

# Cytoplasmic Actin in Neuronal Processes as a Possible Mediator of Synaptic Plasticity

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**ABSTRACT** We have demonstrated that, after permeation with saponin and decoration with S-1 myosin subfragment, the cytoplasmic actin is organized in filaments in dendritic spines, dendrites, and axon terminals of the dentate molecular layer. The filaments are associated with the plasma membrane and the postsynaptic density with their barbed ends and also in parallel with periodical cross bridges. In the spine stalks and dendrites, the actin filaments are organized in long strands. Given the contractile properties of actin, these results suggest that the cytoplasmic actin may be involved in various forms of experimentally induced synaptic plasticity by changing the shape or volume of the pre- and postsynaptic side and by retracting and sprouting synapses.

Recent discoveries are implicating the cytoskeleton and the cytoplasmic ground substance in a number of intracellular functions. The cytoplasmic ground substance consists of a protein-rich polymerized phase forming a complex microtrabecular lattice of contractile, ready solubilized proteins (28) that are in dynamic equilibrium with monomers of their subunits and therefore are ready to undergo phase transitions (18). Actin is the main component of the cytoskeletal microfilaments, and actin and myosin were shown to be part of the ground substance in a number of nonmuscle cells (for review see references 5 and 10) including the neurons (for review see reference 32). By analogy with muscles, it is assumed that the prime function of contractile proteins in neurons is transduction of chemical to mechanical energy, which may be essential for neuronal physiology.

In our previous work on synaptic plasticity in the visual cortex (6) and in the dentate fascia (7, 8), we observed changes in dendritic spines and synaptic contacts, the mechanism of which might involve microfilaments and microtrabeculae of the cytoplasmic ground substance. Likewise, various other experimental interventions or even physiological activity *per se* could induce changes in the cytoskeletal system of neurons which might be the underlying mechanism of what are, in general terms, known as plastic reactions of the central nervous system (CNS). With this assumption in mind, we have investigated the organization of actin filaments in dendritic spines, dendrites, and axon terminals in the dentate fascia of the hippocampus, a region which is known to react with distinct plastic morphological and physiological changes to increased electrical activation. Actin can be identified ultrastructurally by decorating with the myosin S-1 subfragment. This method was introduced by Ishikawa (14) and was substantially improved by adding tannic acid to the fixative (1).

## MATERIALS AND METHODS

All animals used were 25-g mice of the HS/IBG strain from the Institute for Behavioral Genetics, University of Colorado, Boulder, CO. Under urethane anesthesia, mice were perfused transcardially under constant pressure of 3 lb/in<sup>2</sup> with 0.1% glutaraldehyde in stabilization buffer (0.1 M PIPES; 5 mM MgCl<sub>2</sub>; 0.1 mM EDTA at pH 6.9), followed by 0.1% saponin in the same buffer. After finishing the perfusion, brains were quickly removed and blocks prepared from the upper blade of the dentate fascia. These blocks were incubated for 2 h in a mixture of 1% S-1 myosin subfragment and 0.1% saponin, and then fixed for 1 h in 2.25% glutaraldehyde with 0.2% tannic acid added, both in the above stabilization buffer. Osmication was done with 0.75% OsO<sub>4</sub> in cacodylate buffer (pH 6.0 for 1 h) on ice followed by block staining with uranyl acetate, dehydration in alcohol, and embedding in epon. Silver sections were cut and mounted on formvar-coated bar grids, stained according to Sato (30), and viewed with the JEM 100 electron microscope. Control blocks were treated identically except for omission of the S-1 myosin subfragment from the incubation medium. The controls and S-1 subfragment-treated blocks were always from the same animal so that the perfusion procedure was identical for control and experimental blocks.

### *Trimming and Orientation of the Blocks*

Blocks were isolated from the upper blade of the dentate gyrus of the hippocampal formation (Fig. 1*a*). From these blocks, thick sections (1 μm) were prepared and stained with toluidine blue. The final trim from which the thin sections were cut included the entire width of the molecular layer of the dentate fascia. The border against the hippocampus proper is formed by the obliterated hippocampal fissure (characterized by the presence of a number of blood vessels) and was at one side of the thin section. The perikarya of granule cells were at the opposite side of the thin section, thus facilitating orientation of the dentate molecular layer under the electron microscope (Fig. 1*b*).

