. 7). Some varicosity sharing occurred between spines of different dendrites; and some sharing occurred between synapses on spines and synapses on nonspiny dendritic shafts (Table 2). Synapses on branched and unbranched spines of the same cerebellar dendritic segment also shared a single presynaptic varicosity. In the hippocampus, less varicosity sharing was observed and was only seen between spines of different dendrites. These spines often had different shapes and synaptic morphologies such that spines with macular synapses could share varicosities with spines having perforated synapses. Together these results suggest that the presynaptic axon does not trigger the formation of a spine or shaft synapse.



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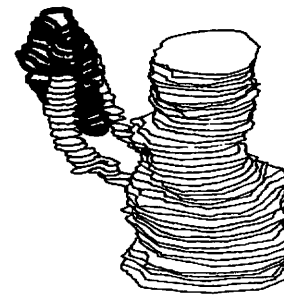

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Fig. 6. Presynaptic varicosities (thick lines) forming one synapse on one dendritic spine of a cerebellar spiny branchlet (thin lines).

Fig. 7. Neighboring spines from a single Purkinje spiny branchlet sharing the same presynaptic varicosity. Bar = 1 μm.

TABLE 2. Sharing of Presynaptic Varicosities by More Than One Synapse

	Percent varicosities shared		
	Two spines		
	Same dendrite	Diff dendrite	Spiny and nonspiny
Cereb	11–21	0–4	3–11
Hippo	0	5–13	0

It is also unlikely that the presynaptic axon alone determines dendritic spine shape, as spines of different shapes formed synapses on the same presynaptic varicosity. Therefore, it is reasonable to assume that extrinsic factors are not solely responsible for induction of dendritic spine morphology.

INFLUENCE OF SMOOTH ENDOPLASMIC RETICULUM ON DENDRITIC SPINE SHAPE: A HYPOTHESIS

In this section we discuss some advantages for neuronal organization that spiny dendrites provide, and we discuss how the smooth endoplasmic reticulum (SER) might be a limiting factor in determining the size and shapes that dendritic spines can assume. Dendritic spines allow their dendrites to reach distant axons without occupying the space between adjacent synapses (Fig. 8). Therefore, axons, other dendrites, and glia can occupy the space between synapses on adjacent dendritic spines. A nonspiny dendrite with the same reach to axons would occupy all the space between the

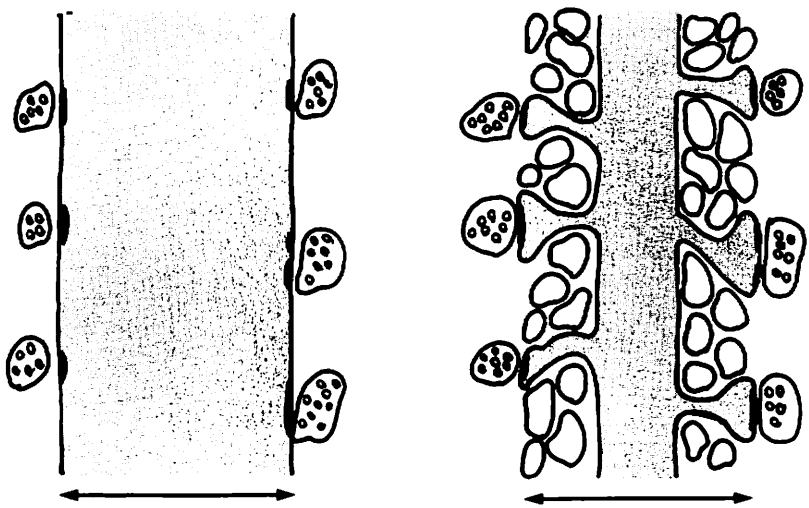


Fig. 8. Spiny and nonspiny dendrites with the same "axonal reach." Open contours next to the spines represent axons, glia, and other dendritic processes that could pass freely next to the spiny dendrite but not through the nonspiny dendrite.

