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Three-dimensional analysis of dendritic spines

II. Spine apparatus and other cytoplasmic components

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Summary. A total of 342 dendritic spines (193 from the visual and 149 from the cerebellar cortex of the mouse) were analyzed in serial and several hundred of thousands of them in single sections, with respect to the presence and organization of the spine apparatus and other cytoplasmic components. The continuity of the spine apparatus with the smooth endoplasmic reticulum of the dendritic trunk was shown in three-dimensional reconstructions. The dense material of the spine apparatus was divided into "inner dense plate" and "outer dense plate". The close relationship between the outer dense plate and the postsynaptic density suggests that the spine apparatus functions as a postsynaptic protein synthesizing centre. The material from the outer dense plate could be used for a dynamic extension of the synaptic active zone. An extraspinous spine apparatus of the axon initial segment was partially reconstructed. Polyribosomes were found in all large spines of the visual cortex but were not so frequent in small spines and in Purkinje cell dendritic spines. Microfilamentous network and intermediate filaments occurred in the spines. The smooth endoplasmic reticulum of Purkinje cell dendritic spines was reconstructed. No spine apparatus and dense material were present in these spines.

Key words: Pyramidal cell – Purkinje cell – Dendritic spine – Spine apparatus – Smooth endoplasmic reticulum

Introduction

The study of dendritic spines has advanced considerably in recent years. The finding that the dendritic spines are dynamic structures which significantly change their shapes in response to stimulated synaptic inputs (Fifková and Van Harreveld 1977; Coss and Globus 1978; Coss et al. 1980; Berard et al. 1981; Fifková and Anderson 1981; Anderson and Fifková 1982; Boycott 1982; Brandon and Coss 1982; Coss and Brandon 1982; Crick 1982; Fifková et al. 1982; Burgess and Coss 1983) has diverted attention from shape morphology to their inner ultrastructure.

Matus et al. (1982) and Caceres et al. (1983) found a high concentration of actin in dendritic spines by means of immunocytochemical methods and thus verified the observation of Le Beux and Willemot (1975a, b) who demonstrated by means of heavy meromyosin labelling that actin-like filaments are ubiquitously distributed throughout the neuron including spines and the postsynaptic density.

Apart from finely filamentous wispy material, now known to contain actin and supposed to be responsible for changes in the shape of the spine, there is another more distinct cytoplasmic component in dendritic spines, the spine apparatus. The spine apparatus is a derivate of smooth endoplasmic reticulum composed of two or more apposing cisternae (sacs) and plates (bands) of dense material between them. It has been first described in spines of the cerebral cortex by Gray (1959) but later was found also in spines of other parts of the brain, for example in the hippocampus (Hamlyn 1962; Westrum and Blackstad 1962), basal nuclei (Hall 1968; Adinolfi 1971; Pappas and Waxman 1972; Wilson et al. 1983) and thalamus (Colonnier and Gillery 1964). This organelle is characteristic for dendritic spines of the mammalian forebrain. It has been found, but rarely, also on spines of the medulla oblongata (see Scheibel and Scheibel 1968 for references) and in spines of the cerebellar cortex (Sotelo 1971), i.e., in the hindbrain.

The spine apparatus, however, is not unique for dendritic spines, for it was occasionally observed in the dendritic trunks (Westrum 1970; Gray and Guillery 1963; Jones and Powell 1969a) and often was found in the axon initial segments (Palay et al. 1968; Peters et al. 1968; Jones and Powell 1969b; Westrum 1970; Kosaka 1980) being sometimes termed a "cisternal organelle" in these localizations.

To my knowledge, no comprehensive attempt has yet been made to study the spine apparatus in detail in series of sections, with the exception of the study of Wilson et al. 1983. It is the purpose of the present paper to ascertain from serial section analysis of the spine apparatus other forms of smooth endoplasmic reticulum and other cytoplasmic components in dendritic spines of visual and cerebellar cortices, also the relationships between the spine apparatus and the filamentous network in dendritic spines, and to discuss their possible functional significance.

Materials and methods

Young adult albino mice were anesthetized with chloralhydrate and perfused through the heart with a phosphate-buffered 1% glutaraldehyde/1% paraformaldehyde solution (Palay and Chan-Palay 1974). Tissue blocks from the

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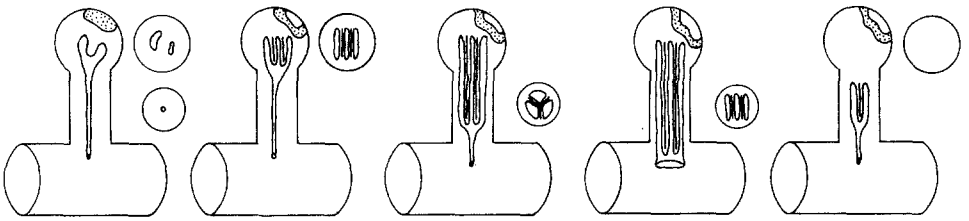


Fig. 1. Most representative locations of spine apparatus in dendritic spine, in parallel and perpendicular sections

cerebral visual cortex, identified according to Valverde and Esteban (1968) and Caviness (1975), and from the cerebellar cortex (vermis), were post-fixed with phosphate-buffered 2% osmium tetroxide, block-stained with uranyl acetate, dehydrated in ethanol, passed through propylene oxide and embedded in an Epon-Durcupan mixture. After selection of specific areas (laminae I and II of the visual cortex and the molecular layer of the cerebellar cortex), performed in toluidine blue-stained semithin sections, short (up to 60 sections) series were cut perpendicularly to the surface of the cortex, picked up on slot grids bearing a carbon-coated collodium film and stained with uranyl acetate and lead citrate. These series were photographed in a Tesla BS 500 electron microscope at initial magnifications of $\times 4{,}000$ or $\times 6{,}000$, then studied at final magnifications of $\times 29{,}000$ or $\times 39{,}000$. In addition to the series, several hundreds of thousands of dendritic spines were studied in single sections, of which some were 0.2 μm thick, to facilitate the study of filamentous and tubular structures.