## RESULTS

Dendritic spines, dendrites, and axon terminals were examined along the entire width of the dentate molecular layer which extends from the perikarya of the granule cells to the obliterated hippocampal fissure (Fig. 1*b*). Actin filaments can be identified at the ultrastructural level by decorating with the S-

1 subfragments that attach themselves to the filament at an angle of  $45^\circ$  and so give rise to an appearance of arrowheads repeating with a periodicity of 35 nm (9). To gain access to the actin filaments, the plasma membrane has to be permeated. This was done with saponin, which is a plant glycoside known to complex with cholesterol and to form globular micelles that disrupt the plasma membrane (25). Since this interaction of saponin with the membrane is independent of fixation with low concentrations of glutaraldehyde, a better tissue preservation can be obtained than if the permeation is done without the prefixation. In comparison to the formerly used glycerin or nonionic detergents such as Triton X-100, saponin leaves the components of the cytoskeleton and the cytoplasmic organelles intact (Fig. 2). Permeation of the neuronal membrane allows the removal of soluble granular cytoplasmic material so that it does not interfere with the appearance of the actin filaments (Fig. 3). In control blocks, the thin filaments observed are likely to be undecorated actin filaments. However, they appear invariably shorter and more branched than the filaments from decorated blocks (Figs. 4, 5, 6). It is possible that the increased branching pattern reflects some damage caused by the osmication, from which the reacted filaments are protected by the S-1 subfragment (22). In preliminary experiments in which we have used the same protocol for the permeation and the S-1 treatment as is reported here, but with a higher osmium concentration (2%), we did not see any long, decorated filaments but rather heavily branched, thicker, and darkly stained filaments. Whether in native neurons actin is present in an F-form

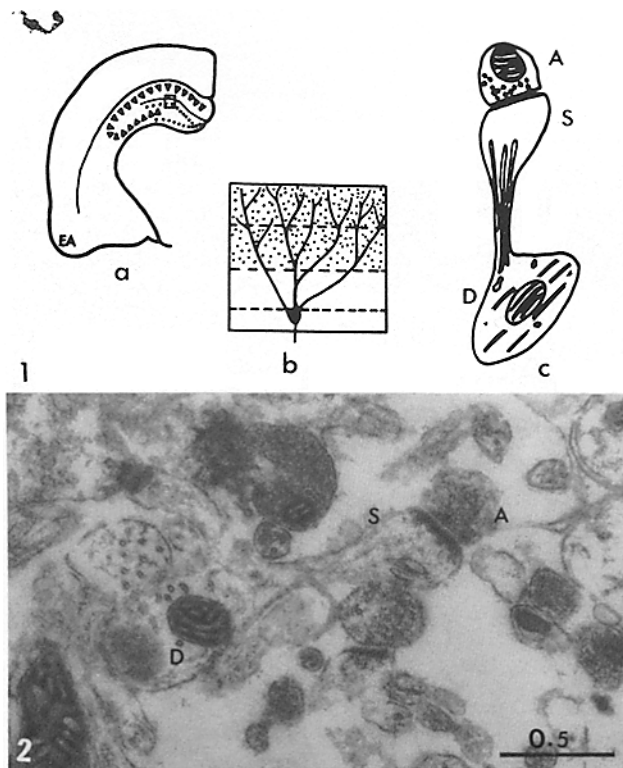


FIGURE 1 (a) Coronal section through a mouse brain at the niveau of the hippocampal formation (dots, dentate fascia; pyramids, hippocampus proper). (b) Dentate granule cell with dendrites extending into the molecular layer (dotted area, region from which the micrographs were taken). (c) Dendritic spine (S) attached to a dendrite (D) with an axon terminal (A) forming a synapse.

FIGURE 2 Segment of a dentate molecular layer of a permeated, nonreacted block. Bar,  $0.5 \mu\text{m}$ .  $\times 30,000$ .

