Ultrastructural Analysis of Spine Plasticity

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Introduction

Dendritic spines are small protrusions that serve as the major site of excitatory synaptic transmission in the brain. The shape and density of spines can be affected by numerous factors, including developmental stage, experimental preparation, temperature, exposure to enriched environments, and neuronal pathology. In addition, physiological models of learning, including long-term potentiation (LTP) and long-term depression (LTD), have indicated that alterations in synaptic ultrastructure may underlie the storage of information in the brain.

Spine Structure and Function

Spine Shape

Dendritic spines can assume striking differences in size, shape, and subcellular composition both within and across brain regions (Figure 1). Most spines are unbranched protrusions that can be classified by their stubby, thin, or mushroom shapes. Branched spines have multiple heads which, in some brain regions, are all innervated by a single axonal bouton (e.g., hippocampal area CA3); in other brain regions the different heads of a branched spine are innervated by different axons (e.g., hippocampal area CA1); and in rare cases some of the heads have no presynaptic partners while other heads are innervated by different axons (e.g., cerebellar Purkinje spiny branchlets).

Localization of Organelles

Smooth endoplasmic reticulum (SER) is an organelle that is likely to be involved in sequestering calcium. Depending on the particular brain regions, few, many, or most of the dendritic spines contain SER (Figure 1). For example, only about 14% of the hippocampal CA1 spines contain SER, and most of it occurs laminated with dense-staining material into a structure known as the spine apparatus (as in Figure 1) in the large, complex spines. In contrast, nearly 100% of the cerebellar Purkinje spines contain SER in a tubular network. Polyribosomes are also present in or near the base of some dendritic spines (Figure 2(d)), suggesting that local protein synthesis of dendritic mRNAs can occur in the spines. In immature neurons, synaptic activity can shift the distribution of polyribosomes from the shaft to spines. Similarly, endosomal compartments including coated pits and vesicles, large vesicles, tubules, and multivesicular bodies are restricted to a subpopulation of dendritic spines that differs from spines that contain SER. Mitochondria rarely occur in dendritic spines and are usually restricted to those that are very large, complex, and highly branched. However, during periods of active synapse formation and remodeling in cultured neurons, mitochondria can localize to smaller dendritic spines.

Postsynaptic Targets

Evaluation of dendritic spine structure readily reveals them to be the major postsynaptic target of excitatory synaptic input (Figure 2(a)). Since all dendritic spines have at least one excitatory synapse on their head, more spines mean more synapses and accordingly more point-to-point connections in a neuronal ensemble involving spiny neurons. The size of the spine head also influences the amount of synaptic input, because larger spines are more sensitive to glutamate, the major excitatory neurotransmitter in the brain. Larger spines have larger postsynaptic densities (PSDs), which can then anchor more glutamate receptors. Thus, one function of spines is the preservation of the individuality of inputs. Occasionally, inhibitory or modulatory axons also form synapses on the heads, necks, or at the bases of dendritic spines. An inhibitory input on spines could act to 'veto' or modify the strength of the excitatory input.

Amplification of Voltage in Spine Head

The constriction in dendritic spine necks poses a small resistive barrier, thereby amplifying the depolarization attained in the immediate vicinity of the synapse, in contrast to that which would be generated if the synapse occurred directly on the wide dendritic shaft (Figure 2(b)). Computer simulations have revealed that most of the spine necks are sufficiently wide and short such that charge transfer to the postsynaptic dendrite is 85–100% complete within 100 ms after the initiation of a synaptic event. The time delay in charge transfer is sufficient, however, to provide a transient amplification of voltage at the spine synapse, which may facilitate opening of voltage-dependent channels in the spine head, such as the calcium channel associated with the *N*-methyl-D-aspartate (NMDA) class of glutamate receptors.