Details concerning the reconstruction technique employed are to be found in Špaček and Lieberman (1974a).

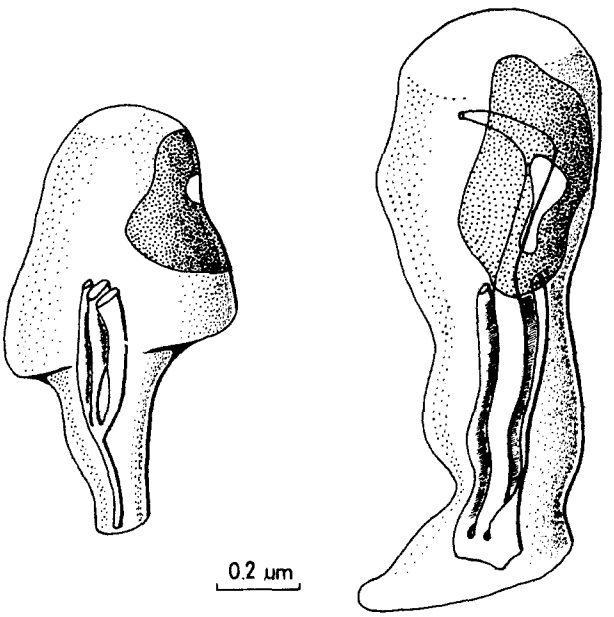


Fig. 2. Graphic reconstructions of mushroom-shaped dendritic spines with spine apparatus

Results

Visual cortex

Spine apparatus. The *cisternae* of the spine apparatus are most distinct when cut perpendicularly in the heads of bigger spines. They are either separated one from another or U-, S-, or fork-shaped, and their number in the spine apparatus is determined by the dendritic spine surface area and volume and by the synaptic active zone surface area. A majority of spines with a complex or multifocal synaptic active zone, mostly of mushroom-shaped type, contain the spine apparatus (Špaček and Hartmann 1983). The most elaborate spine apparatus in my material possessed 8 cisternae. Basic modes of location of the spine apparatus in dendritic spines are shown in Fig. 1. A tubule of smooth endoplasmic reticulum connecting the cisternae of spine apparatus with the endoplasmic reticulum in the dendritic trunk is occasionally found in single sections and regularly in series (Figs. 2, 3). The connecting tubule usually ramifies in the head or in the stalk of spine, but cases where the sacks of the spine apparatus were already formed in the dendritic trunk at the base of the stalk were not rare (Figs. 6, 7). The spine apparatus was sometimes located in the stalk only. The cistern of the endoplasmic reticulum of the dendritic trunk, giving rise to the connecting tubule, was often juxtaposed to mitochondria (Figs. 6, 26).The end-parts of the sacks of the spine apparatus in the spine head may protrude in vesicle-like manner. Occasionally, more than one profile of spine apparatus is found in the head or stalk of spines. As apparent from serial sections, this is due to the corkscrew morphology of one spine apparatus only (cf Scheibel and Scheibel 1968).

The *dense material* between cisternae of the spine apparatus forms plates about 10–20 nm wide. An intermediate dense line with side-arms or a row of fine granules or filaments about 8–10 nm in diameter, tubules about 25 nm in diameter (cf Westrum et al. 1980), and, exceptionally, globules up to 30 nm in diameter, are visible in cross-sections (Figs. 8, 9, 10). The dense plate is occasionally doubled and it can even be Y-shaped in cross-sections through the stalk (Fig. 1). This interposed dense material, well-known from the descriptions of previous authors, has been denominated in this study *inner dense plate* to distinguish it from *outer dense plate,* which is another dense material continuous with one or more inner dense plates but localized on the outside of the spine apparatus. It is not clear whether the outer dense plate actively emerges from an inner dense plate or whether in fact it represents an inner dense plate made naked by a retraction of cisternae. Apart from numerous single sections, outer dense plate was found in serial sections through 40 dendritic spines, and it seems that it is present in most spines equipped with the spine apparatus if not in all of them. Outer dense plate was frequently observed in heads or stalks of spines either in parallel or perpendicular to the spine axis (Figs. 11–14, 16, 17). Its shape was sometimes concave (Fig. 17). Exceptionally it was found at the base of a dendritic spine (Fig. 15). Outer dense plate has a tendency to widen and to round off into a ball-shaped fluff. The same appearance could, however, also result from a section cut tangential to the plate. In its central part, the internal structure of outer dense plate resembles that of inner dense plate. From its periphery, there