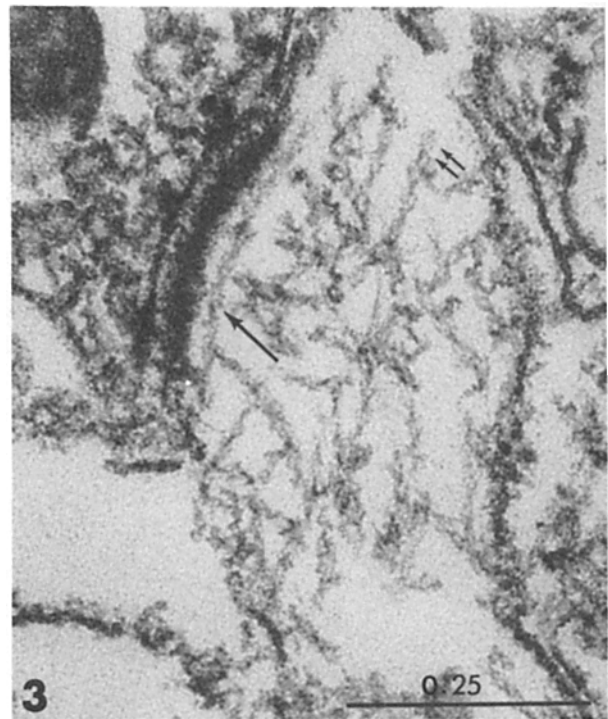


FIGURE 3 Dendritic spine. The decorated actin filaments appear to fill the entire spine. Associated in parallel with the postsynaptic density is one actin filament (single arrow). A double arrow indicates a free barbed end of the filament. Bar,  $0.25 \mu\text{m}$ .  $\times 130,000$ .

or whether the F-form is induced by the S-1 treatment cannot be answered by the present experiments.

### Dendritic Spines

Appendagelike protrusions which emanate from dendrites of the dentate granule cells are called dendritic spines. Each spine has a synapse-carrying head and a stalk by which it is attached to the parent dendrite. The stalk contains a spine apparatus composed of sacs of smooth endoplasmic reticulum that are in continuity with the smooth endoplasmic reticulum of the dendrite (Fig. 1 c). The sacs alternate with plates of dense material which is in association with microtubules of the parent dendrite (33). Dendritic spines are easily recognized in the permeated material. In the spine head, the actin filaments are arranged in the form of a lattice. In cross sections, the filament appears as a dark center with the S-1 subfragment attached under an angle (Fig. 7). Actin filaments display the arrowhead complexes similar to those described in a number of nonmuscle cells. They are associated with the postsynaptic density (PSD) and with the plasma membrane with their barbed ends (i.e., the end of the arrowhead barbs if the filament were decorated with S-1 subfragment [13]) and the arrows are pointing away from it (Figs. 8, 9). The actin filaments appear also to be associated in parallel with the PSD and plasma membrane. This association is formed by fine, periodically occurring strands emanating from the filament towards the plasma membrane (insert of Fig. 10). In instances where the plane of sections reveals the spine apparatus, some filaments are oriented with their arrowhead points and some with their barbs towards the sacs of the spine apparatus (Fig. 11). The actin filaments tend to branch and to be cross-linked. The branched filaments have their arrowheads directed towards the branching points and their barbed ends

