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DENDRITIC SPINES: CELLULAR SPECIALIZATIONS IMPARTING BOTH STABILITY AND FLEXIBILITY TO SYNAPTIC FUNCTION

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INTRODUCTION

Dendritic spines, the tiny protrusions that stud the surface of many neurons, are the location of over 90% of all excitatory synapses that occur in the CNS. Their small size has, in large part, made them refractory to conventional experimental approaches. Yet their widespread occurrence and likely involvement in learning and memory has motivated extensive efforts to obtain quantitative descriptions of spines in both steady state and dynamic conditions. Since the seminal mathematical analyses of D'Arcy Thompson (1992), the power of quantitatively establishing key parameters of structure has become recognized as a foundation of successful biological inquiry. For dendritic spines, highly precise determinations of structure and its variation are again proving to be essential for establishing a valid concept of function. The recent conjunction of high quality information about the structure, function, and theoretical implications of dendritic spines has, in fact, produced a flurry of

new considerations of their role in synaptic transmission (Rall 1970, 1974; Diamond et al 1970; Kawato & Tsukahara 1983; Horwitz 1984; Wilson 1984; Perkel & Perkel 1985; Shepherd et al 1985; Gamble & Koch 1987; Shepherd & Brayton 1987; Wickens 1988; Segev & Rall 1988; Brown et al 1988; Qian & Sejnowski 1989; Holmes 1990; Zador et al 1990; Baer & Rinzel 1991; Larson & Lynch 1991; Koch et al 1992; Koch & Zador 1993).

A powerful working hypothesis is that the structure of dendritic spines sets the boundaries within which synaptic function can be modulated. Theory defines the limits of what can happen within these boundaries and experimental manipulation defines what actually happens to spine morphology. As measurements of spine dimensions and organelle and molecular composition have become more precise, so too have the theoretical models of spine function improved. In the following discussion we evaluate some of the morphological, theoretical, and experimental evidence indicating that dendritic spine structure and composition can influence synaptic efficacy. In this context, we consider how spines might serve the cellular mechanisms that establish specific and enduring memories.

INTEGRATION OF SYNAPTIC INPUT ON SPINY NEURONS

The intrigue of understanding the cellular mechanisms involved in learning and memory has provided a strong impetus for investigating many aspects of neural organization. Several discrete levels of integration, ranging from changes in cellular ensembles to changes in individual molecules, have been proposed as key loci for learning and memory. Neurons are, however, more than globes filled with talented molecules, and the complexity of neuronal structure sets neurons apart from all other cells. Highly branched dendrites receive and integrate input from hundreds, even thousands, of other neurons. Neurons with different functions can be classified according to the shape of their dendritic arbor and the density of spines occurring along their dendrites. The degree of dendritic branching, the length of individual dendritic branches, and the frequency of dendritic spines are all modified by experience and probably represent the growth of new synapses (Greenough & Bailey 1988).

Spiny neurons tend to be the principal input/output cells of a given brain region (Shepherd 1990). As such, they integrate diverse excitatory and inhibitory input, from different regions and from different cell types within a brain region. For example, a spiny pyramidal cell is the principal cell of hippocampal area CA1 (Figure 1). The spines that are located on the proximal two thirds of CA1 pyramidal cell dendrites receive excitatory synapses from both the ipsilateral and the contralateral hippocampus. Distal spines of the same pyramidal cells receive excitatory synapses from the entorhinal cortex.

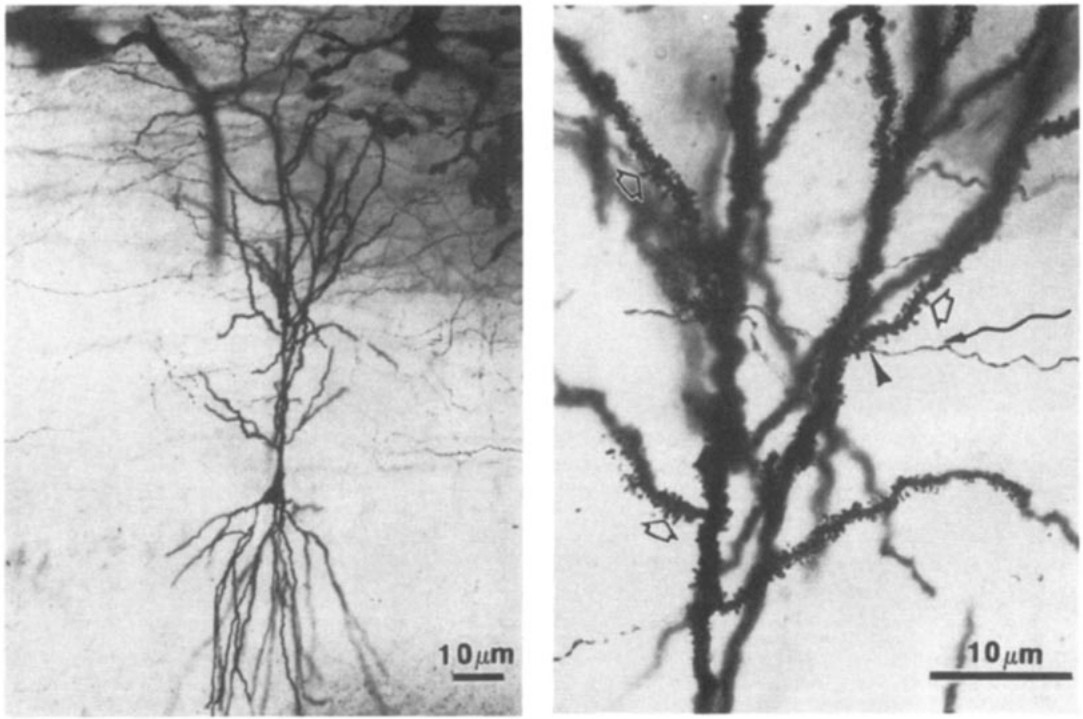

Figure 1 Light micrographs of a Golgi-impregnated spiny pyramidal cell in area CA1 of the rat hippocampus. (*A*) From the apex of the pyramidal cell body emerges an apical dendrite with multiple lateral branches. From the base emerge several basilar dendrites. (*B*) At higher magnification, the dendritic spines (*open arrows*) that stud the surface of these dendrites become just visible, along with the presynaptic axons (*wavy arrow*), which occasionally come into close apposition with a spine (*arrowhead*). For unknown reasons, the Golgi method usually impregnates less than 1% of the cells and axons that are present in the neuropil.

In contrast, nonspiny neurons tend to be the local interneurons of a brain region (Shepherd 1990). Their dendrites are usually spine-free or sparsely spiny and have large swellings or varicosities along their lengths. Both excitatory and inhibitory synapses occur directly onto the dendritic shafts, often with a higher frequency at the varicosities (KM Harris, personal observation). The axons of the nonspiny cells usually remain within a brain region to form inhibitory or modulatory synapses on the dendritic shafts between the spines and on the somata of the spiny pyramidal cells. The excitatory synapses typically have an asymmetric appearance, featuring a thickened postsynaptic density adjacent to a presynaptic axonal bouton containing round, clear vesicles (Peters et al 1991). The inhibitory synapses tend to have a symmetric appearance, owing to the near equal thickening of the pre- and postsynaptic membranes, with both round and flattened vesicles in the presynaptic bouton. Glutamate and aspartate are the predominant excitatory neurotransmitters, whereas GABA is the primary inhibitory neurotransmitter functioning at synapses on spiny neurons throughout the CNS (Shepherd 1990). The presynaptic axons of symmetric synapses also contain several substances that either modulate the inhibitory influence of GABA or alter the excitability of the spiny cell directly. Thus, the dendritic, axonal, and synaptic morphologies of spiny and nonspiny neurons differ dramatically, along with their electrophysiological and biochemical properties, indicating that the roles played by spiny neurons are likely to differ from those of nonspiny neurons in an ensemble that involves both. When integration of the diverse excitatory, inhibitory, and modulatory actions drives the spiny cells past threshold, they usually send a signal to the next brain region, where its neurons undergo similar integrative activities.