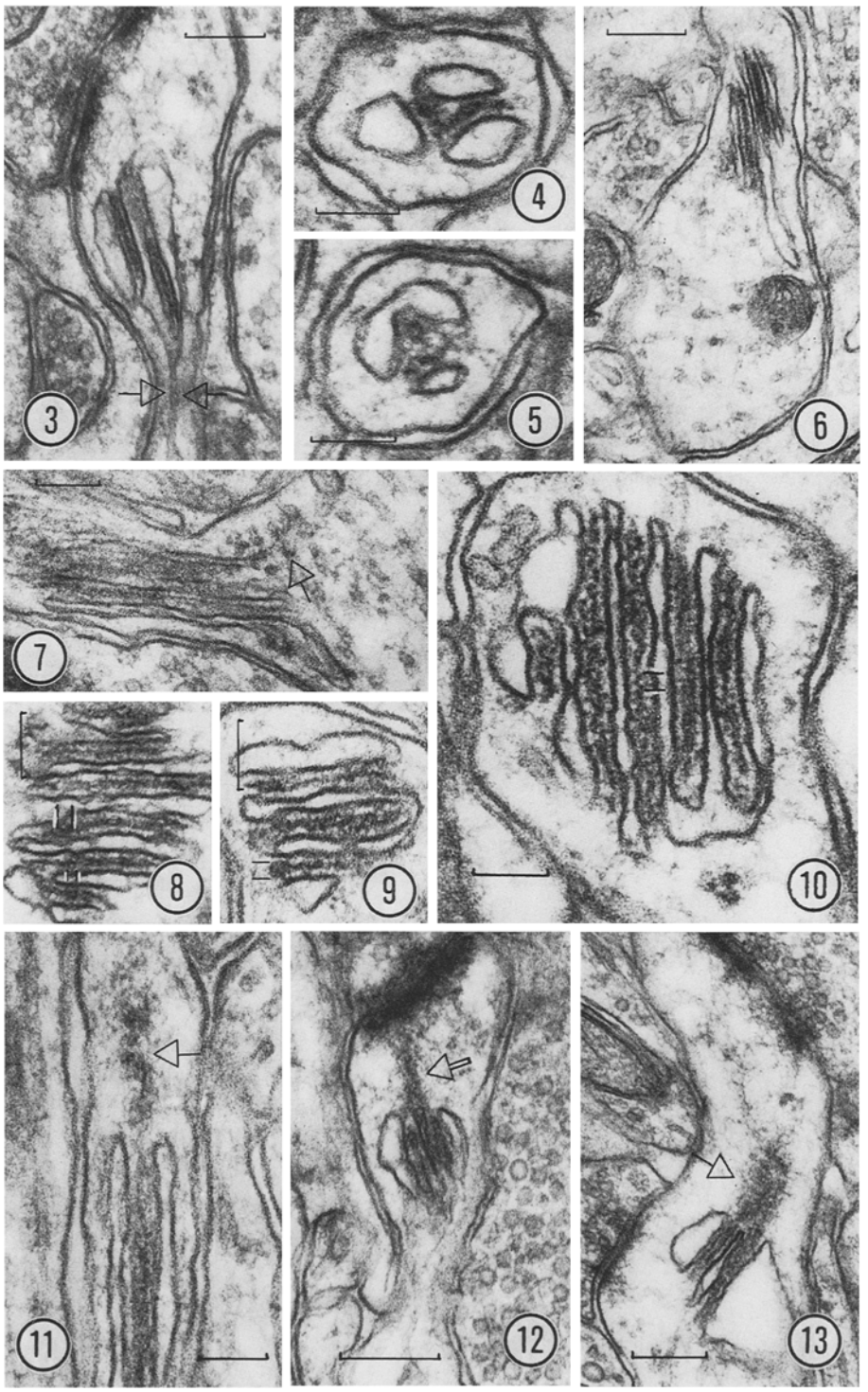


Fig. 3. Spine apparatus with the connecting tubule of smooth endoplasmic reticulum in the stalk (*arrows*). Scale = 0.2 μm

Figs. 4, 5. Perpendicular sections through spine apparatus in dendritic spine stalks. Scale = 0.1 μm

Fig. 6. Cistern of endoplasmic reticulum in the dendritic trunk continuous with spine apparatus located in dendritic spine stalk. Mitochondrion occurs in the vicinity of the cistern. Scale = 0.2 μm

Fig. 7. Spine apparatus located in the base of a dendritic spine and a cluster of polyribosomes (*arrow*). Scale = 0.1 μm