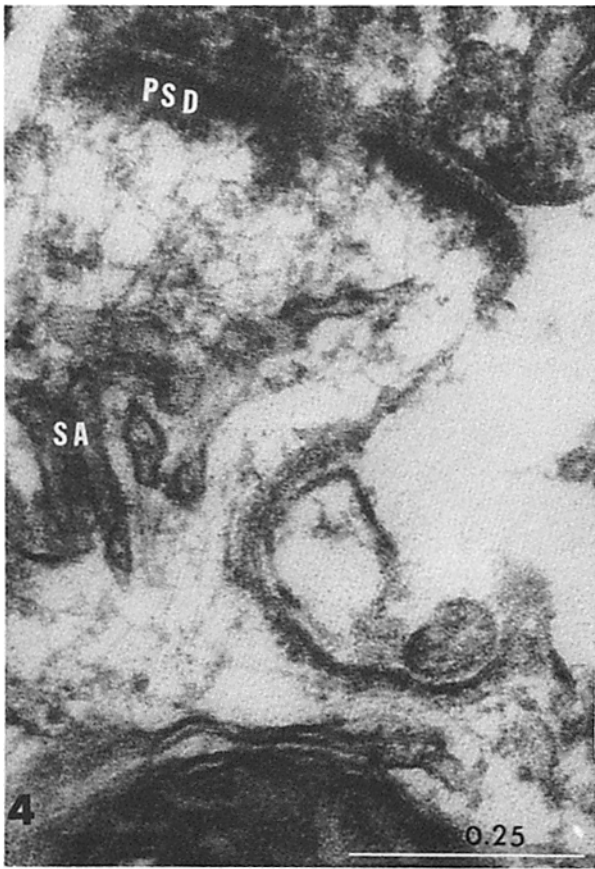


FIGURE 4 Spine from a control block. Note the thin filaments between the *PSD* and the spine apparatus (*SA*). Bar, 0.25  $\mu\text{m}$ .  $\times 125,000$ .

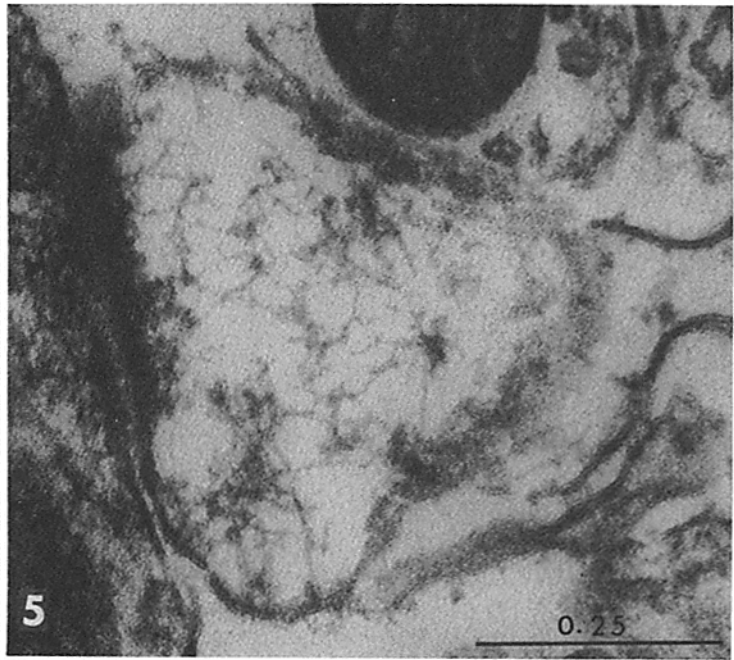


FIGURE 5 Spine head from a control block. The actin filaments appear shorter and more profusely branched than in the reacted spine in Fig. 1 which has the same enlargement. Bar, 0.25  $\mu\text{m}$ .  $\times 130,000$ .

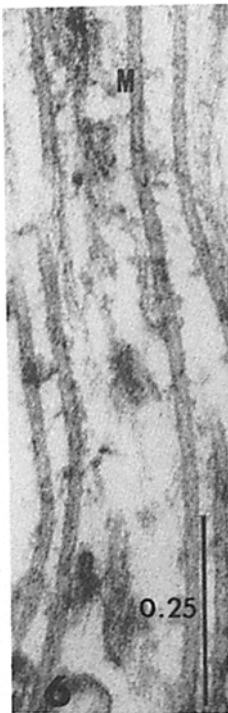


FIGURE 6 Dendritic shaft of a control block (*M*, microtubule). Bar, 0.25  $\mu\text{m}$ .  $\times 100,000$ .

unattached (Figs. 8, 12). They seem to fill entirely the spine head and in the spine stalk they are lengthwise oriented, forming a braided structure which is similar to that frequently seen in the dendrites (Fig. 13).