STRUCTURE OF DENDRITIC SPINES

As the first postsynaptic element encountered by the excitatory neurotransmitter, dendritic spines are uniquely situated to be a fundamental integrative unit. The synaptic strength at different spines determines the pattern of activity of individual cells and ultimately of the neuronal ensemble. Dendritic spines are so small and intermingled within the complex neuropil that contemporary electrophysiological and biochemical techniques cannot directly evaluate the activity and composition of individual living spines within this neuropil. However, recent confocal microscopy has revealed that individual dendritic spines do persist over periods of several hours in hippocampal slices in vitro (Hosokawa et al 1992). This observation establishes a necessary prerequisite for spines as fundamental integrative units, namely that once formed they are relatively persistent structures.

Most of what we suspect about spine function is based on computer

Table 1 Range in spine dimensions and their association(s) with presynaptic axons in six brain regions[a]

Brain region	Total length (μm)	Neck diameter (μm)	Neck length (μm^3)	Total volume (μm^3)	Total surface (μm^2)	PSD area (μm^2)	PSD: head surface area	Maximum number of boutons per spine	Maximum number of branches per spine
Cerebellum	0.7–3	0.1 –0.3	0.1–2	0.06 –0.2	0.7–2	0.04–0.4	0.17 ± 0.09	2	5
Hippocampal CA1	0.2–2	0.04–0.5	0.1–2	0.004–0.6	0.1–4	0.01–0.5	0.12 ± 0.06	3	3
Visual cortex	0.5–3	0.07–0.5	—	0.02 –0.8	0.5–5	0.02–0.7	0.10 ± 0.04	2	2
Neostriatum	—	0.1 –0.3	0.6–2	0.04 –0.3	0.6–3	0.02–0.3	0.13	2	2
Hippocampal CA3	0.6–6.5	0.2 –1	0.1–1	0.1 –2	1 –3	0.01–0.6	0.09 ± 0.04	3	16
Hippocampal Dentata	1 ±0.6	0.2 ±0.1	0.8	—	—	—	—	—	3

[a] For the first five brain regions all of the data are from three-dimensional analyses of serial EM reconstructions. Cerebellar spines are from the Purkinje spiny branchlets (Harris & Stevens 1988), comparable to Spacek & Hartman (1983). Spines of hippocampal CA1 pyramidal cells are from Harris & Stevens (1989); for visual cortex from pyramidal cells, from Spacek & Hartman (1983). Spines in the neostriatum are from Wilson et al (1983), and spines in hippocampal area CA3 are from the proximal portion of the pyramidal cells (Chicurel & Harris 1992). The data for hippocampal area dentata are from three-dimensional analyses of Golgi-impregnated cells viewed with high-voltage EM and measured on stereo pair images of Hama et al (1989). The volume and surface area of the spine head is typically about 90% of the total. Only branched spines contacted more than one excitatory axonal bouton; all unbranched spines synapsed with just one bouton. In neostriatum and visual cortex, a second inhibitory synapse occurs on the neck of about 8% of the spines Difiglia et al 1982, Wilson et al 1983, De Zeeuw et al 1990). In most brain regions less than 10% of the spines are branched; in contrast, about 90% of the proximal CA3 spines are branched. "Maximum" refers to the maximum number viewed to date.

simulations that vary the structural dimensions and the locations of active molecules in simulated spines. For simplicity, the theoretical models have used ideal geometries, such as spheres with variable dimensions for the heads, connected to cylinders with variable lengths and widths for the necks. For some spines, these descriptions are adequate, and the theoretical conclusions are generally interpretable (e.g. Wilson et al 1983, Harris & Stevens 1988, Brown et al 1988).

Physiological evidence readily shows that different excitatory synapses can have very different efficacies (Manabe et al 1992, reviewed in Lisman & Harris 1993). If dendritic spine structure participates in defining the differences in synaptic efficacy, then the heterogeneity of synaptic strength should

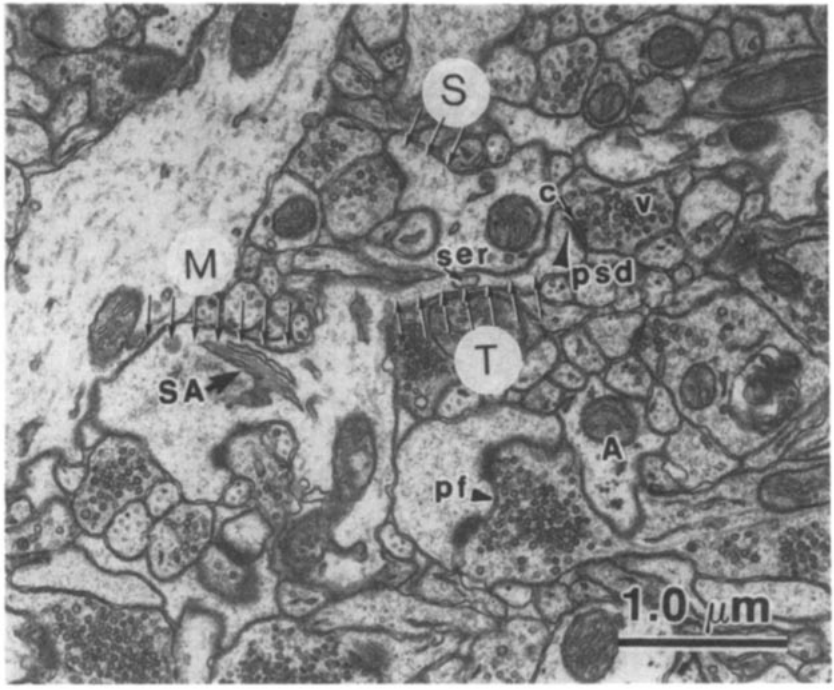

Figure 2 Electron micrograph of a section through dendritic spines in stratum radiatum of hippocampal area CA1. In this fortuitous section, three spines were sectioned parallel to their longitudinal axis, revealing spines of the stubby (*S*), mushroom (*M*), and thin (*T*) morphologies. The postsynaptic density (*psd*) occurs on the spine head (see *T*) immediately adjacent to the synaptic cleft (*c*) and to the presynaptic axonal bouton that is filled with round vesicles (*v*). This *T* spine contains a small tube of smooth endoplasmic reticulum (*ser*) in its neck. In the *M* spine, a spine apparatus (*SA*) is visible. A perforated postsynaptic density (*pf*) is evident on the head of another mushroom spine. Near to this spine is a large astrocytic process (*A*), identified by the black glycogen granules and clear cytoplasm.