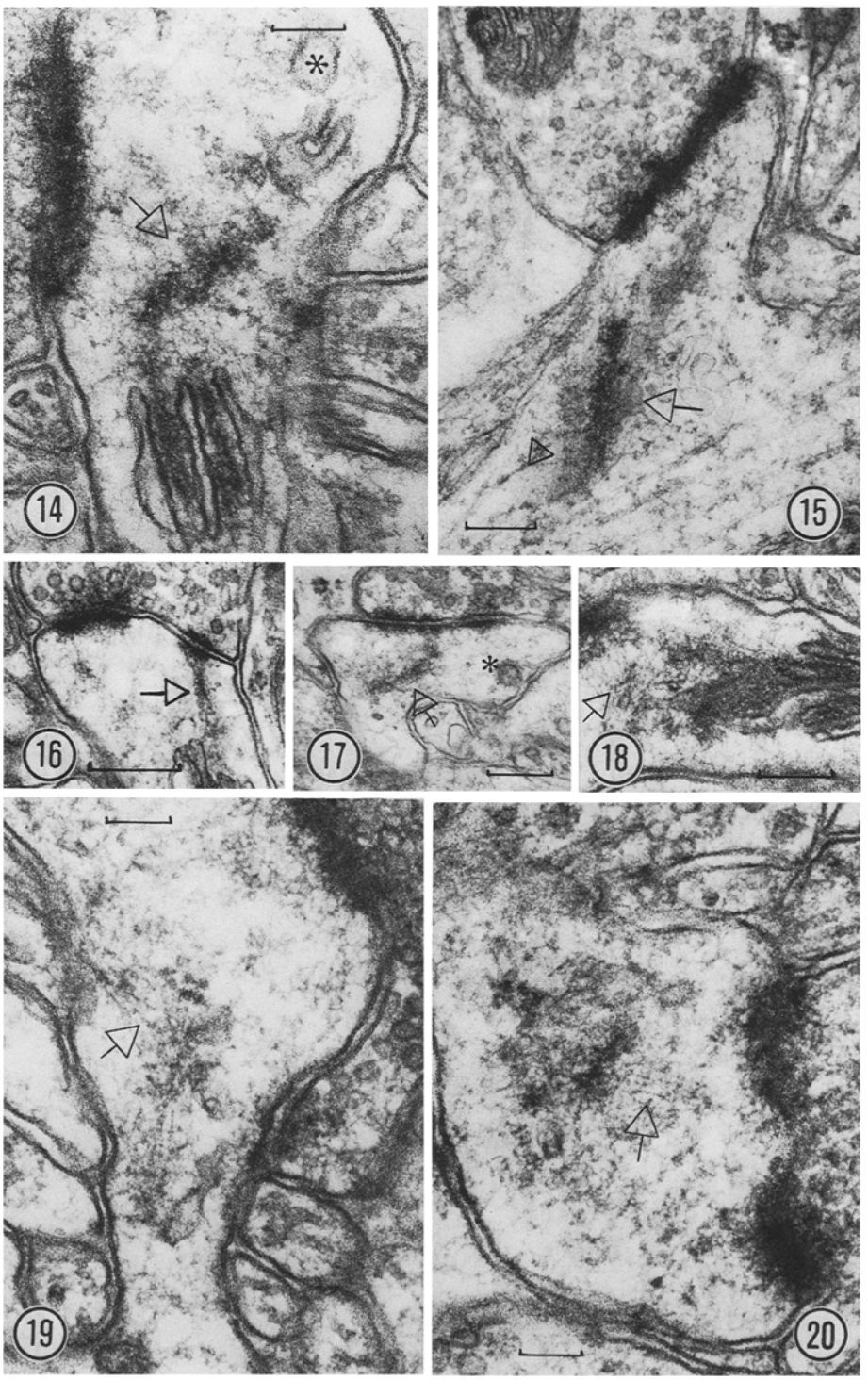
Figs. 8–10. Spine apparatus with elaborated cisternae and inner dense plates. Thin tubules and a globule (**Fig. 9**) are marked with lines. Scale = 0.1 μm

Fig. 11. Spine apparatus located in dendritic spine stalk. Outer dense plate (*arrow*) arises from inner dense plate. Scale = 0.1 μm

Figs. 12, 13. Outer dense plate (*arrows*) in apparent relationship to the postsynaptic density. Scale = 0.2 μm

radiate thin filaments to continue with a three-dimensional microfilamentous lattice filling the dendritic spine head (Figs. 18–21). When localized near synaptic active zone, the filaments radiate directly into the postsynaptic density (Figs. 12, 17, 18). Sometimes such a radiation was found from outer dense plate into the attachment plaque between dendritic spine and axon terminal or glial process (Fig. 16).

Dendritic spines small in volume and surface area, and with simple synaptic active zone, have no differentiated spine apparatus (Špaček and Hartmann 1983). Irrespective of whether in single or serial sections, the detection of smooth endoplasmic reticulum was very difficult in small spines, even when using initial magnification higher than that referred to in Methods. Some of these small spines seem to be quite free of smooth endoplasmic reticulum, whereas others contain a very thin, often discontinuous (perhaps an artefact) tubule widened in the spine head (Fig. 22). Dense material similar to outer dense plate was only exceptionally observed in spines lacking spine apparatus.



Figs. 14–17. Outer dense plate (*arrows*) in dendritic spine heads and base (**Fig. 15**). A regular latticework of fine filaments next to outer dense plate is apparent in Fig. 15 (*empty arrowhead*), terminal parts of cisternae of spine apparatus in **Fig. 14** (*asterisk*), attachment plaque in **Fig. 16** (*arrow*), coated vesicle in **Fig. 17** (*asterisk*). Scale = 0.1 μm (Fig. 14), 0.2 μm (Figs. 15, 16, 17)

Figs. 18–20. Fine filaments radiating from peripheral parts of outer dense plate (*arrows*). Scale = 0.2 μm (Fig. 18), 0.1 μm (Figs. 19, 20)

Extraspinous spine apparatus (cisternal organelle) up to 5 μm in length was regularly found in axon initial segments and occasionally in dendritic trunks near the base of a spine. In the initial segment, the spine apparatus was most frequently in juxtaposition to the postsynaptic density. It usually did not exceed three cisternae. Fig. 23 demonstrates it in partial reconstruction.

Other cytoplasmic components. Coated vesicles and *multivesicular bodies* are occasionally observed in dendritic spine heads. The latter are particularly frequent in the dendritic trunks near the base of the spine stalk (cf Harding and Powell 1977). Mitochondria and microtubules were very occasionally found in the proximal part of the spine stalk.