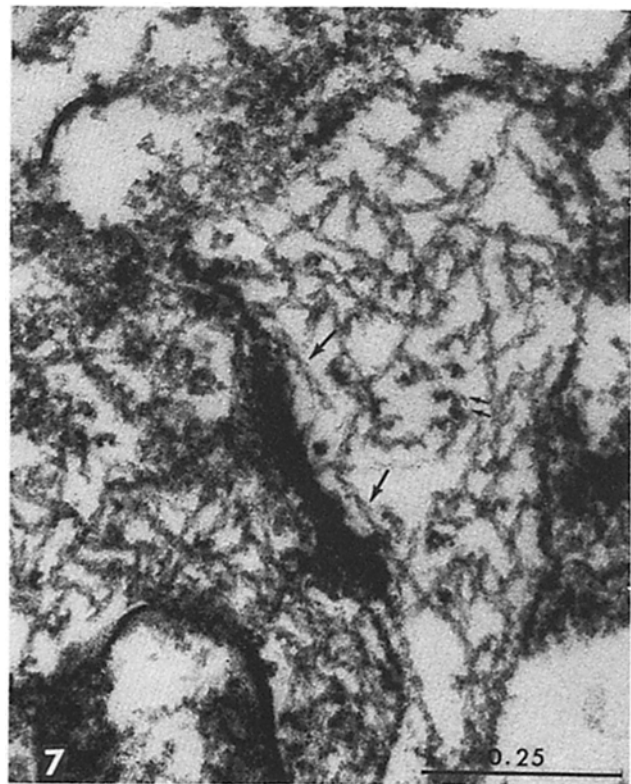


FIGURE 7 A spine head. Two filaments oriented in parallel with the postsynaptic density (single arrows). Cluster of cross sectioned filaments (double arrow). Bar, 0.25  $\mu\text{m}$ .  $\times 125,000$ .

## Dendrites

Dendrites appear to have actin filaments organized either in fascicles or in a lattice form (Figs. 14, 10), similar to that observed in the dendritic spines. However, the filament network in dendrites is far less dense than that of a spine. The association of individual actin filaments with the PSD of the axodendritic synapse as well as the dendritic plasma membrane is similar to that of a spine (Figs. 9, 15).

## Axon Terminals

In axon terminals, actin filaments are arranged in a network similar to that in the dendritic spines (Figs. 11, 15). Whether there are regularly occurring associations between the filaments and synaptic vesicles could not be established with certainty in the present material.

## DISCUSSION

Actin was biochemically isolated from synaptosomes of mammalian brains, from cultured sympathetic ganglia (for review see reference 32), and also from the postsynaptic density of the CNS (3, 16, 21, 33). At the light microscope level, actin was demonstrated, with fluorescent antibodies, in neurites and growth cones of avian dorsal root ganglia cells (17, 20) and in microspikes of the murine neuroblastoma cells (4, 27). At the electron microscope level, actin was demonstrated with S-1

subfragment in neuroblastoma cells (4, 27), isolated Deiters neurons (23), and in sensory hair cells (9). However, no one has brought any evidence for the presence of actin filaments at the ultrastructural level in neuronal processes *in situ*. The present results show for the first time the existence of cytochemically labeled actin filaments in the form of clear arrowhead complexes *in situ* in dendrites, dendritic spines, and axon terminals of the CNS. The only paper that has attempted to demonstrate actin filaments in neuronal processes of the *substantia nigra* did not establish any clear arrowhead complexes (19). The authors of that paper claimed that actin filaments in neurons may never show a typical arrowhead pattern because of the decreased reactivity of the nonmuscle actin and because of the thin sectioning required for electron microscopy. Thin-sectioned perikarya of Deiters neurons displayed decorated

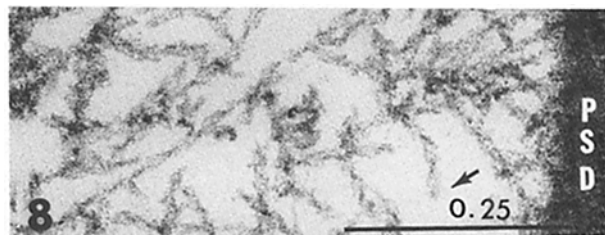


FIGURE 8 Segment of a spine head with the PSD. An arrow points to a free barbed end of a filament. Note the number of actin filaments associated with PSD. Bar, 0.25  $\mu\text{m}$ .  $\times 125,000$ .