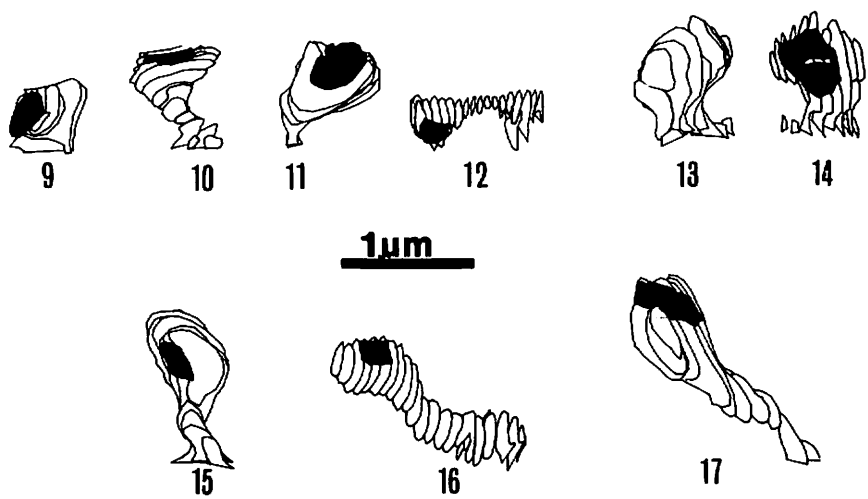
synapses, and therefore, fewer neuronal processes could fit into a limited brain volume of nonspiny dendrites. We propose that dendritic spines facilitate reach to distant axons while reducing the volume that a neuron must occupy for that reach. In this way, dendritic spines enhance the efficiency of packing neuronal processes next to one another.

If this is a major advantage of dendritic spines, then why are not all dendritic spines very thin and long in order to maximize reach and minimize brain volume occupied? We suggest that spine sizes and shapes reflect the amount of SER found inside the spines. Other studies suggest that SER sequesters calcium in the dendritic spines (Fifkova et al., 1983; Andrews et al., 1984; and Landis, this volume). The volume of SER found inside a spine might be responsive to synaptic activity or other processes in the dendrite or extracellular space that change ionic concentrations within the spine. As the SER swells or shrinks in response to changes in ionic concentrations, so does the spine. A spine cannot be smaller or thinner than its intrinsic SER volume plus some additional cytoplasmic volume surrounding the SER (see Stevens, this volume, for a broader version of this hypothesis extended to varicose dendrites and axons; and Sasaki-Sherrington et al., 1984; Jacobs and Stevens, 1986a,b). This hypothesis does not exclude involvement of

cytoplasmic and membrane proteins as part of the mechanism of shape change occurring in response to changes in spine and SER volume. It also does not exclude changes in synaptic receptor densities occurring in parallel response to changes in synaptic activity.

The Shape of SER in Spines Mimics Spine Shape

In the hippocampus and the cerebellum, spines of different sizes and shapes were found near or adjacent to one another on the same dendritic segment. Example reconstructions of spines from each region are illustrated in figures 9–17. Synapses were usually located along the side of the spine heads. All of the CA1 spines had one synaptic junction. Some of these synapses were perforated by electronlucent regions (Fig. 14). In the cerebellum, most of the spines were lollipop-shaped, and all of these had a single macular synaptic junction. Some of the cerebellar dendritic spines were branched, having two or more necks and heads attached by a common base to the parent dendrite. Some of the heads of the branched spines had no synapses and ended in a glial sheath with no evidence of a presynaptic varicosity. For a more detailed description of synaptic morphology in these two regions, see Harris and Landis (1986).



Figs. 9–14. Reconstructions of dendritic spines (thin lines) in area CA1 with their synaptic junctions (thick dark regions).

Fig. 15–17. Reconstructions of dendritic spines from spiny branchlets of cerebellar Purkinje cells. For all of these spines, the dendritic segment to which they were attached would have run parallel to the calibration bar.

In single sections of the cerebellar dendritic spines, the SER appears to form disconnected cisterns (Fig. 18). Three-dimensional reconstructions of the SER showed that it formed a continuous, looped cistern that was always connected to the SER of the parent dendrite. When viewed in three-dimensional reconstructions, the shape of the reconstructed SER mimicked the shape of the dendritic spines in which it was found (eg., Figs. 19,20).