Sharing of Postsynaptic Potential

A long-standing hypothesis has been that the narrow dimensions of the spine neck would attenuate current

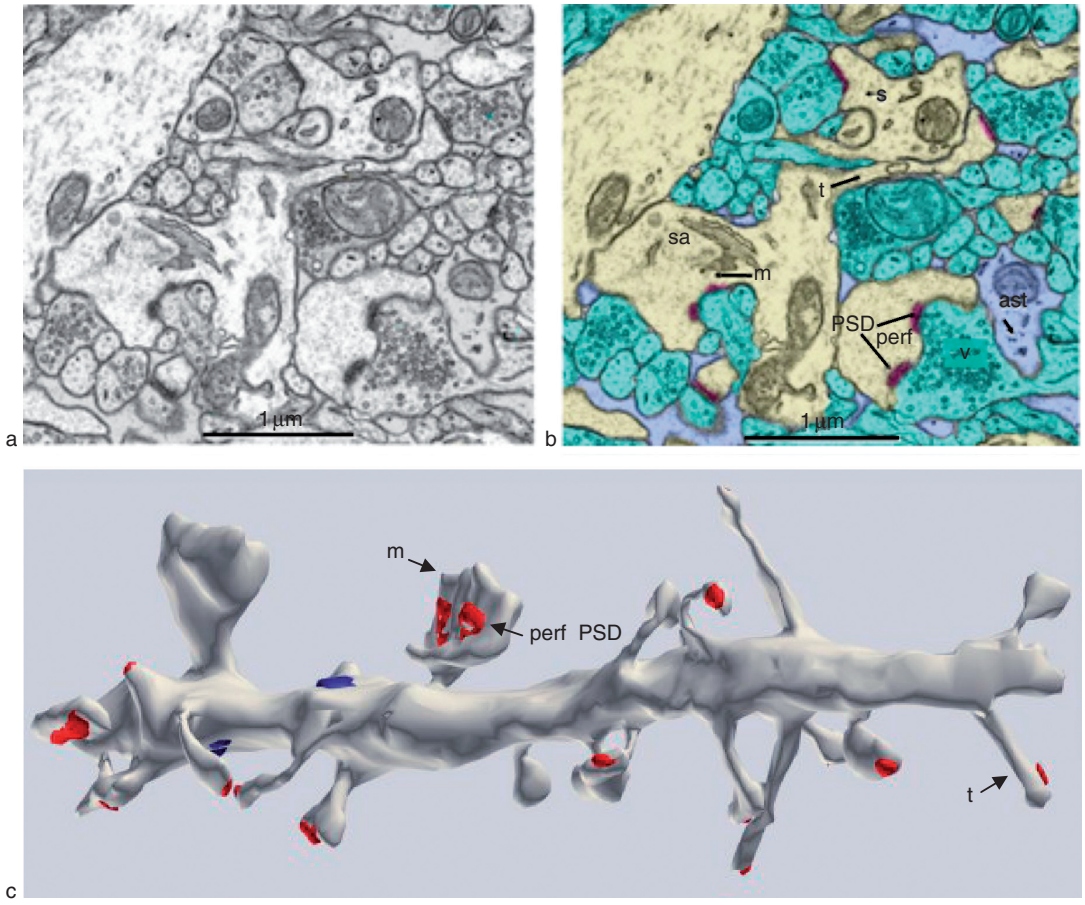

Figure 1 Dendritic spine shape. (a) Electron micrograph of a section through dendritic spines in stratum radiatum of hippocampal area CA1. (b) The same micrograph that has been color-coded to identify dendrites (yellow), axons (green), and astroglia (purple). In this fortuitous section, three spines 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 and to a 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 PSD (perf PSD) is evident on the head of another mushroom spine. Near to this spine is a large astrocytic process (ast) identified by the glycogen granules and clear cytoplasm. (c) A 3-D reconstruction of a dendrite showing a variety of spine and synapse shapes.

flow between the spine head and the dendrite. Morphological evidence suggests, however, that most spine necks are not thin and long enough to significantly reduce the charge transferred to the parent dendrite. Current 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 release of 10–20 quanta would likely be fully transmitted to the postsynaptic dendrite. Thus, the constriction in the spine neck is not sufficient to prevent addition of voltage changes among co-activated synapses. Other models endow the spine with active membrane that would further enhance the sharing of postsynaptic potentials among neighboring spines (Figure 2(c)).