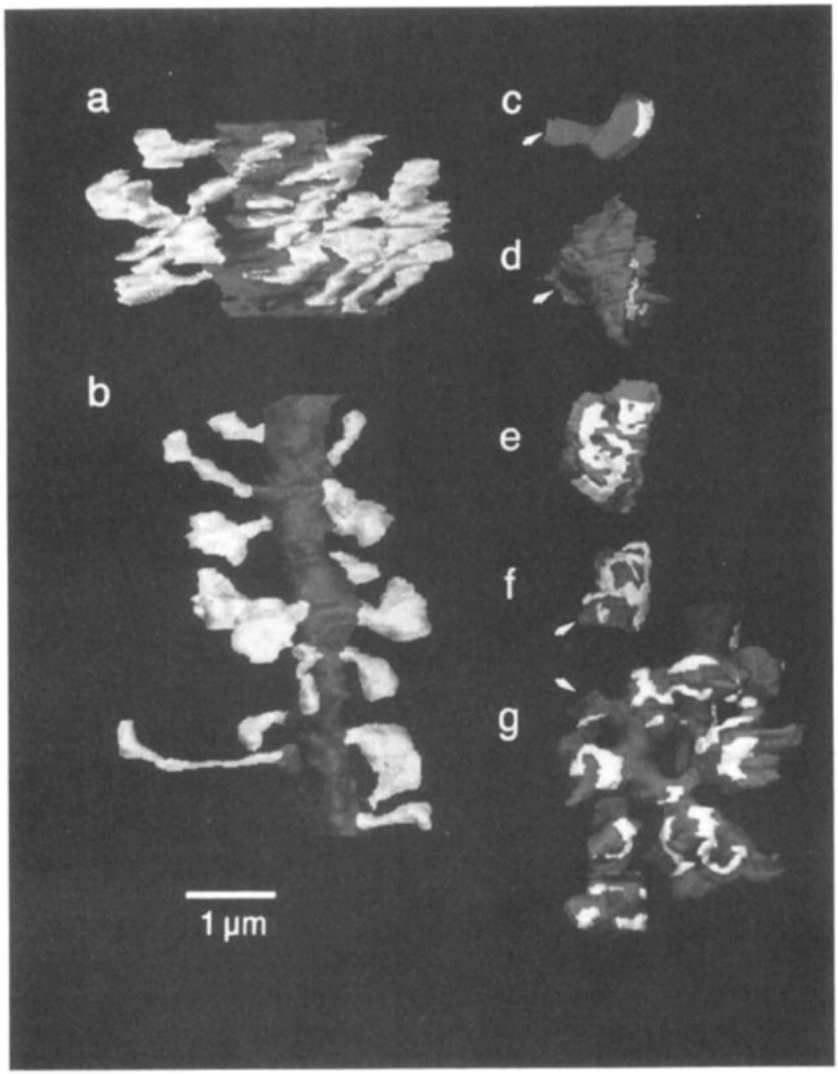

Figure 3 Three-dimensional reconstructions of dendritic segments from (*a*) a cerebellar Purkinje spiny branchlet and (*b*) a hippocampal CA1 pyramidal cell. The dendritic shaft is illustrated in gray, and the individual spines are illustrated in white. In *c–g* the spines are gray, the PSDs are white, and the small white arrows indicate where the spines joined their parent dendrites. (*c*) Profile of a typical cerebellar spine with a macular PSD. (*d*) A side view and (*e*) a top view of a short mushroom-shaped CA1 spine. This spine has a complex PSD with several segments and perforations. (*f*) A single headed spine in hippocampal area CA3, also with a complex PSD, and (*g*) a highly branched spine from hippocampal area CA3 which has multiple PSDs.

be evident in spine structure. Indeed, there are dramatic differences in spine and synaptic shape (Figure 2). Spines necks can be long or short, fat or thin, straight or bent, cylindrical or irregular, and branched or unbranched in all combinations. Spine heads can be small or large, and spherical, oval, or irregular in shape. This heterogeneity in spine structure occurs both among spines on a single dendrite and across different cell types. Figure 3 reveals large differences in the three-dimensional shape of dendritic spines, and Table 1 summarizes the variability in spine dimensions across several brain regions. The greater-than-tenfold differences in spine dimensions shown in this summary easily provide sufficient heterogeneity in spine structure to account for the large heterogeneity in synaptic strengths. Despite these gross differences in spine structure, it is possible to construct rather distinct categories of dendritic spine shapes (e.g. thin, mushroom, stubby, branched) both within and across brain regions (Jones & Powell 1969, Peters & Kaiserman-Abramof 1970, Harris et al 1992).

The diversity in spine morphology may reflect dynamic states during the life history of individual spines and/or different synaptic efficacies occurring along a single dendrite at a particular time. The distinct categories also might well represent specific spine functions or the stages through which individual spines must pass to achieve a "mature" state.

COMPOSITION OF DENDRITIC SPINES AND THEIR SYNAPTIC COMPLEX

Dendritic spines must be considered within the context of the overall synaptic complex, which includes the spine, the postsynaptic density, the synaptic cleft, the presynaptic axonal bouton and its vesicles, and the neighboring astrocytic processes. Morphological and biochemical evidence shows that multiple organelles and molecules are localized within dendritic spines. The specific composition of spines and their synapses may result in further discrimination in the functions of spines. Here we summarize the composition of dendritic spines and their synapses and refer the reader to other articles and reviews for more detail.

Postsynaptic Density (PSD)

One of the most conspicuous ultrastructural features in the CNS is the postsynaptic density (PSD) (Peters et al 1991). The PSD is a structure about 50 nm thick that is apposed to the cytoplasmic side of the postsynaptic membrane. It is found at virtually all excitatory synapses, including those occurring on the heads of dendritic spines (Figure 2). Three-dimensional reconstructions have shown PSDs to be either disc (macular) shaped or highly irregular in shape with perforations, which are electron lucent regions within

the PSD (Cohen & Siekevitz 1978, Spacek 1985a, Harris & Stevens 1989). Some PSDs on a single spine head are segmented into discrete zones (Geinisman et al 1992; Figures 2 *pf* and 3*e,f*). In all brain regions, spine dimensions are proportional to the total area of the PSD or segments of the PSD added together (Westrum & Blackstad 1962; Peters & Kaiserman-Abramof 1970; Wilson et al 1983; Harris & Stevens 1988, 1989; Harris et al 1992; Chicurel & Harris 1992). In freeze-fracture preparations, the extracellular half of the synaptic membrane of dendritic spines has an aggregate of particles, ranging in size from 6–17 nm, with mean densities of about 2800 particles/μm^2 on hippocampal dendritic spines and 3600 particles/μm^2 on cerebellar dendritic spines (Harris & Landis 1986). It has been proposed that these particles are anatomical representations of the molecules involved in synaptic function.