Clusters of *polyribosomes* were a regular finding in serial sections through 20 dendritic spines of the mushroom-shaped type. Polyribosomes were found in 100% of analyzed heads (Fig. 24), 65% of stalks and under 70% of the spine bases (Fig. 25). They were not so frequent in another 25 analyzed spines of the thin and stubby type (Ta-

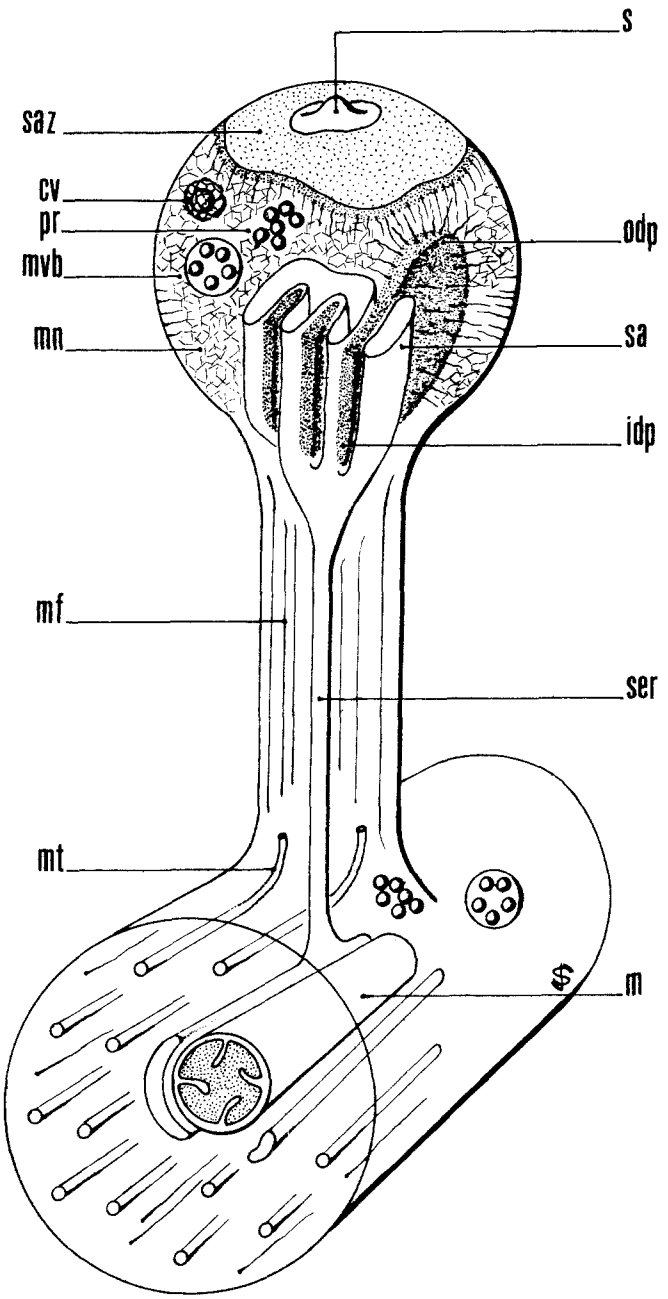


Fig. 21. Schematic illustration of the ultrastructure of a dendritic spine with spine apparatus; *s* spinule; *saz* synaptic active zone; *cv* coated vesicle; *pr* polyribosomes; *mvb* multivesicular body; *mt* microtubules; *odp* outer dense plate; *mn* microfilamentous network; *sa* spine apparatus; *idp* inner dense plate; *ser* smooth endoplasmic reticulum; *m* mitochondrion

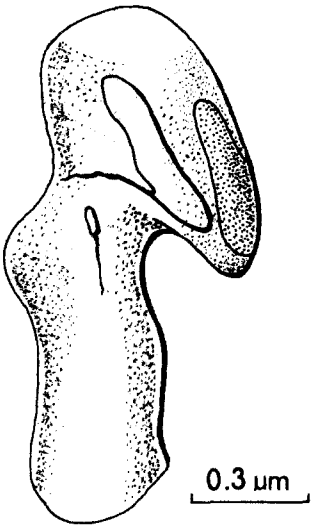


Fig. 22. Graphic reconstruction of dendritic spine lacking spine apparatus. The tubule of smooth endoplasmic reticulum is incomplete (fixation artifact)

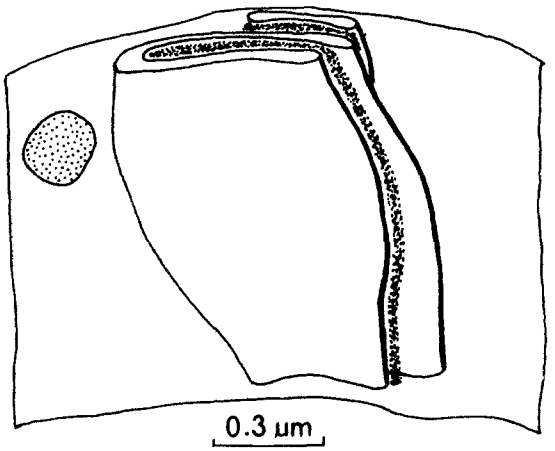


Fig. 23. Graphic reconstruction of extraspinous spine apparatus juxtaposed to synaptic active zone in the axon initial segment