Biochemical Compartmentalization

Compartmentalization of calcium has now been demonstrated in the heads of dendritic spines under a variety of conditions. Two features of spines help to achieve localization of this second messenger in spine heads at least for a short time: (1) the spine neck could provide a narrow diffusion path and (2) a rise in spine calcium could cause release from SER, the intracellular calcium stores thereby amplifying the calcium signal. Biochemical compartmentalization in spine heads may also serve an important role in restricting calcium from the postsynaptic dendrite, thereby preventing excitotoxic cell damage such as microtubule breakdown and mitochondrial swelling. Since dendritic spines rarely have microtubules or mitochondria, high calcium concentrations in the spine head

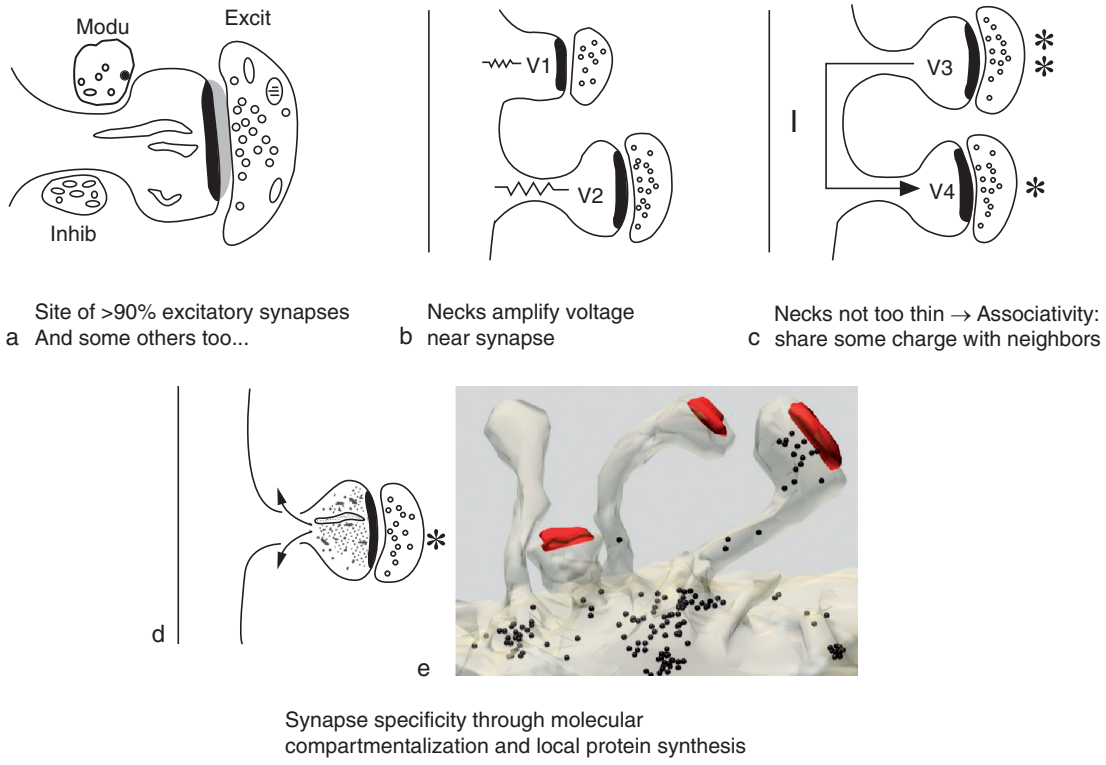

Figure 2 Dendritic spine function. (a) Spines exist as principal sites of excitatory synaptic transmission. Spines exist to (b) amplify electrical potential at the synapse and (c) promote associativity among neighboring synapses. Spine shape and resistance of the spine neck may influence potential (V) generated by synaptic activation. (d) Spines exist as molecular compartments. Smooth endoplasmic reticulum (tubules), calcium, and a myriad of other signaling mechanisms (stippling) are recruited in response to synaptic activation (asterisk). (e) Three-dimensional reconstruction of thin spines emerging from a dendrite. Polyribosomes (black dots) are most frequent at the base of dendritic spines, although they can also occur within them.