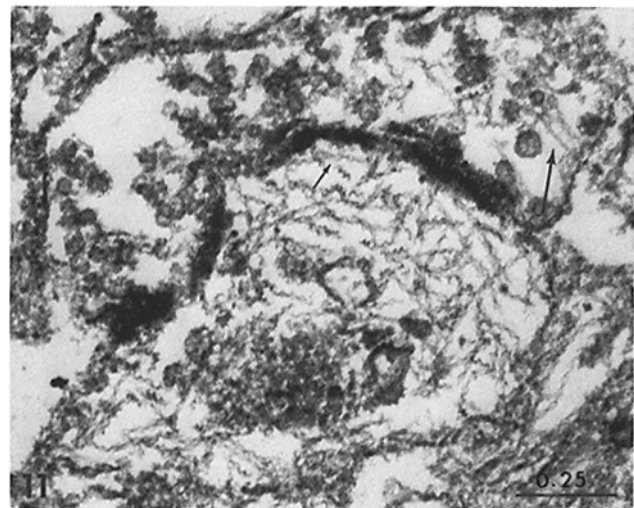


FIGURE 11 Spine head with a spine apparatus (SA) and an axon terminal. A small arrow indicates an actin filament in parallel with PSD. Large arrow points to actin filaments in the axon terminal. Bar, 0.25  $\mu\text{m}$ .  $\times 66,000$ .

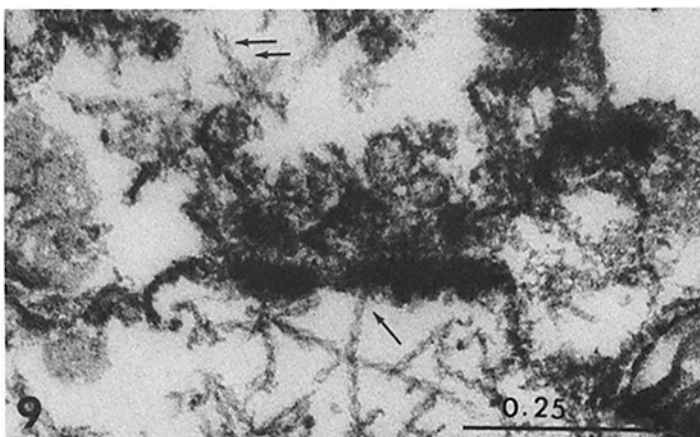
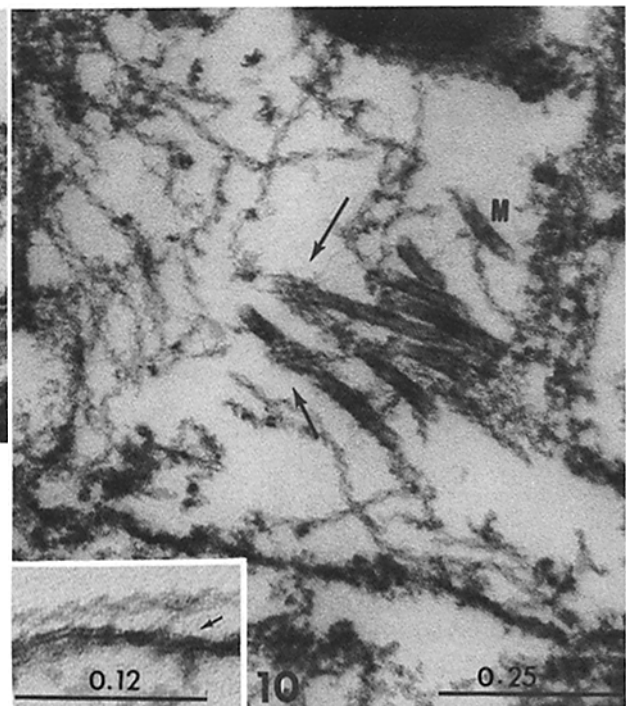


FIGURE 9 Axodendritic synapse. Single arrow points to an actin filament associated with its barbed end with the PSD. Actin filaments in axon terminals (double arrow). Bar, 0.25  $\mu\text{m}$ .  $\times 100,000$ .