More than 30 proteins that are highly enriched in PSDs have been identified in subcellular fractions from the brain (Kelly & Cotman 1978; Carlin et al 1980, 1981, 1983; Siekevitz 1985; Wu et al 1986; Wu & Siekevitz 1988; Kennedy et al 1990; Walsh & Kuruc 1992). These proteins have been grouped into five classes: (*a*) neuroreceptor glycoproteins (e.g. binding sites for excitatory amino acids and GABA, ion channels for Ca^{2+} and K^+ and others), (*b*) protein kinases [calcium/calmodulin-dependent protein kinase type II (CaM-kinase II), protein kinase C (PKC), and the associated regulatory protein calmodulin], (*c*) structural and mechanochemical proteins (tubulin, actin, brain spectrin/fodrin, myosin, dynamin, mapII, adducin, dystrophin, microtubule associated protein 2 (MAP2), neurofilament proteins), (*d*) proteins involved in endocytosis (elongation factor 1 alpha; N-ethylmaleimide sensitive factor; BiP, a resident protein of the ER), and (*e*) proteins involved in the glycolytic pathway (e.g. possibly glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase). These constituents must be regarded with caution, as the PSD-enriched preparations are known to have some contamination from mitochondrial and other membranes as well as from polyribosomes. Under some conditions, 50% of the total PSD fraction contains what has been referred to as the major PSD protein (Goldenring et al 1984), which is CaM-kinase II (Carlin et al 1981, Kennedy et al 1983, Kelly et al 1984). The CaM-kinase II molecule has aroused considerable interest because it can switch from a calcium/calmodulin-dependent state to a calcium/calmodulin-independent, autophosphorylating state after a brief exposure to calcium and calmodulin. Lisman & Goldring (1988) have postulated that this switch in the state of the CaM-kinase II could mediate short-term changes in synaptic efficacy through phosphorylation of certain proteins (e.g. MAP2 or tubulin) in the PSD, though many of the specific substrates for CaM-kinase II in the PSD remain to be identified (Kennedy 1992). It has long been thought that changes in the structure of the PSD reflect alterations in synaptic efficacy (Cohen & Siekevitz

1978, Nieto-Sampedro et al 1982, S kevitz 1985). The molecular composition of the PSD certainly provides many candidate molecules that could work in consort to mediate the plasticity of synaptic structure and electrophysiology.

Organelles

All spines contain smooth endoplasmic reticulum (SER in Figure 1*C*) (Peters et al 1991; Harris & Stevens 1988, 1989; Spacek 1985a,b), an organelle known to be involved in membrane synthesis (Hall 1992) and to store calcium. The volume of the SER is proportional to spine volume and PSD area and occupies about 10–20% of the total spine volume (Harris & Stevens 1988). The more complex spines contain sacs of SER laminated with dense-staining material into a structure known as the spine apparatus (Gray 1959; Figure 2 *sa*). The spine apparatus appears to be similar to the Golgi apparatus in both its overall structure and its intimate association with the SER, though the Golgi apparatus is typically restricted to the soma and proximal dendrites. Whether the spine apparatus performs similar functions to those of the Golgi apparatus, e.g. modification of proteins to form proteoglycans and vesicle formation (Hall 1992), is not known.

The SER is also thought to be involved in the sequestration and intracellular release of calcium, like the sarcoplasmic reticulum of muscle cells (e.g. Hall 1992). X-ray microanalysis of cerebellar dendritic spines has revealed a preferential localization of calcium in the spine SER (Andrews et al 1988), and precipitates of calcium-oxalate occur in the SER of hippocampal and cortical dendritic spines (Burgoyne et al 1983, Fifkova et al 1983). The inositol triphosphate (IP3) receptor has been identified on the SER in spines and dendrites (Mignery et al 1989, Walton et al 1991). Since the IP3 receptor is activated by calcium in the cytoplasm, release of the stored calcium could be triggered by a brief rise in intracellular calcium, as discussed below.

Polyribosomes have been revealed through three-dimensional reconstructions in more than three quarters of visual cortical spines (Spacek 1985b) and in at least one head of nearly all the highly branched CA3 dendritic spines (Chicurel & Harris 1992). In addition, polyribosomes have been detected both within spines and at the base of spines in the dendrites of hippocampal area dentata and area CA1 neurons (Steward & Levy 1982, Steward & Reeves 1988). The frequency of polyribosomes in the vicinity of dendritic spines increases during synaptogenesis (McWilliams & Lynch 1978, Steward 1983, Steward & Falk 1985) and with rearing of rats in an enriched environment (Greenough et al 1985). The mRNAs that encode for MAP2 and CaM-kinase II, and the brain cytoplasmic mRNA (BC1) are prominent in dendritic laminae throughout the CNS, suggesting that these two proteins (and probably others) are locally synthesized within dendrites (Garner et al 1988, Burgin et al 1990, Tiedge et al 1991, reviewed in Steward & Banker 1992). The preferential

positioning of polyribosomes near to or within dendritic spines indicates that spines and their synapses may be recipients of proteins that are synthesized locally in the dendrites or spines and reinforces the view of spines as autonomous components. This local synthesis of proteins may provide a cellular mechanism whereby new proteins can be specifically targeted in response to synaptic activation (Steward & Banker 1992).

Mitochondria rarely occur in dendritic spines and are typically restricted to the very complex or very large dendritic spines such as those found in the cerebral cortex (Ebner & Colonnier 1975, 1978; Westrum et al 1980), in the branched spines of hippocampal area CA3 (Hamlyn 1962, Amaral & Dent 1981, Chicurel & Harris 1992), or in spines of the olfactory bulb that have both pre- and postsynaptic functions (Cameron et al 1991). Similarly, multivesicular bodies are restricted to large spines (Chicurel & Harris 1992) and the base of dendritic spines (KM Harris, personal observation). The function of the multivesicular bodies has not been clarified for spines; however, studies in other neuronal systems (Rosenbluth & Wissig 1964, Schmied & Holtman 1987, Bailey et al 1992) support their role in the endolysosomal system and involvement in synaptic turnover and plasticity. Coated vesicles are occasionally found in dendritic spines of the adult brain; their frequency also increases with synaptogenesis, and it has been proposed that they may facilitate the formation of new synapses (McWilliams & Lynch 1981).

Cytoskeleton and Cytoplasm

The cytoskeleton of dendritic spines is characterized by a loose network of filaments (Gray 1959). It is distinguished from the dendritic cytoskeleton by the near absence of microtubules, except for an occasional microtubule in the largest and most complex spines (Westrum et al 1980, Chicurel & Harris 1992). The filamentous network of spines is comprised of actin and actin-regulating proteins (Landis & Reese 1983, Fifkova 1985, Cohen et al 1985). The actin filaments of the spine neck are longitudinally situated, whereas those in the head are organized into a lattice surrounding the SER or spine apparatus. This organization of the actin filaments suggests that they provide the scaffolding for the basic spine structure. Other molecules found in the spine cytoplasm that may interact with the actin cytoskeleton, usually in a calcium-dependent manner, include calmodulin, myosin, brain spectrin (fodrin), and MAP2. The organization of the actin filaments within spines does not seem to differ dramatically across the brain regions studied to date. However, some of the actin-associated proteins are heterogeneously distributed and together with local calcium concentrations may contribute to the diversity in spine structure described above. Surprisingly, the growth-associated protein GAP-43, normally thought to be involved in growth cones,

neurotransmitter release, and the function of presynaptic axons (Benowitz & Perrone-Bizzozero 1991), has occasionally been found in dendritic spines or appendages of neostriatal neurons (DiFiglia et al 1990).