are less likely to have these detrimental effects. Not all spine morphologies would be expected to restrict diffusion, and only some spines have internal stores. A recent study that utilized caged glutamate to activate NMDA receptors and a low-affinity Ca^{2+} indicator (to minimize the perturbation of endogenous Ca^{2+} buffering) indicates that small spines with narrow necks exhibit large, isolated increases in $[Ca^{2+}]_i$. However, the necks of larger spines allow for a greater efflux of Ca^{2+} into the dendritic shaft at the base of the spine. Perhaps only a subset of spines, or alternatively all spines, but only at a restricted time during their history, achieve the compartmentalization of calcium, whether in spines or along a short segment of dendrite and its associated spines.

The specific localization of voltage-dependent calcium channels is also an important factor in determining which components of the dendritic arbor will compartmentalize and utilize relatively high concentrations of calcium. Whether spines preferentially sequester other second messengers remains to be determined. Certainly those tethered to the PSD are prevalent in spines, but further work is needed to determine how they are targeted to spines and whether compartmentalization in the spine head is a crucial element in their regulation. Recent evidence suggests that synaptic activity can create a bidirectional barrier to the diffusion of proteins, indicating a synapse-specific mechanism to amplify the biochemical signals necessary for establishing plasticity (Figure 2(d)). In addition, the presence of polyribosomes in some spines could provide the necessary machinery for generating plasticity-related proteins (Figure 2(d)).

Enhanced Connectivity

With the evolution of the brain, the increase in synaptic density had to be accompanied by a significant reorganization of the neuropil. Dendritic spines permit dendrites to synapse with neurons 1–2 μm away, which allows for increased synaptic density in densely packed neuropil (Figure 3(a)). Even some invertebrate neurons exhibit spinelike structures, indicating that dendritic spines appeared well before the evolution of the complex mammalian brain. Consider the simple case of an orthogonal relationship between dendrites and axons (Figure 3(b)). There can only be two

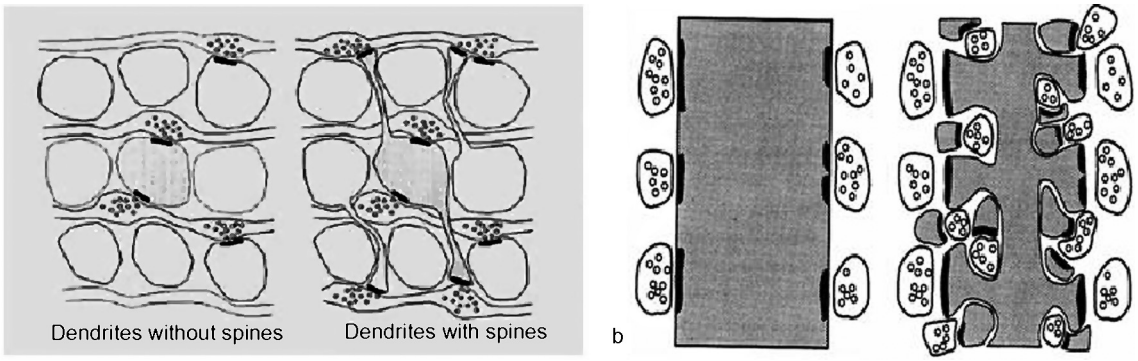

Figure 3 Enhanced connectivity between neurons. (a) Spines increase the packing density of synapses. Schematic illustrates a cross-section through two dendrites (shaded), one without and one with dendritic spines. Convolution and interdigitation of dendrite, axon, and spine membranes support more synapses. (b) The presence of spines also allows for an increase in synaptic density without increasing the overall volume of the brain.

synapses on either side of the dendrite in any given plane without the presence of spines. Dendrites with spines can reach beyond their immediate perimeter to connect with axons in nearby rows, thereby at least doubling the density of possible connections. In addition, the shape of dendritic spines allows efficient interdigitation between neighboring processes, thus achieving the high synapse packing density in the neuropil.