In our reconstructions of SER in hippocampal dendritic spines, "stubby" spines (eg., Fig. 9) or "thin" spines had SER of a tubular shape like that described for the cerebellar spines. In larger "mushroom-shaped" spines, the SER formed flattened cisternae laminated with dense-staining material

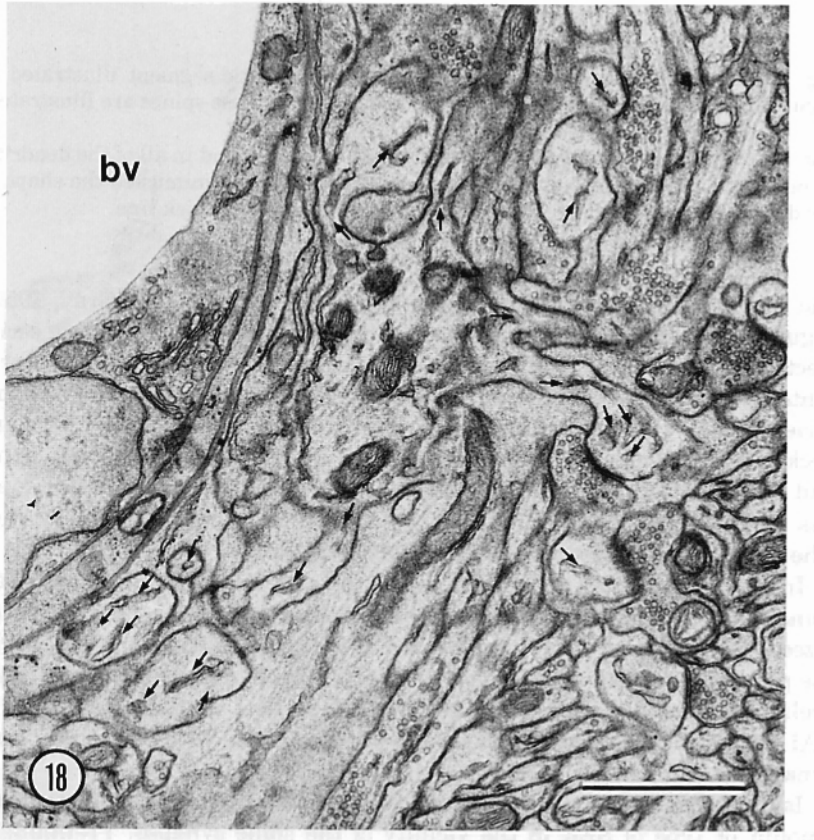


Fig. 18. Dendritic segment of a cerebellar Purkinje cell that was located near to a blood vessel (bv). Arrows indicate portions of smooth endoplasmic reticulum in every dendritic spine, which was found through serial reconstructions to be attached to this dendritic segment. Bar = 1 μm.