Synaptic Plasma Membrane and Cleft Material

The plasma membrane of dendritic spines is similar in appearance to the membrane surrounding the rest of the neuron and the presynaptic axonal bouton and vesicles. It is characterized by a lipid bilayer, which when cross-sectioned can be readily discerned in osmium-stained material (Peters et al 1991). Between the pre- and postsynaptic membranes is the synaptic cleft, a region where the extracellular space widens slightly to about 10–20 nm and is filled with a dense-staining material. The plasma membrane also contains many integral proteins, of which some are specific to the synapse and others are generally found throughout the neuron. For example, two G proteins (G_i & G_o) that are involved in the opening of Ca^{2+} and K^+ channels are found in the synaptic plasma membrane fraction (Wu et al 1992). Although the composition of the synaptic cleft has not yet been delineated, it is likely comprised of cell surface molecules involved in cell-cell adhesion (McDonald 1989, Akiyama et al 1990). Emerging evidence suggests that the synaptic plasma membrane fraction contains integrin-type adhesion receptors (Bahr & Lynch 1992) and neural cell-adhesion molecules (NCAMS) (Persohn et al 1989). In addition, peptides that block a subclass of the integrins disrupt the stabilization of synaptic potentiation (Staubli et al 1990, Xiao et al 1991), suggesting an important role in structural plasticity. Several lines of evidence have led to the hypothesis that the basal lamina protein agrin may be responsible for the aggregation of synaptic proteins on the surface of muscle fibers (Ferns & Hall 1992). Isoforms of this protein are produced throughout the CNS, where they may perform similar synaptic functions (McMahan et al 1992). Whether similar proteins are specifically found in the dense material of the CNS synaptic cleft remains to be determined.

Presynaptic Vesicles

The boutons associated with dendritic spines have numerous round clear vesicles (Figure 2) which contain glutamate (Storm-Mathisen et al 1983; Otterson et al 1990a,b; Clements et al 1990). On the presynaptic membrane is the presynaptic grid (Aghajanian & Bloom 1967, Vrensen & Cardozo 1981), which is characterized by dense projections on the cytoplasmic side of the membrane and which may be the equivalent of the actin-like filaments (Landis 1988) thought to be the "vesicle docking" sites (Schwartz 1992).

The full composition of the vesicles and the presynaptic bouton is very complex and beyond the scope of this review (Maycox et al 1990, Verhage et al 1991). However, it is noteworthy that certain kinds of structural data

can be brought to bear on physiological issues (e.g. Clements et al 1992, Larkman et al 1992). For example, the dimensions of the presynaptic and postsynaptic elements are tightly linked. The total number of vesicles is closely correlated with spine volume, SER volume, and the area of the PSD on dendritic spines (Harris & Stevens 1988, 1989). These correlations hold for a large range in vesicle number, from 38–1234 and 3–1606 for cerebellar and CA1 spine synapses, respectively. These data suggest that a coordinating process coregulates the dimensions of these pre- and postsynaptic structures (Lisman & Harris 1993).

Astrocytes

Astrocytes are identified by the presence of dark glycogen granules and astrocytic fibrils in the cytoplasm, which is typically light in electron micrographs (Peters et al 1991). In some brain regions, such as the cerebellum, the astrocytic processes have been found through EM reconstruction to surround the synaptic complex, involving dendritic spines and their presynaptic axonal boutons (Spacek 1985c). In other brain regions, such as the hippocampus and neocortex, the tiny astrocytic processes that occur in the vicinity of the spines do not surround the entire complex, though their presence becomes obvious through immunolabeling and EM reconstruction (Aoki 1992; KM Harris, unpublished observation).

Astrocytes perform many important functions for the synapses involving the regulation of the extracellular milieu and uptake of potassium and glutamate (Kuffler 1967, Barres 1991). In cultures of dissociated cerebral cortex, astrocytes and astrocytic processes surround a thick layer of neuropil that is full of synapses on dendritic spines and shafts; in contrast, the neuropil of astrocyte-poor cortical cultures is very thin, and few synapses form (Harris & Rosenberg 1993). Neurons in the astrocyte-poor cultures are 100-fold more sensitive to glutamate-induced toxicity (Rosenberg & Aizenman 1989, Rosenberg et al 1992); in fact, cell death occurs at glutamate concentrations that normally occur in the extracellular fluid of a healthy brain. It was proposed that the astrocytes provide a physical buffer in vivo like that seen in vitro, allowing the astrocytes to clear the extracellular fluid of glutamate in the immediate vicinity of the synapses. Astrocytes in the vicinity of hippocampal dendritic spines reportedly proliferate during synaptic plasticity, suggesting an increased need for glutamate regulation at the larger synapses (Sirevaag & Greenough 1987, Wenzel et al 1991). Astrocytes also may regulate calcium in response to stimulation by glutamate (Cornell-Bell et al 1990a,b). Finally, it has been shown that growth of cerebellar dendritic spines is induced by an astrocyte-secreted factor even in the absence of presynaptic axons (Seil et al 1992). Together, these observations suggest an elaborate functional relationship between dendritic spines and their astrocytic partners.

FUNCTIONS OF DENDRITIC SPINES

As Postsynaptic Targets

Ultrastructural evaluation of dendritic spines reveals them to be the major site of excitatory synaptic input. Occasionally, inhibitory/modulatory synapses form on the heads, on the necks, or at the bases of dendritic spines (Figure 4*a;* Colonnier 1968, DiFiglia et al 1982, De Zeeuw et al 1990, Dehay et al 1991, Fifkova et al 1992), which could act to "veto" or modify the strength of the excitatory input (Qian & Sejnowski 1990). Because most dendritic spines have a single excitatory synapse on their head, more spines means more synapses and accordingly more point-to-point connections in a neuronal ensemble involving spiny neurons. Thus, one function of the spine is to preserve the individuality of inputs.

Ramon y Cajal originally postulated that spines could increase the surface area available for new synapses to form. Most of the dendritic shaft between spines, however, does not have synapses, and ample room is available for more synapses to occur even in the absence of more dendritic spines (Gray 1959, Harris & Stevens 1988). Spines allow dendrites to reach multiple axons as they weave through the neuropil (Figure 4*b;* Swindale 1981). For nonspiny dendrites to attain the same radius of access to the axons, they must be thicker than spiny dendrites (which typically they are) and must occupy a significantly greater volume of the neuropil (Figure 4*c*). Spiny dendrites thus allow more synaptic connections to be compacted into a limited brain volume, and hence they can be considered the microscopic parallel to sulci and gyri in the brain. Since their discovery around the turn of the century, however, it has been suspected that dendritic spines do more than simply connect neurons. In fact, both Ramon y Cajal (1893) and Tanzi (1893) suggested that changes in dendritic spine number and/or morphology could provide a cellular basis for learning and memory.

Spines and LTP

A major driving force for establishing a functional description of spine morphology has been the desire to understand the morphological substrate for the profound synaptic plasticities seen in the hippocampus and cortex. One of the most extensively investigated has been long-term potentiation (LTP), a long-lasting enhancement of the post-synaptic response resulting from repetitive or appropriately patterned activation of the neurons (reviewed in Madison et al 1991, Bliss & Collingridge 1993). LTP is widely considered to be a cellular mechanism of at least some forms of learning and memory. In spite of continuing controversy over the exact sequence of events, a growing consensus holds that changes in the properties of both the pre- and postsynaptic elements are involved (Kullman & Nicoll 1992, Larkman et al 1992, Bliss &