Synaptic Activity and Spine Organization

Spine Stability and Turnover

The stability of spines largely depends on the level of synaptic activity and the developmental stage of the neuron. In immature neurons, spines and filopodia turn over rapidly as connections are formed and stabilized. Only a small amount of glutamate is required to stabilize a spine. Excitotoxicity resulting from too much glutamate can result in the disappearance of spines, while a block of synaptic transmission can result in robust spinogenesis, particularly on mature neurons following traumatic injury or exposure to cold temperatures as occurs during hibernation and some surgical and experimental procedures. The appearance and disappearance of spines may reflect the capacity of mature neurons for recuperative synaptogenesis or a form of synaptic scaling, a homeostatic mechanism that ensures that the output of a postsynaptic cell remains constant despite alterations in synaptic input. In contrast, young neurons do not exhibit spinogenesis with blocked transmission, but rather insert more receptors to maintain synaptic homeostasis.

Brain regions also differ in the amount of synaptic turnover that occurs with the modulation of sensory experience. Recent technological advances have allowed dendritic spines of the somatosensory cortex to be imaged *in vivo* for periods of time ranging from a few hours to a few months. Small spines (thin or filopodial) appear and disappear in an experience-dependent manner associated with synapse formation or elimination, whereas larger spines persist for months. The effect of sensory experience on synapse number depends on the rates of synapse formation and elimination at different developmental stages, and the percentage of stable spines increases with age.

In the visual cortex of adult mice, *in vivo* imaging reveals that the majority of spines (75–90%) are stable under baseline conditions. In another study, rats reared in the dark until postnatal day 30 have a significantly lower spine density compared to light-reared controls and also have shorter, rounder spines. Although an increase in spine head diameter is observed in dark-reared animals that are exposed to light for 10 days, the low spine density is not reversed. Presuming that an increase in the number of receptors present in the PSD accompanies the increase in spine head diameter, the parent dendrite could still preserve the level of synaptic input even in the absence of spinogenesis.

In the neocortex, cerebellum, and hippocampus, localization of different afferent inputs on the same dendritic arbor is dictated by the laminar structure of these brain areas. However, in the lateral nucleus of the amygdala, inputs from the cortex and thalamus are interspersed on the same dendritic branches and afferent specificity is determined by spine morphology. Large spines are contacted by thalamic afferents and exhibit larger Ca^{2+} transients following activation than smaller spines contacted by cortical afferents. The increased Ca^{2+} signaling observed in large spines is mediated by R-type voltage-dependent Ca^{2+} channels that are preferentially localized to thalamic inputs. Thus, in the amygdala, organization of afferent inputs

can be regulated at the level of an individual spine by altering its morphological and molecular properties.

Perisynaptic Astroglia and Spine Structure

Traditionally, astroglia have been thought of as 'support' cells for neurons. However, recent evidence suggests that astroglia may play a much larger role in the maintenance and plasticity of synapses. Release of the cytokine tumor necrosis factor alpha (TNFα) by glial cells enhances excitatory synaptic strength by increasing the surface expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors on dendritic spines while reducing inhibitory synaptic strength by causing the endocytosis of γ-aminobutyric acid ($GABA_A$) receptors. Since astroglial processes can project extensively throughout the neuropil, astroglia are in a position to scale synaptic responses at the level of entire neuronal networks. In addition to affecting the physiological properties of synapses, astroglia can also regulate the structure of dendritic spines in response to synaptic activity. Transient interactions between cell surface molecules such as the ephrinA3 ligand and the EphA4 receptor regulate the structure of excitatory synaptic connections through neuro-glial cross-talk. Activation of EphA4 by ephrin-A3 induces spine retraction, and inhibiting ephrin/EphA4 interactions distort spine shape and organization. In addition to Ephs and ephrins, astrocytic membranes contain other contact-mediated factors that influence synaptic maturation. Astroglial processes tend to be found around larger and presumably more mature synapses, while areas that have undergone recent spinogenesis tend to be devoid of astroglia. Thus in the mature brain, astroglia may be involved in enhancing the stability of spines and preventing the extension of new dendritic protrusions. Interactions between cell surface molecules and the release of various soluble factors by astroglia may be of crucial importance to the turnover and structural alteration of spines observed with synaptic plasticity.