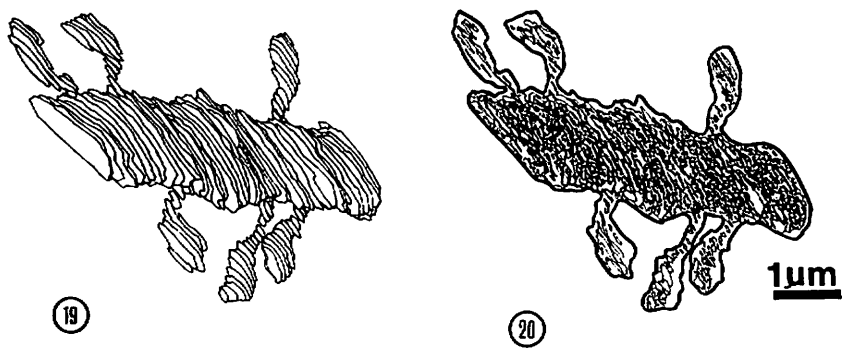


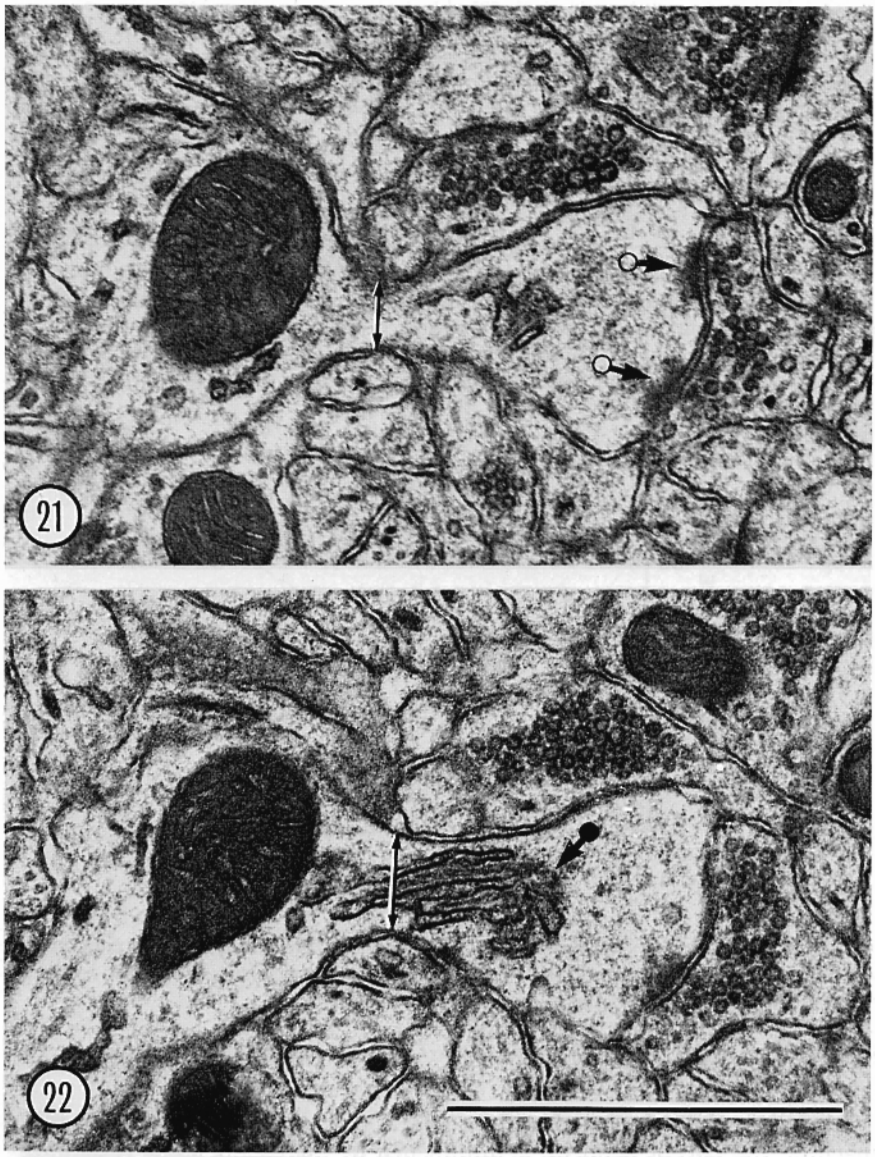
Fig. 19. Partial reconstruction of the cerebellar dendritic segment illustrated in Figure 18. This segment had 54 dendritic spines. Six of these spines are illustrated.

Fig. 20. The smooth endoplasmic reticulum (thin lines) found in all of the dendritic spines was connected to the SER of the parent dendrite and mimicked the shape of the dendritic spines that are outlined from Figure 19 with a thick line.

and has been referred to as a "spine apparatus" (Fig. 21, 22; Gray, 1959). Figures 21 and 22 also illustrate an important reason for using serial electron microscopy to study dendritic spine dimensions and ultrastructural content. If one were to measure the spine neck diameter on section 9 of this series, one would mistakenly assume that this spine had a very thin spine neck and a bulbous head. One would also wrongly assume that this spine had no well-organized spine apparatus. It only becomes clear that this spine has a wide neck, which is nearly filled with a laminated spine apparatus, when the spine is viewed through serial sections.

In the visual cortex, the spine apparatus is typically found in dendritic spines that have a perforated postsynaptic density (Spacek, 1985). We analyzed 84 spines from five reconstructed dendritic segments in area CA1 for the presence of spine apparatuses and perforated synaptic junctions. These preliminary analyses presented in Table 3 show that the spine apparatus of CA1 dendritic spines is also preferentially associated with perforated postsynaptic densities.