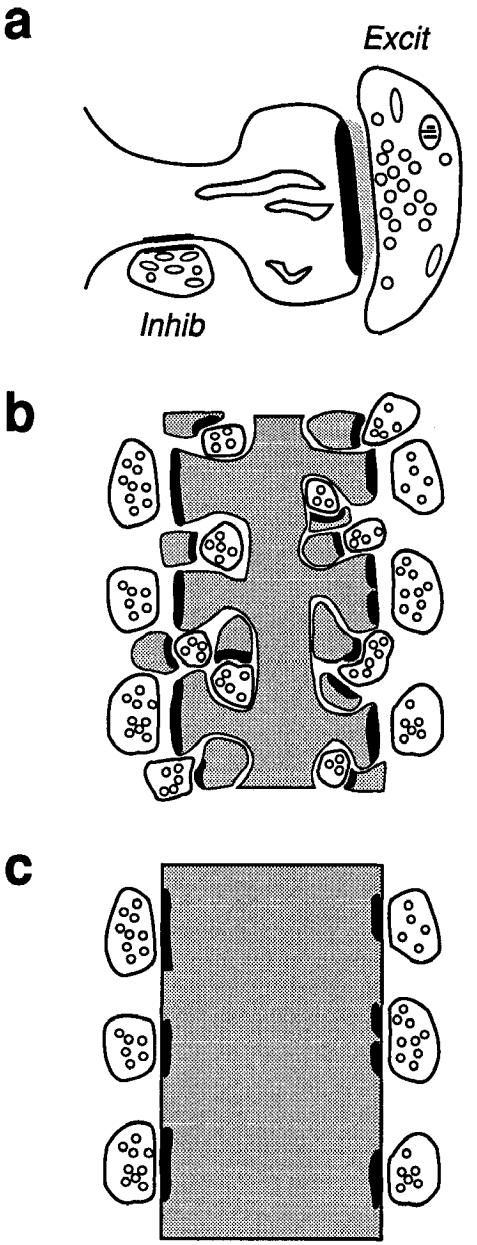

Figure 4 Functions of dendritic spines. (*a*) Sites of excitatory synaptic input (*excit*) and occasionally inhibitory/modulatory (*inhib*) synaptic input. (*b*) Longitudinal section through a spiny dendrite illustrating its reach to many axonal boutons and the interdigitation of other processes between the spines. (*c*) Nonspiny dendrite with the same "axonal reach" as the spiny dendrite in *b*, but no other processes can occupy the space between the synapses.

Collingridge 1993, Lisman & Harris 1993). In the next four sections we consider how the structure of dendritic spines could contribute to the cellular mechanisms that mediate the induction, associativity, specificity, and endurance of LTP. We propose that if spines serve these roles for LTP, they could similarly facilitate learning and memory.

ROLE SPINES MAY SERVE IN THE INDUCTION OF LTP Induction of LTP requires entry of calcium into the postsynaptic cell (Madison et al 1991). To achieve this calcium entry at most of the synapses where LTP is induced, glutamate must be released from the presynaptic terminal at (or near) the same time that the postsynaptic element is depolarized. The postsynaptic depolarization is necessary to relieve a magnesium block in the calcium channel that is associated with the N-methyl-D-aspartate (NMDA) receptor (Madison et al 1991). The constriction in dendritic spine necks, if it poses a resistive barrier, results in an amplification of the depolarization attained in the immediate vicinity of the synapse, relative to that which would be generated if the synapse occurred directly on the dendritic shaft (Figure 5*a;* Perkel 1982, Turner 1984, Coss & Perkel 1985, Brown et al 1988). Thus, spine neck constriction could facilitate induction of LTP by allowing the voltage-dependent channels to open in response to a lower synaptic activation than would be required to depolarize synapses on nonspiny dendrites.

Results from ontogenetic studies on LTP, the NMDA receptors, and dendritic spines in the rat hippocampus lend support to this hypothesis. At birth, no potentiation is elicited from tetanic stimulation in area CA1, but by postnatal days 3–4 posttetanic potentiation, lasting less than a minute, can be induced (Harris & Teyler 1984). By days 5–7 a more enduring potentiation can be induced, but it lasts for only about 45 minutes post tetanus (Harris & Teyler 1984, Bekenstein & Lothman 1991). By days 10–11 the potentiation endures for 2.5 hours, and by day 15 some animals show persistent LTP (for at least 9 hours in vitro). These findings cannot be explained simply by the development of NMDA receptors: In area CA1, the NMDA receptors are present at about 75% of adult values from birth through day 7 (Insel et al 1990, McDonald & Johnston 1990, McDonald et al 1990). The development of a minimum number of dendritic spines may be required for the induction of LTP, as spines are first present at days 5–7, when a nonpersistent form of LTP is first induced (Minkwitz 1976; Pokorny & Yamamoto 1981a,b; Harris et al 1989). Notably, with maturation more spines have constricted necks, and LTP can be induced at lower stimulus intensities than those required at the younger ages (Harris & Teyler 1984, Bekenstein & Lothman 1991). Spines may similarly facilitate the effectiveness of the maturing NMDA receptors and ontogeny of LTP in the cortex (e.g. Mates & Lund 1983; Wilson &

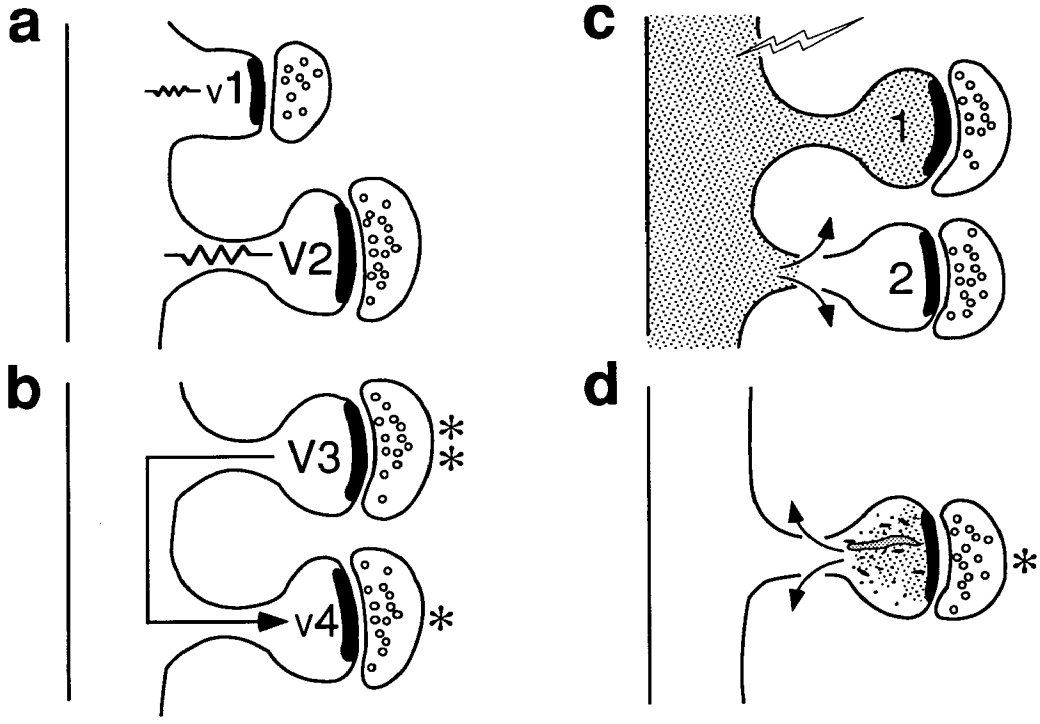

Figure 5 Functions of dendritic spines, continued. (*a*) Amplifying potential (*V*) in spine head. This amplification could contribute to the induction of LTP. (*b*) Sharing postsynaptic potential among neighboring spines. This coactivation could facilitate the associativity of LTP; **, strongly activated synapse; *, weakly activated synapse. (*c*,*d*) Biochemical compartmentation. This compartmentation could contribute to the specificity and endurance of LTP. (*d*) illustrates a tube of SER as an intracellular store of calcium that can be released upon synaptic activation. The small dots represent calcium concentration and the larger black shapes represent stimulus-activated molecules. (See text.)