Structural Synaptic Plasticity at Dendritic Spines

Long-Term Potentiation

LTP is a cellular model of learning and memory that has been extensively investigated regarding changes in spine and synapse structure. Two key structural changes would contribute to a potentiated synaptic response: an increase in the number of spines or an increase in the size of synapses with LTP. Several studies in the hippocampus over the last 30 years have attempted to link structural alterations of the synapse to the increase in synaptic strength, but the results remain controversial, particularly in the adult brain. Numerous studies involving *in vivo* induction of LTP in the dentate gyrus of adult rats have led to differing conclusions about the amount of structural plasticity that occurs. Early studies describe an input-specific increase in spine volume and a shortening of spines accompanied by an enlargement of the spine neck. Overall spine density and branched spines in particular are increased 30 min after the *in vivo* induction of LTP in the dentate gyrus, although the increase in branched spines may be due to high-frequency stimulation alone, even in the absence of LTP. While there has been some speculation that branched spines might arise from the splitting of individual potentiated spines, careful three-dimensional (3-D) reconstructions of the neuropil have revealed that branched spines never synapse on the same axonal bouton. In addition, the number of neuronal and dendritic processes that pass between the branches argues against the idea of spine splitting. An alternative hypothesis suggests that branched spines arise from the formation of new protrusions called filopodia which can be initiated by the enhanced synaptic activity, navigate between neighboring processes to find a presynaptic partner, and then become dendritic spines. In any case, the majority of morphological alterations that have been attributed to *in vivo* induction of LTP have included more subtle changes such as increases in the concavity of spine heads and an increase in PSD area, as well as an increase in the ratio of perforated to nonperforated PSDs. Because perforated PSDs tend to occur in mushroom spines, this change could indicate an alteration in spine morphology if potentiation results in a shift from thin spines to mushroom spines.

Uncertainty also exists about the extent of structural plasticity that occurs following the induction of LTP in area CA1 of the adult rat hippocampus. Early studies that analyzed single EM images suggest that there is an increase in shaft and sessile synapses following induction of LTP. However, it was not specified whether these synapses were symmetric (inhibitory) or asymmetric (excitatory). More recent studies specifically aimed at detecting changes in synaptic density failed to detect a reliable increase in synapse number after induction of LTP. Although changes in synaptic density have been difficult to detect, recent evidence suggests that spines can get bigger with LTP. When LTP is induced via stimulation of the Schaffer collaterals, a small fraction of spines transiently expand within minutes of tetanic or theta burst stimulation, but return to their initial size within a few minutes. The percentage of expanding spines varies directly with stimulus strength, presumably

reflecting an increase in number of presynaptic fibers recruited. Even with a stimulation intensity that evokes a maximal field excitatory postsynaptic potential (fEPSP), no more than 10% of spines respond. Thus, the relatively small fraction of expanding spines that synapse with the presynaptic terminals activated by field stimulation reflects the challenges present in detecting plasticity-related changes in the adult brain. Even serial section electron microscopy and 3-D reconstruction of dendrites 2 h post-LTP did not reveal net changes in the density, shape, or size of spines. One possibility is that synaptic proliferation occurs immediately after LTP induction, but with time, competition between recently potentiated spines and their nonpotentiated neighbors results in the elimination of the nonpotentiated spines; thus, the dendrite maintains its optimal number of synaptic connections. Under such a scenario, individual spines could exhibit dramatic plasticity while the overall population number and average size of dendritic spines would appear unchanged.