Is it possible that one of the spine's functions is to package an appropriate amount or type of SER in the vicinity of the spine synapse? Preliminary results from area CA1 show that larger dendritic spines contain more SER than smaller dendritic spines (Fig. 23). Larger cerebellar spines also contain more SER than smaller cerebellar spines. (Harris and Stevens, manuscript in preparation).



Figs. 21 and 22. CA1 dendritic spine with perforated postsynaptic density (open circles) and a well-developed spine apparatus (solid circles). In both figures the spine is clearly attached to the parent dendrite, but the dimensions of the neck (double-headed arrow) are dramatically different in each section, illustrating the importance of measuring spine dimensions through serial sections. Bar = 1 μm.

TABLE 3. Association of Perforated Synapses With Spine Apparatuses[a]

Synapse morphology	SER morphology				
	SA	Org in spine	Org at base	Tube	Totals*
Perforated	6	2	1	2	11
Curved partial perforation	1	1	2	0	4
Macular	0	0	11	58	69

[a]Abbreviations: SA, a laminated spine apparatus with dense staining material separating the flattened cisternae of SER; Org, cisternae of SER that are lined up next to one another that appear to be organized but are not flattened, and do not have dense staining material—this organized SER was seen either inside the spines or at the spine bases; Tube, a single tube of SER with no evidence of organization among cisternae.
*$\chi^2 = 61.14$; $P < .0001$.

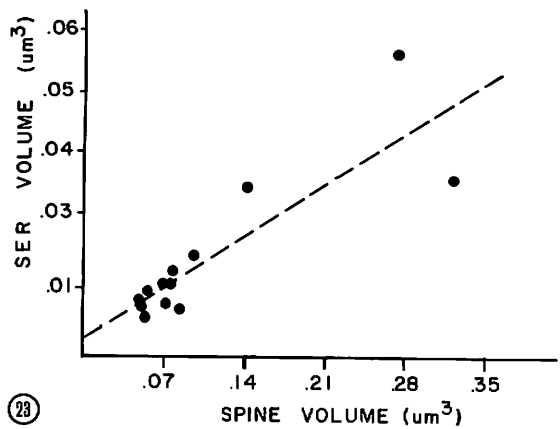


Fig. 23. The volume of SER within CA1 dendritic spines is positively correlated with the total spine volume. These are preliminary results from spines on a single reconstructed dendritic segment.

Biophysical Effects of SER on Synaptic Current Passing Through Dendritic Spines

Modeling of dendritic spines has shown that the peak synaptic current reaching the parent dendrite is diminished by increasing spine resistance and is enhanced by reducing spine resistance (Rall, 1974). Spine resistance can be increased by lengthening or narrowing the neck, by increasing cytoplasmic resistivity, or by occluding the spine head or neck with intracellular organelles. The greatest effect of the intracellular organelle, the SER,

occurs on spines with neck diameters in the range of 0.1–0.2 μm (Fig. 24, modified from Fig. 8 of Wilson, 1984). For example, if 50% of a spine neck with a diameter of 0.1 μm were occluded by SER, synaptic transmission through the neck would be 58% less than if no SER occluded the neck. In contrast, synaptic transmission would be reduced only 17% if the neck diameter were 0.2 μm and 50% occluded by SER.

These calculations illustrate the importance of obtaining accurate measures of spine dimensions and the volume of SER occluding portions of the spines. To obtain these measures we have developed a procedure to edit three-dimensional reconstructions of dendritic spines into their heads, necks, and bases (Fig. 25, 26) Complete reconstructions of the spines were first rotated and viewed from several angles to evaluate where the head and neck junction should be positioned and where the spine base begins. Then the length and diameter of the spine neck where it was thinnest were determined. The volume of each spine compartment was subsequently computed, and the volume of SER occluding them was determined.