Racine 1983; Kleinschmidt et al 1987; Komatsu & Toyama 1988, 1989; Perkins & Teyler 1988; Tsumoto et al 1989; Insel et al 1990; Tsumoto 1992).

SPINES PERMIT ASSOCIATIVITY IN LTP It has been shown that weak tetanic stimulation is insufficient to induce LTP, but that when the weak stimulation at one set of synapses is coupled with strong stimulation at another set of synapses on the same cell, LTP is induced at both sets of synapses (McNaughton et al 1978, Levy & Steward 1979, Barrionuevo & Brown 1983, Kelso & Brown 1986, Kelso et al 1986, Larson & Lynch 1986, Sastry et al 1986, Brown et al 1991). The weak and strong stimulation must occur within 100 ms of one another to potentiate the synapses at the weak site, and for this reason it is thought that the "associative messenger" is the polarization state of the postsynaptic membrane (Madison et al 1991).

A longstanding hypothesis has been that the narrow dimensions of the spine neck attenuate current flow between the spine head and the dendrite (Rall 1970, 1974; Coss & Perkel 1985). Morphological evidence suggests, however, that most spine necks are not thin and long enough to significantly reduce the charge transferred to the parent dendrite, if the conductance changes at the synapse are less than 5 nS (Wilson et al 1983; Wilson 1984; Brown et al 1988; Harris & Stevens 1988, 1989). Recent electrophysiological evidence from hippocampal CA1 cells suggests that the mean synaptic conductance for a minimal evoked response is 0.21 ± 0.12 nS, such that the current generated by the release of 10–20 quanta would likely be fully transmitted to the postsynaptic dendrite (Bekkers et al 1990). Thus, spines should permit the addition of voltage changes among coactivated synapses via the dendrites connecting them (Figure 5*b*), thereby allowing the associativity observed with LTP. Other models endow the spine with active membrane (Miller et al 1985, Perkel & Perkel 1985, Shepherd et al 1985, Rall & Segev 1988, Segev & Rall 1988). These models suggest that the excitable membrane might facilitate communication between spines; such facilitated communication would enhance the associativity of the postsynaptic potential among spines.

POSSIBLE EFFECT OF SPINES ON THE SPECIFICITY OF LTP LTP has long been known to be specific to the inputs that are activated during tetanic stimulation (Bliss & Lomo 1973; reviewed in Wigstrom & Gustafsson 1988). In the early experiments, input pathways from different brain regions were tested. LTP was subsequently shown also to be restricted to the stimulated axons within a single input pathway. In these experiments, two subsets of axons were first shown to converge on the same CA1 dendrites, but at different synapses. Then one set of axons was tetanized and LTP was induced. LTP was restricted to those axons that were tetanized, and LTP was not evoked in the nontetanized

subset of axons. This specificity is partly explained by the requirement for glutamate to be released from the presynaptic axon at the same time that the postsynaptic area of the synapse is sufficiently depolarized (i.e. during the tetanus). However, if the molecules relevant to LTP at the tetanized synapses were to diffuse rapidly to the neighboring synapses, then they might also modify those synapses, resulting in a nonspecific spread of the potentiation. The subcellular localization of specific molecules and their regulating organelles within spines may be important factors in establishing the specificity of LTP.

Several modeling studies have predicted that changes in the concentration of calcium and other molecules occurring in the spine will not necessarily transfer to the dendritic shaft, and vice versa (Gamble & Koch 1987, Brown et al 1988, Wickens 1988, Holmes 1990). Recently, visualization of events within living spines in vitro has provided direct confirmation of these predictions. Two sets of experiments examined whether calcium diffuses freely between the dendrites and the spines (Guthrie et al 1991, Muller & Connor 1991). Guthrie et al (1991) exploited the ability of cobalt to quench fura 2 fluorescence to test whether specificity is accomplished by a physical diffusion barrier between spines and their dendritic shaft. As cobalt diffused along a dendritic shaft from a distant region of locally induced entry (*lightning bolt* in Figure 5*c*), the loss of fluorescence occurred virtually simultaneously in the dendrite and the adjacent spines (Figure 5*c, spine 1*). That is, there was essentially no absolute physical barrier to diffusion of small ions from the dendritic shaft into the spine. Nonetheless, when a parallel experiment was done with calcium as the diffusing ion, large rises in calcium in the shaft did not occur in many of the adjacent spines (24/74), indicating that calcium in the dendrite, in contrast to cobalt, is indeed isolated from some spines (Figure 5*c, spine 2*). Muller & Connor (1991) employed synaptic activation to demonstrate that stimulation of spine synapses results in sustained elevation of spine calcium that long outlasts changes in the dendritic shaft (Figure 5*d*). Were stimulation-activated release of calcium to occur from the SER in the spine, the calcium concentration in the spine head would be amplified. Thus, the local concentration of postsynaptic calcium has emerged as a candidate for the mechanism by which specificity could be achieved.

Mathematical modeling provides plausible insights into how such calcium compartmentation could occur in the absence of an absolute barrier to diffusion between spines and dendrites (Zador et al 1990). Three conditions could achieve localization of this second messenger: (*a*) the spine neck could provide a narrow diffusion path that limits calcium ion flux into or out of some spine heads; (*b*) even a small rise in spine calcium could cause a controlled release from intracellular calcium stores, thereby amplifying the calcium signal; and

finally (*c*) only a very few calcium pumps would be required to extrude the few calcium ions that might diffuse through the limited volume of the spine neck from even micromolar concentrations in the dendritic shaft (*arrows* in Figure 5*c*) or from the spine head to the dendrite (*arrows* in Figure 5*d*). Not all spine morphologies would be expected to restrict diffusion. Similarly, it is possible that the distribution of intracellular calcium stores and pumps is not the same on all spines. For example, only the large, mushroom-shaped dendritic spines have laminated spine apparatuses, whereas the smaller, thin spines have a thin tube of SER (see Figure 2 above). Perhaps a subset of spines, or alternatively all spines, but only at a restricted time during their developmental history, achieve the compartmentalization required to confer this specificity.

ROLE OF SPINES IN THE ENDURANCE OF LTP The hallmark of LTP in mature animals is its longevity; it can last for hours to days to weeks depending on the exact experimental conditions (Bliss & Gardner-Medwin 1973, Barnes 1979, Racine et al 1983, Staubli & Lynch 1987). Considerable attention has been devoted to understanding the cellular mechanisms mediating this endurance. From the postsynaptic perspective, the local compartments that spines create in the vicinity of the synapses may allow the concentration of calcium and other molecules relevant to LTP (Figure 5*d*) to remain high enough for sufficient time to stabilize changes in the synaptic machinery leading to persistent LTP.