Table 1. Frequency of polyribosomes in dendritic spines. Visual cortex

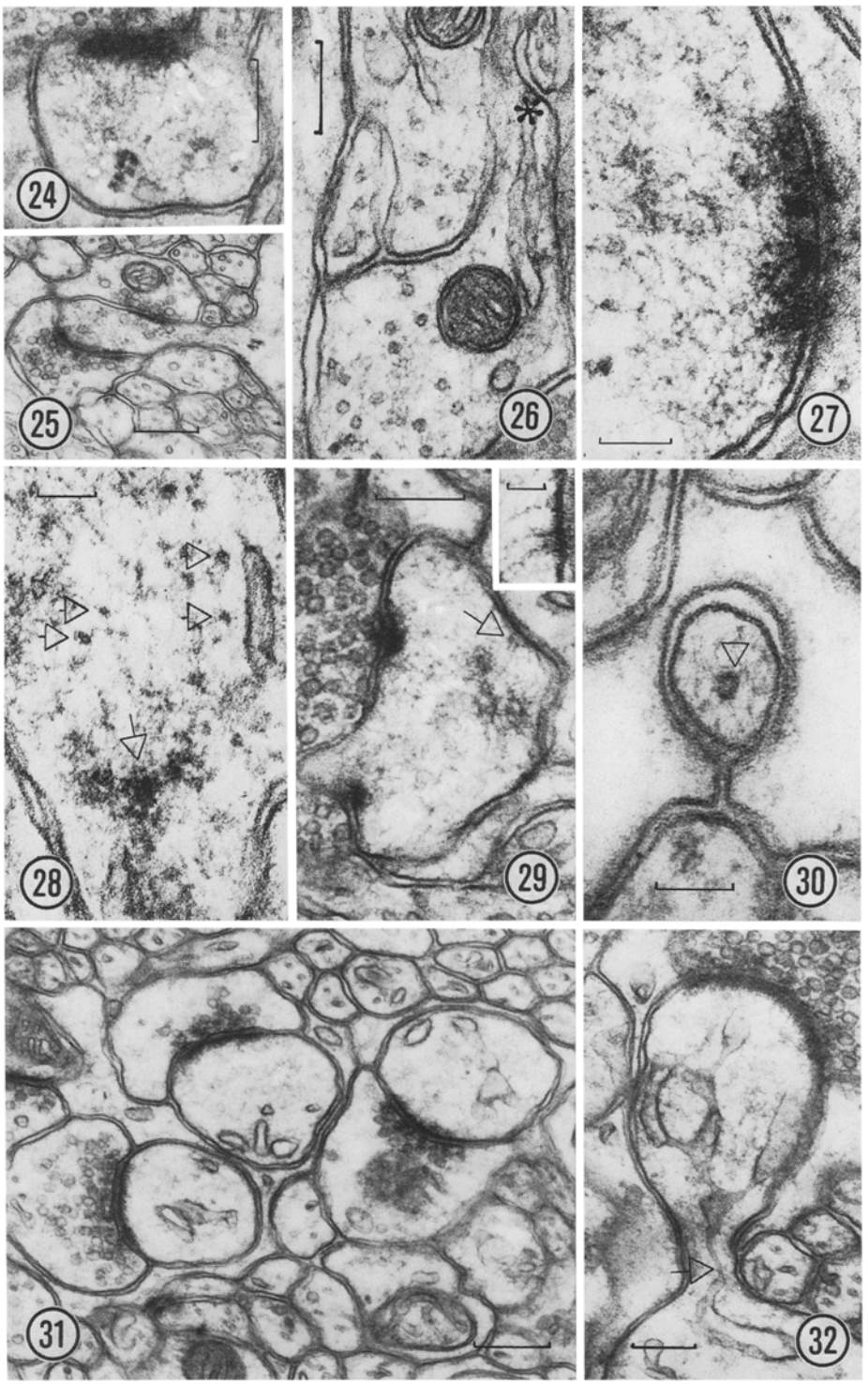
	Type of dendritic spine			
	mushroom-shaped (%)	thin (%)	stubby (%)	total (%)
Presence of spine apparatus	100	35	0	67.5
Presence of ribosomes:				
head	100	75	40	82.2
stalk	65	30	0	42.2
base	70	60	40	62.2

ble 1). However, there seems to be no relationship between the presence or absence of spine apparatus and polyribosomes.

A *filamentous network* is the most characteristic component in all dendritic spines. It is organized as a three-dimensional latticework in the heads (Figs. 27, 28), whereas filaments in the stalks usually run in parallel. Very fine microfilaments 2–4 nm in diameter are the main constituent of the network; microfilaments about 8 nm and intermediate filaments about 10–12 nm are not so numerous. Continuity between the filamentous network, the postsynaptic density, inner dense plate and outer dense plate was observed. Some filaments of the network were anchored into the plasma membrane (Fig. 29). Microtubules were not found in the spine heads.