Measures of spine necks at their thinnest portions have been completed for 77 cerebellar spines and 54 hippocampal spines. Most cerebellar spine neck diameters were 0.15–0.2μm (full range = 0.06–0.77 μm. These values of spine neck diameter are in the critical range for the graphs in Figure 24 where small changes in neck dimensions or intra-neck resistivity can result

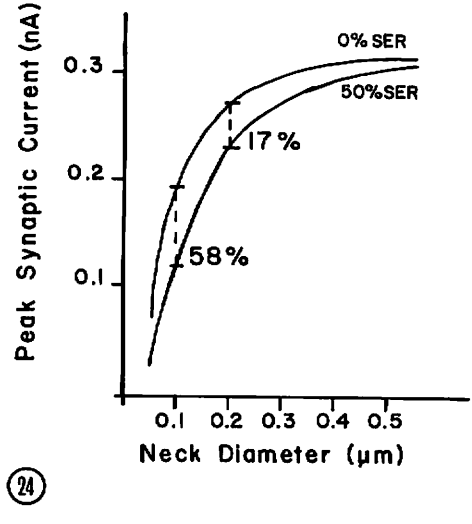


Fig. 24. Effect on peak synaptic current reaching the spine base when smooth endoplasmic reticulum partially occludes spine necks of different diameters. The curves of this figure are for a 3-μm-long spine and were traced from Wilson (1984).

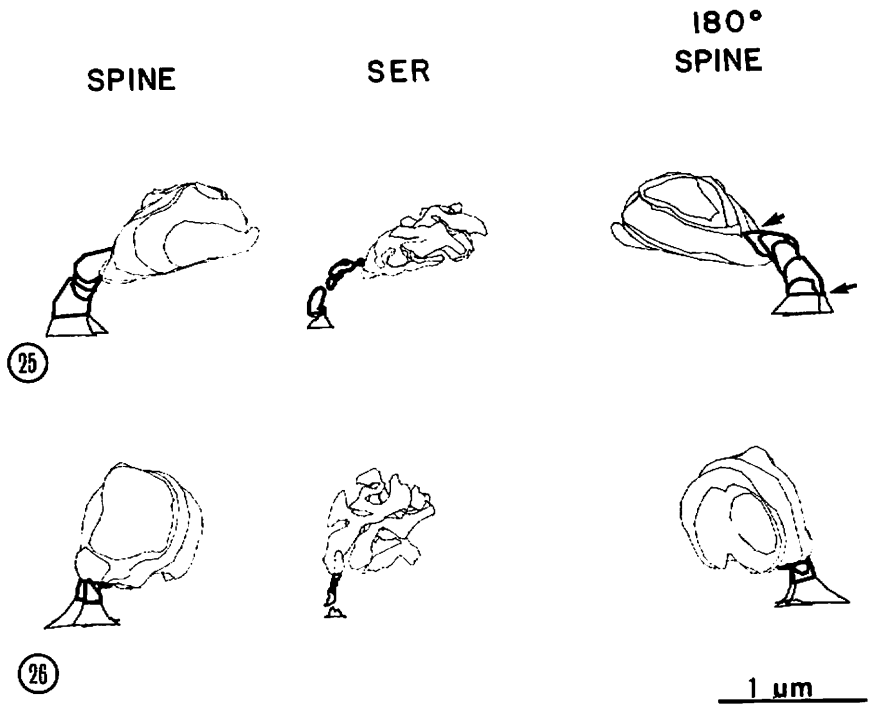


Fig. 25 and 26. Edited cerebellar dendritic spines and their smooth endoplasmic reticulum. The arrows indicate the junctions of the spine head (thin lines) with the neck (thick lines), and the junction of the spine neck with the slightly wider base (medium thick lines). The neck is defined here as the thinnest region of the spine of relatively uniform diameter. The shape of the SER in these spines mimics the spine shape. These edited necks were used to determine the percent of neck volume occupied by SER shown in Figure 27.

in dramatic changes in synaptic current flow (Wilson, 1984; see also Perkel, 1982 to 1983).

The model presented in Figure 24 is for a long spine (3 μm) with resistivity of the SER and spine cytoplasm assumed to be 50 ohm-cm. The model does not account for interactions between spine neck length and possible differences in intra-neck resistance. It illustrates, however, that small *decreases* in neck diameter or *increases* in neck resistance could dramatically *reduce* current flow through cerebellar spines. Small *increases* in cerebellar spine neck diameters would not dramatically change the flow of current through the spine.