Study of the ontogeny of LTP also supports this hypothesis. During development, LTP does not persist longer than 2.5 hours until postnatal day 15 (Harris & Teyler 1984, Jackson et al 1993). Different 15-day-old animals express one of two patterns of potentiation: some animals show enduring potentiation like that seen in adults, whereas others show an elevated response for 2.5 hours, which then decays to baseline by 4 hours posttetanus. These findings suggest that day 15 may be a threshold age for expressing persistent LTP. At day 15 about half of the synapses that will be found in the adults have been formed (Harris et al 1992). Spines of the thin, mushroom, and stubby shapes are all present at about equal frequencies. By the time the animals are young adults (days 48–60), however, the majority of synapses are on small, thin spines. These observations suggest that a sufficient number of spines with constricted necks may be required for persistent LTP. They also illustrate the importance of potentially dynamic changes in spine structure during development.

CHANGES IN DENDRITIC SPINE STRUCTURE WITH LTP Average spine and synaptic dimensions have been compared in preparations that have undergone

plasticity with those in preparations that have not. Several other reviews have considered the changes in spine morphology that accompany synaptogenesis during development, behavioral changes associated with learning and memory, and pathological changes associated with neural dysfunction (Scheibel & Scheibel 1968, Huttenlocher 1975, Coss & Perkel 1985, Greenough & Bailey 1988, Calverly & Jones 1990). Considerable accumulated evidence suggests that changes in spine and synaptic structure occur during LTP (for review see Wallace et al 1991). Where tested, the reported changes in spine and synaptic morphology have been specific to the tetanized input (Van Harreveld & Fifkova 1975, Fifkova & Van Harreveld 1977, Desmond & Levy 1988a). Controversy remains as to whether new spines and synapses form or if the geometries of existing spines and synapses change (Wallace et al 1991, Harris et al 1992). For example, in hippocampal area dentata, some results suggest that dendritic spines swell during LTP (Van Harreveld & Fifkova 1975, Fifkova & Van Harreveld 1977), and others suggest a change in the morphology of existing PSDs during LTP (Desmond & Levy 1986, 1988b, 1990). In contrast, results from a study utilizing serial EM reconstructions suggest that during LTP the total spine number doubles and the number of branched spines and spines with wide necks increases (Andersen et al 1987a,b; Trommald et al 1990). In hippocampal area CA1, no significant changes in overall spine density have been detected, although there is evidence for spine "rounding" and an increase in the frequency of stubby dendritic spines (Lee et al 1980, Chang & Greenough 1984). Except for extremely fortuitous sections (such as the one shown in Figure 2 above), the morphology of most spines cannot be identified on a single section, and therefore no data exist in these studies on the fate of the predominant thin, mushroom, and branched dendritic spines during LTP (Harris et al 1992).

As implied by the description of dendritic spine composition above, several molecular mechanisms exist that could mediate rapid short-term and long-term changes in spine and synaptic morphology. For example, glutamate and its analogues activate proteolysis of brain spectrin (fodrin) by the neuron-specific protease, calpain I (Siman & Noszek 1988). Degradation of fodrin, a structural protein of the (spine) cytoskeleton (Perlmutter et al 1988), could allow the spine to undergo shape changes (Siman et al 1990), possibly in response to growth of the synapse. The state of actin polymerization is regulated by calcium concentration and determines the viscosity of the spine cytoplasm (Fifkova 1985). The actin filaments are transient structures that can change rapidly in response to the calcium-activated second messenger systems involving stimulation of phosphorylation by calmodulin. Actin could serve to stabilize spine structure through its binding to the subplasmalemmal cyto-

skeleton or to alter spine structure through "contraction" (Crick 1982, Katsumaru et al 1982, Eccles 1983).

Spines Might Prevent Neuronal Pathology During Normal Synaptic Transmission and Plasticity, Such as LTP

Spines are nearly absent or have gross distortions in the cerebral cortex and hippocampus of individuals suffering from severe mental retardation (Marin-Padilla 1972, 1974, 1976; Purpura 1974, 1975a,b; Huttenlocher 1975, Williams et al 1990), epileptic seizures (Scheibel et al 1974), and the neuropathology associated with hypoxia, ischemia, and stroke (Fischer et al 1974, 1980; Rothman & Olney 1986; von Bossanyi & Dietzmann 1990). Under normal conditions, the compartmentation of calcium in dendritic spines could allow its concentration to achieve levels that can activate the second messenger systems. Such intracellular calcium levels could be toxic in the dendrite, but the spines contain a sufficiently small volume that the endoplasmic reticulum and cytoplasmic calcium buffers can return the calcium concentration to basal levels shortly after synaptic activation.

In experimental animal models of seizures and hypoxia/ischemia, a characteristic sequence of ultrastructural lesions occurs (Olney et al 1979, 1983; Evans et al 1983; von Lubitz & Diemer 1983; Sloviter & Dempster 1985; Siman & Card 1988; Allen et al 1989; Remis et al 1989; Yamamoto et al 1990). Notably, spines are lost in one of the first steps during this process, and the dendrites and their organelles become grossly swollen during these early stages (i.e. the rapid toxicity on a time scale of minutes to hours). Subsequently the animals or cells are returned to normal conditions, and over a prolonged time the neurons die (i.e. the delayed toxicity). Excitotoxicity induced by seizures or hypoxia/ischemia may be forms of neuronal pathology that result from excessive use of the same synaptic mechanisms that are normally used in LTP and learning and memory. A compelling hypothesis is that the cellular changes involving dendritic and cellular swelling during the phase of rapid toxicity are mediated through the influx of large amounts of sodium, chloride, and water following excessive activation of the glutamatergic receptors. The delayed toxicity may well result from a prolonged elevation in calcium throughout the neuron, which can cause hyperexcitability, proteolysis of neurofilaments, irreversible mitochondrial damage, and breakdown of membrane phospholipids with release of arachidonic acid and oxygen-free radicals (Rothman & Olney 1986, Choi 1988, Meyer 1989, Meldrum & Garthwaite 1990). These findings support the speculation that dendritic spines promote synaptic stability and plasticity under normal conditions and additionally protect the dendrites and postsynaptic cells from changes in molecular composition that might otherwise be pathological.

PROSPECTUS

Here we have described how the structure of dendritic spines could facilitate not only the stability and reliability of excitatory synaptic transmission but also the functioning of cellular mechanisms that mediate the induction, associativity, specificity, and endurance of LTP. We have also discussed how spines may serve to prevent cytotoxicity during normal synaptic transmission and plasticity. Furthermore, evidence suggests that the morphology of spines and their synaptic complexes changes with both LTP and cytotoxicity. This evidence is based largely on the comparison of static images from experimental and control neurons. Though powerful in its own right, such a statistical or population approach cannot follow dynamic changes in individual spines.

Foreseeable advances in light microscope technology will undoubtedly allow the life histories of individual spines to be followed at a gross level. The formation of new spines or the resorption of existing spines is clearly in the realm of this level of resolution. Gross changes in the volume of the spine head will also be detectable. However, the following key features of spine and synaptic structure are all below the resolution of light and require ultrastructural analysis: (*a*) spine neck diameter, which could modulate ionic flux; (*b*) irregularities in spine surface area, which could affect channel number and capacitance; (*c*) PSD area, which predicts the availability of several molecules involved in synaptic transmission; (*d*) SER volume, which could regulate the ionic and other molecular composition of the cytoplasm; and (*e*) presynaptic vesicles, the size and distribution of which may predict availability of neurotransmitter for release. Since theory predicts that even subtle changes in spine structure can influence synaptic transmission and plasticity, it will be important to establish the degree to which these alterations occur. Obviously, significant progress in spine research will best be made when single experiments can make use of the full range of temporal and spatial resolution of both light and electron microscopy.

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