Cerebellar cortex

Dendritic spines on the tertiary spiny branchlets of Purkinje cell dendrites do not contain a differentiated spine apparatus. Branching and focally dilated tubules of *smooth endo-*



Figs. 24, 25. Clusters of polyribosomes in the head (**Fig. 24,** visual cortex) and in the base (**Fig. 25,** cerebellar cortex) of dendritic spines. Scales = 0.2 μm (Fig. 24) and 0.3 μm (Fig. 25)

Fig. 26. Dendritic mitochondrion in the vicinity of smooth endoplasmic reticulum which is continuous with a connecting tubule (*asterisk*) in a spine neck. Scale = 0.2 μm

Fig. 27. Microfilamentous network in dendritic spine head continuous with the postsynaptic density. Scale = 0.1 μm

Fig. 28. Intermediate filaments (*empty arrowheads*) in dendritic spine head; outer dense plate (*arrow*). Scale = 0.1 μm

Fig. 29. Microfilaments anchoring in plasma membrane (*arrow*). Scale = 0.2 μm and 50 nm (*inset*)

Fig. 30. Perpendicular section through dendritic spine stalk of a spiny branchlet of Purkinje cell. The tubule of smooth endoplasmic reticulum is marked by *empty arrowhead*. Scale = 0.1 μm

Figs. 31, 32. Dendritic spine heads of a spiny branchlet of Purkinje cell with the tubular profiles of smooth endoplasmic reticulum. Continuity with the hypolemmal cistern marked by *arrow*. Scale = 0.3 μm (Fig. 31), and 0.2 μm (Fig. 32)

plasmic reticulum are a very distinct organelle in the spine heads (Figs. 31, 32). Usually one thin tubule connects smooth endoplasmic reticulum of tubules in the head with the hypolemmal cistern in the dendritic trunk (cf Palay and Chan-Palay 1974) (Figs. 30, 32). To reconstruct thin tubules, some of them arteficially disrupted, appeared difficult (Fig. 33), but superimposition of their profiles from serial sections provides good information concerning the organization of smooth endoplasmic reticulum in dendritic spines and its close affinity to mitochondria in the dendritic trunk (Fig. 34).

Polyribosomes were found in 6 heads and under 10 bases of a total of 45 serially analyzed dendritic spines, which represents 13.3 and 22.2%, respectively.

The *microfilamentous network* was at places slightly denser, but no formations were found similar to the outer dense plate of dendritic spines in the visual cortex. Multivesicular bodies and complex vesicles were not observed.

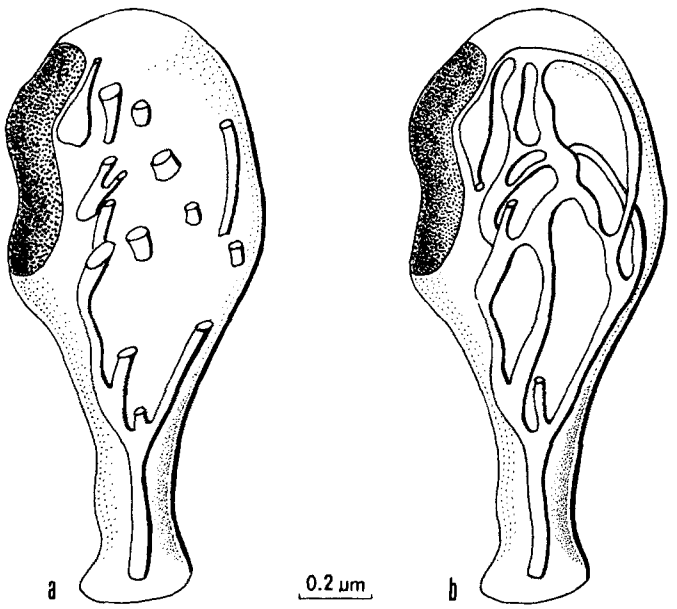


Fig. 33a, b. Graphic reconstruction of smooth endoplasmic reticulum in Purkinje cell dendritic spine. The incomplete tubules of the real reconstruction (**a**) are completed to obtain the likely image (**b**)

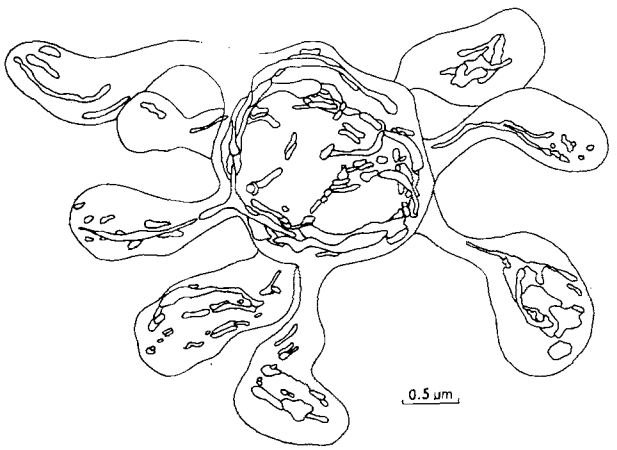


Fig. 34. Superimposed profiles of smooth endoplasmic reticulum in a spiny branchlet of Purkinje cell (made from 8 serial sections; cf. Fig. 14 of Part I., Špaček and Hartmann 1983). The empty places between the profiles of smooth endoplasmic reticulum in the branchlet were occupied by mitochondria

Discussion

Comments on methods

Sensitive impregnation methods, e.g., that of Thiéry et al. (1983), were developed for the detection and high voltage study of smooth endoplasmic reticulum in thick sections. The ultrastructure of other cell components is not so well preserved as in the standard fixation. Neither is one able to distinguish in such an impregnation those very thin tubules of smooth endoplasmic reticulum which have become narrowed to microtubule diameter, due to fixation.

In material fixed by standard methods, a classification of different kinds of filaments by reason of their diameter only is an inadequate method. Immunocytochemical methods are now necessary for such studies. Perhaps the tannic acid or albumin pretreatment ought to be used as an additional protective technique for enhanced microtubular preservation (Gray 1975). Hence, the part of this study dealing with the filamentous network is reported with reservations. However, it was not the main goal of this paper to study in detail the fine structure of dendritic spine cytoplasmic filaments.