FIGURE 10 Dendritic shaft. Arrows indicate actin filament and microtubules (M). Bar, 0.25  $\mu\text{m}$ .  $\times 100,000$ . The insert shows a parallel association of an actin filament with the plasma membrane. Bar, 0.12  $\mu\text{m}$ .  $\times 200,000$ .



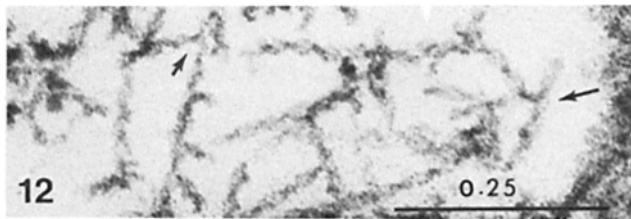


FIGURE 12 Segment of a spine head. Arrows indicate the branching points of actin filaments. Bar, 0.25  $\mu\text{m}$ .  $\times 132,000$ .

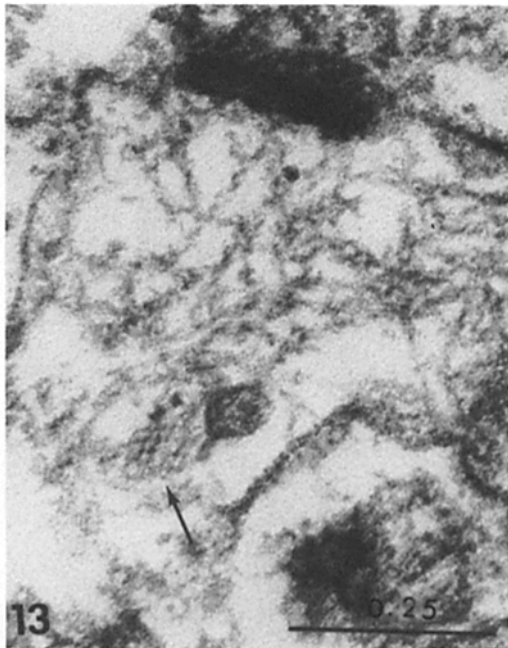


FIGURE 13 An arrow indicates a strand of actin filaments in a spine stalk. Bar, 0.25  $\mu\text{m}$ .  $\times 110,000$ .

actin filaments, however, for a short distance only (2–3 arrowheads [23]) which indicated fragmentation of the filaments. This lack of success in filament preservation in the earlier work may have been partly due to the fact that the membrane permeation was done in unfixed tissues with glycerin and partly because of the destructive effect of osmium (22). Long strands of actin filaments similar to those observed in present preparations were demonstrated in the sensory hair cells of the inner ear, where they form the main body in the stereocilia and a complex of loosely organized filaments in the cuticular plate (9). In our preparations, the filaments never form a dense network under the plasma membrane as they do in microspikes and neurites of neuroblastoma cells (12, 17). Interaction between actin filaments and the plasma membrane of dendritic spines and dendrites appears to occur either in the parallel or the perpendicular direction as described in the microvilli (24). The well-known property of actin to bind to other proteins and to itself (26) was observed in our preparations in the form of “Y”-shaped branching patterns, similar to those described in the kidney cells (31). Likewise, we have noticed that branched filaments have their arrowheads directed towards the branching points and their barbed ends unattached to any membrane.

In vitro experiments have shown that isolated cytoplasm of a variety of cells, when exposed to  $\text{Mg}^{2+}$  and ATP, becomes markedly gelled. Electron microscopy of these gels showed that they are made up of cross-linked actin filaments. Addition



FIGURE 14 Dendritic shaft. In between two arrows is a long fascicle of actin filaments. Note the lower density of cytoplasmic actin filaments here than in spines. Bar, 0.25  $\mu\text{m}$ .  $\times 100,000$ .