We have measured the volume of spine necks and SER occluding the necks of 19 cerebellar dendritic spines. To obtain these measures, the recon-

structed spines were graphically "resectioned" into their heads, necks, and bases. Two of these "resectioned" spines are illustrated in Figures 25 and 26. The volume of neck SER was then compared to the total volume in the spine neck compartment. The percent occlusion was computed and compared to the spine neck diameter (Fig. 27). These preliminary results show that the amount of spine neck occluded by SER is 7–27%. Thin spine necks, less than 2 μm in diameter, exhibit the full range of this occlusion, suggesting that SER occlusion might reduce current flow through some of these spines and have little or no effect on other larger spines.

Hippocampal spines and other cerebellar spines are being analyzed to obtain a broader sample of spine dimensions and SER content in the spines. Other features such as synaptic area and vesicle content of the presynaptic varicosity are being analyzed to learn whether these structural representatives of synaptic activity might also distinguish spines with different amounts of SER.

CONCLUSIONS

We have presented some evidence that spine distribution can be influenced by extrinsic factors such as the density of axons in the local neuropil. We have shown that spine shape, however, is not likely to be predetermined by the presynaptic axons, because spines of differing shapes can share the same presynaptic varicosity and because spine and shaft synapses can share the same presynaptic varicosity. We have begun to analyze whether the smooth endoplasmic reticulum, the only organelle consistently found inside dendritic spines, might play a role in limiting dendritic spine shape. We

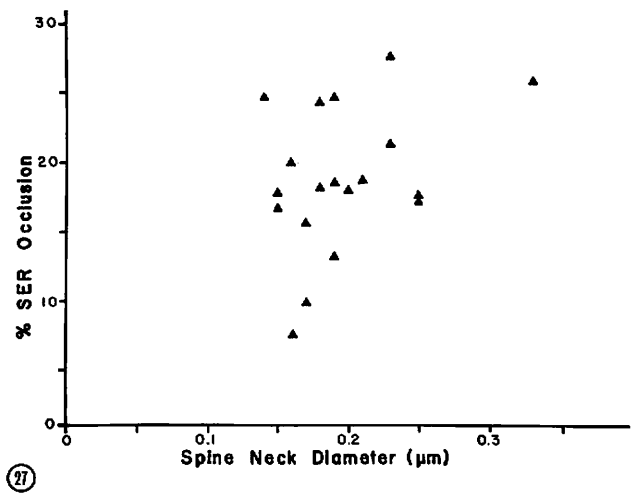


Fig. 27. Percent of neck volume occluded by SER in 19 cerebellar dendritic spines.

have found that the shape of SER mimics the overall shape of the dendritic spines. Hippocampal spines with perforated synapses frequently have laminated SER that forms a spine apparatus, while hippocampal and cerebellar spines with simple macular synapses have only simple tubes of SER. Preliminary results suggest that the volume of SER occupying the spine is proportional to the total spine volume. The volume of the SER occupying the spine neck might alter the amount of synaptic current passing through the dendritic spines. These anatomical studies were greatly facilitated by the use of a computer-assisted system for three-dimensional reconstruction from serial electron microscopy. Further biophysical modeling will be necessary to test how much SER occlusion in spines of different dimensions will effect synaptic current flow through the spine. Experimental studies will be required to determine if spine SER is involved in the calcium cascade following synaptic transmission or changes shape within the spines in response to synaptic activity.

ACKNOWLEDGMENTS

We thank Ms. Judy Trogadis for training K.M.H. in the use of the serial reconstruction system. We thank Ms. Linda Kirschner and Mr. Nariman Shambayati for developing the software for displaying and editing three-dimensional reconstructions in the Image Graphics Lab of the Neuroscience Department at Children's Hospital. Dr. Max Snodderly is appreciated for his many helpful suggestions and discussions of this work. Mr. William McIntosh is thanked for photographic assistance on some of the figures. Dr. Robert Goldstein of the Eye Research Institute of the Retina Foundation is thanked for allowing K.M.H. to use their RSl statistical package (Bolt, Beranek, and Newman, Cambridge, MA). This research was supported by NINCDS grant #NS21184 to K.M.H. and a research grant from the Medical Research Council of Canada to J.K.S.

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