For comments concerning the methods for three-dimensional reconstruction see Špaček and Hartmann 1983.

Table 2. Supposed functional significance of spine apparatus

Author	Supposed function of spine apparatus
Hamlyn (1962)	Mediation of postsynaptic changes associated with memory and learning
Gray and Guillery (1963)	Participation in synaptic activity
Jones and Powell (1970)	Participation in synaptic activity
Tarrant and Routenberg (1977, 1979)	A source of repository of macromolecules for the postsynaptic membrane
Manina (1979)	Synthesis of proteins, processing and storage of information associated with learning
Westrum et al. (1980)	Synthesis, depot or releasing point of proteins
Burgoyne et al. (1983)	Ca^{++} sequestering organelle
Fifková et al. (1983)	Ca^{++} sequestering organelle
Wilson et al. (1983)	Participation in control of the size and shape of dendritic spine

A possible function of spine apparatus and its relation to the shape of the dendritic spine

Possible functions of the spine apparatus so far suggested are summarized in Table 2.

The findings reported in the present study support some of the functional hypotheses put forward.

1. Outer dense plate emerging from the inner dense plate as a product of the spine apparatus and radiating into the surrounding filamentous network resembles focal densities (the homologues of the Z disc) of smooth muscle cells, especially of the tumorous ones (personal observation; Krstić 1978). The radiating organization suggests a *contraction centre* into which the microfilamentous network is anchored and which plays a role in changing the shape of the dendritic spine.

2. Regarding the striking affinity of outer dense plate for the postsynaptic density, an alternative possibility appears more probable, i.e., that the spine apparatus might be a *postsynaptic protein synthesizing centre*, its outer dense plate being a material to be used for a rapid dynamic extension of the synaptic active zone. This possibility seems particularly attractive considering that both the spine apparatus and the outer dense plate are regularly present near the large complex synaptic active zones where the need of an increased postsynaptic protein synthesis is great.

3. Mitochondria of the parent dendrite, apposed to smooth endoplasmic reticulum connected with the spine apparatus, might be a source of Ca^{2+} contained in its cisternae, and a donor of energy for the postsynaptic protein synthesis.

It is supposed that a shortening (and an eventual widening) of the spine stalk through swelling of the spine head

decreases the electrical resistance and thus enhances the synaptic effectiveness (Coss and Globus 1978; Crick 1982). If the size of the synaptic active zone is determined by presynaptic stimulation, if it is now experimentally proved that the shape of the dendritic spine can be modified by stimulation (experience) in response to variations of the presynaptic activity, and if it is known that the size of the dendritic spine is determined by the size of the synaptic active zone (Špaček and Hartmann 1983), then the question arises as to whether the different shape types of dendritic spines in the cerebral cortex (mushroom-shaped, thin, stubby) are stable structures or whether they are just transient structures expressing by their shapes an instantaneous state of the synaptic activity occurring on them. In the light of up-to-date knowledge, this latter possibility seems more probable. As a consequence of increased stimulation, a percentage of large spines of mushroom-shaped type should then increase to the detriment of small spines of thin and stubby type in the stimulated area of the cerebral cortex. According to our previous quantitative study in the visual cortex, a majority (86%) of large dendritic spines with a large complex synaptic-active zones developed spine apparatus (Špaček and Hartmann 1983). For this reason, a frequency of spine apparatus should also increase in the stimulated area. According to Manina (1979), spine apparatus really increases up to 200% in number during the learning process.

Although some dendritic spines (e.g., those of the spiny branchlets of Purkinje cells or complex spines of the thalamic nuclei) are large enough, they lack the spine apparatus. One possible explanation might be that their synaptic active zones, being of the simple type (Špaček and Lieberman 1974a; Špaček and Hartmann 1983), do not require rapid rearrangements of the postsynaptic density, perhaps due to the mode of their stimulation.

Ribosomes in dendritic spines

The presence of ribosomes in dendritic spine heads was mentioned by Peters and Kaiserman-Abramof (1970). Recently Steward and Levy (1982), Steward (1983) and Steward and Fass (1983) reported, in addition to the clusters of polyribosomes in dendritic spine heads, also the presence of polyribosomes in the stalks and under their bases in the dentate gyrus. In normal animals, up to 21% of the analyzed spines contained polyribosomes, less than in our material. I found polyribosomes also in dendritic spines of the thalamic ventrobasal nucleus and the dorsal lateral geniculate nucleus. It cannot be excluded that some of the globular particles present in the inner dense plate (Fig. 9) are in fact ribosomes. It is generally accepted that whereas the polyribosomes attached to the endoplasmic reticulum are concerned mainly with the production of "export" protein, those lying free in the cytoplasm are engaged in the synthesis of proteins for endogenous cellular needs. In the case of dendritic spines, such proteins could represent contractile proteins and specific postsynaptic proteins.

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Note added in proof: Subsequent to the time of this paper's submission for publication, the following paper has appeared:
Dyson SE, Jones DG (1984) Synaptic remodelling during development and maturation: junction differentiation and splitting as a mechanism for modifying connectivity. Dev Brain Res 13:125–137.
The authors demonstrate in E-PTA stained material an involvement of the spine apparatus in a process of the synapse reorganization. Their suggestions are in several aspects very close to those presented in my paper.