Although the mature brain presents several challenges to describing the morphological changes that underlie synaptic plasticity, the immature nervous system has provided more consistent results. Dynamic changes in local dendritic segments that were targeted for potentiation-induced changes have been observed in organotypic slice cultures. Significant local outgrowth of filopodia-like dendritic processes is seen 20 min after delivery of a tetanic stimulus, and some of the activity-induced filopodia later turn into spine-like structures. When LTP is induced by pairing postsynaptic depolarizations with single presynaptic stimuli and transmitter release is blocked everywhere but in the small area being imaged, two to nine new spines are formed in the nonblocked region, emphasizing the localized specificity of structural changes associated with LTP. Similarly, stimulation of individual spines with uncaged glutamate induces a localized increase in spine head diameter that is apparent within 1–5 min, and 30% of the stimulated spines remain enlarged 70–100 min after stimulation. The magnitude of potentiation correlates with early or long-lasting spine enlargement, suggesting a high level of fidelity between physiological and morphological changes. Spine and PSD enlargement are also associated with the translocation of polyribosomes into spines following the induction of LTP in acute PN15 hippocampal slices. Given that the maintenance of LTP is dependent on the synthesis of new proteins, the presence of polyribosomes in a subset of spines may provide an indication of which spines underwent potentiation.

Although quite a bit of uncertainty still surrounds the set of structural changes associated with LTP, it appears likely that the effect is selective to potentiated synapses and that changes in a tissue will be correlated with the numbers of stimulated axons and dendrites. Nevertheless, there is evidence that LTP-inducing stimuli can result in increases in spine size and number and may involve the remodeling of the PSD and the translocation of organelles into activated spines. All of these changes could form the basis of long-lasting changes in synaptic strength.

Long-Term Depression

In contrast to LTP, LTD is a long-lasting reduction in synaptic transmission that may also play an integral role in the processing and retention of information. Although the literature concerning structural changes associated with LTD is much less abundant, there does appear to be a correlation between a reduction in synaptic response and a decrease in the number and size of dendritic spines. Low-frequency stimulation (900 pulses at 1 Hz) results in the shrinkage of dendritic spines in acute hippocampal slices and the retraction of spines in organotypic hippocampal slice cultures. While these results indicate a strong link between structural and synaptic plasticity, much work remains to be done to fully elucidate the morphological correlates of LTD.

See also: Developmental Synaptic Plasticity: LTP, LTD, and Synapse Formation and Elimination; Endocytic Traffic in Spines; Eph Receptor Signaling and Spine Morphology; LIM Kinase and Actin Regulation of Spines; Long-Term Depression: Cerebellum; Long-Term Potentiation and Long-Term Depression in Experience-Dependent Plasticity; Long-Term Potentiation (LTP).

Further Reading

Bliss T, Collingridge G, and Morris R (eds.) (2004) *Long-Term Potentiation: Enhancing Neuroscience for 30 Years*. New York: Oxford University Press.

Ethell IM and Pasquale EB (2005) Molecular mechanisms of dendritic spine development and remodeling. *Progress in Neurobiology* 75: 161–205.

Harris KM and Kater SB (1994) Dendritic spines: Cellular specializations imparting both stability and flexibility to synaptic function. *Annual Review of Neuroscience* 17: 341–371.

Harris KM and Stevens JK (1988) Dendritic spines of rat cerebellar Purkinje cells: Serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* 8: 4455–4469.

Harris KM and Stevens JK (1989) Dendritic spines of CA1 pyramidal cells in the rat hippocampus: Serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* 9: 2982–2997.

Nimchinsky EA, Sabatini BL, and Svoboda K (2002) Structure and function of dendritic spines. *Annual Review of Physiology* 64: 313–353.

Peters A, Palay SL, and Webster HDF (1991) *The Fine Structure of the Nervous System: Neurons and Their Supporting Cells,* 3rd edn. New York: Oxford University Press.

Sorra KE and Harris KM (2000) Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines. In: Eichenbaum HB (ed.) with Harris KM and Sorra KE (special issue eds.) *Dendritic Spines of the Hippocampus. Hippocampus* 10: 501–511.

Stuart G, Spruston N, and Hausser M (eds.) (2001) *Dendrites.* New York: Oxford University Press.

Yuste R and Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual Review of Neuroscience* 24: 1071–1089.

Relevant Website

http://synapses.clm.utexas.edu – synapse web.