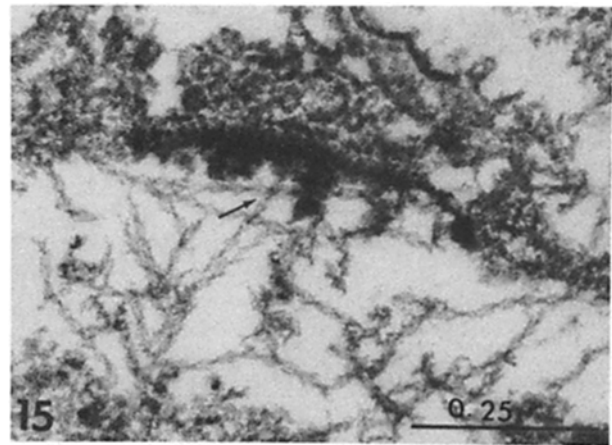


FIGURE 15 Axodendritic synapse. An arrow points to a crossing of two filaments both associated with the postsynaptic density. Bar, 0.25  $\mu\text{m}$ .  $\times 100,000$ .

of  $\text{Ca}^{2+}$  to these actin-containing gels leads to their contraction, indicating the contractile nature of the cytoplasm (15). The properties of the nonmuscle actin suggest that the actin-myosin interaction in nonmuscle cells, including neurons, may be regulated in a manner analogous to that of a muscle (27, 32). In the light microscope, fluorescent antibodies against myosin have shown that myosin is concentrated in locations where actin is found. However, thick myosin fibers have not been demonstrated with the electron microscope in nonmuscle cells or neurons. Whether this reflects their rarity, their destruction during permeation and fixation, or their true absence in the cell remains uncertain (15). This, and the excess of actin over myosin leads to the speculation that most of the actin in nonmuscle cells is involved in support-providing functions that do not require myosin (27). However, given that only a few

myosin molecules are needed to generate the small forces involved in nonmuscle contractility, then even in the absence of myosin fibers, the myosin molecules could be provided by the cytoplasmic ground substance (28). Thus, actin may have a dual role as a structural and contractile protein in neurons and may play a role in the motility of spines, dendrites, and axon terminals that may subservise different forms of neuronal plasticity.

Studies on neuronal plasticity have unequivocally demonstrated that different experimental interventions in the developing and mature nervous system may induce rearrangement of the synaptic pattern of various brain regions by sprouting new synapses, retracting others, or changing the shape or dimensions of the existing ones. Given the functional importance of such modifications, it appeared essential to search for the mechanism or mechanisms underlying such a change. The capacity of the neuron to change the shape, configuration, volume, density, and length of its synapses may have a common denominator which could be linked to the ubiquitously present actin in the neuronal processes. It can be surmised that the signals to neurons that were modified by various experimental treatments may change the physicochemical properties of the cytoplasm which may induce the assembly or disassembly of the actin lattice with a consequent contraction or relaxation of the element involved. The dentate fascia of the hippocampus displays synaptic plasticity known as long-term potentiation (a prolonged increase in the synaptic strength induced by brief, high frequency bursts of stimuli to its afferent pathway) which has received considerable attention as a physiological model of neuronal plasticity (2). In the stimulated dentate fascia, we have observed an increased volume of the spine head (8), and widening and shortening of the spine stalk (7). These morphological changes would reduce the length constant and resistance of the spine and consequently increase its conductance, as has been mathematically predicted (29). The concentration of actin filaments in dendritic spines, as compared to the dendrites, is surprisingly high; especially in the spine stalk region where the filaments form a lengthwise organized network similar to that seen in dendritic shafts. Such an arrangement of contractile elements in the cytoplasm could be responsible, under certain conditions of excitation, for changes in the length and width of the spine stalk and thus, for changes in electrical properties of the system. It has been shown in neurons that during excitation the inward flow of  $\text{Na}^+$  is followed by a flow of  $\text{Ca}^{2+}$  (for review see reference 11) which may affect an intraneuronal pool of  $\text{Ca}^{2+}$  similar to that of the muscle sarcoplasmic reticulum. In the spine, calcium could be stored in the sacs of the spine apparatus and thus, be readily available when a synaptic potential invades the spine head. This possibility is currently under investigation.

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