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Dendritic Spine Geometry: Functional Implication and Regulation

Minireview

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Dendritic spines are tiny protrusions on dendritic shafts where most excitatory synapses are located. Recent advances in imaging technologies have given us great insight into the function of spines as biochemical compartments. Here we review recent evidence suggesting that the geometry of dendritic spines controls postsynaptic calcium signaling and is bidirectionally regulated during synaptic plasticity.

The geometry of dendritic spines is diverse. Even within the same cell, no two spines look alike. In addition, changes in their structure and density are associated with various physiological processes, such as neuronal development, hibernation, and estrus cycles, as well as pathological conditions such as mental retardation. Since the discovery of dendritic spines, their structure has been extensively studied, both with Golgi staining at the light microscopic level and through the use of electron microscopy. These techniques, however, give only static images of dendritic spines. Recent advances in technologies such as two-photon microscopy and in the development of labeling techniques using GFP and other molecular probes enable us to visualize dendritic spines in action and update our view of spines as signaling compartments indispensable for neuronal function.

The Geometry of Dendritic Spines and Ca^{2+} Dynamics

It is now well established that dendritic spines act as biochemical compartments restricting increases in Ca^{2+} concentration to individual synapses. Ca^{2+} is a crucial signaling ion for synaptic plasticity, and an important aspect of spine function is the synapse-specific implementation of plasticity. Additionally, geometric parameters, such as spine neck length (Majewska et al., 2000b) along with the expression of different molecules within the spine, can shape the size and duration of synaptic Ca^{2+} transients, influencing plasticity at the synapse. But because of the spine's small size and due to the fact that indicators commonly used to monitor Ca^{2+} activity can severely alter Ca^{2+} function, it has been difficult to elucidate the exact relationship between spine geometry and spine Ca^{2+} dynamics.

Studies have shown that during synaptic stimulation, the primary mode of calcium entry is through NMDA channels (Sabatini et al., 2001; Yuste et al., 2000). Using two-photon uncaging of glutamate to stimulate single, identified dendritic spines, Noguchi et al. confirm and extend previous findings showing that both AMPA (I_{AMPA}) and NMDA (I_{NMDA}) currents scale linearly with spine head size (Matsuzaki et al., 2001; Noguchi et al., 2005). The relationship is steeper for I_{AMPA}, resulting in small spines having few AMPA receptors, but a substantial number of NMDA receptors and acting as "silent" synapses. Paradoxically, although, I_{NMDA} increases with spine volume, the postsynaptic calcium increase is lower in larger spines (Nimchinsky et al., 2004; Noguchi et al., 2005, see below).

Ca^{2+} clearance in spines and Ca^{2+} extrusion is thought to be achieved by active diffusion of Ca^{2+} to the dendrite (Majewska et al., 2000a). The relative contribution of these two pathways has been controversial. Initially, heterogeneity in efflux pathways was reported for CA1 pyramidal spines, with some spines showing efflux dominated by diffusion to the dendrite ("diffusers") and others showing stronger extrusion ("pumpers"; Majewska et al., 2000a). Similar heterogeneity was also confirmed for layer 5 pyramidal neurons in visual cortex (Holthoff et al., 2002). In these studies, the authors constructed mathematical models that extrapolated to the endogenous case, because they used large amounts of exogenous high-affinity dye that could alter calcium mobility in the cytoplasm, exaggerating the role of diffusion. A contradictory study, which used an indirect fluctuation analysis to determine the diffusional coupling between spine and dendrite and lower concentrations of high-affinity indicator found that spine geometry effectively isolated spines from the parent dendrite, leaving extrusion mechanisms solely responsible for the clearance of Ca^{2+} from the spine (Sabatini et al., 2002). Interestingly, measurements of time scales of calcium signaling, calcium amplitudes, and extrusion rates, obtained in the three studies were in agreement. The discrepancies in the perceived role of diffusion in spine efflux could be accounted for by the different methods employed in these studies or by the heterogeneity of the dendritic spine population involved (Helmchen, 2002; Holthoff et al., 2002). Sabatini et al. examined spines located on thin, higher-order dendrites far from the soma and excluded short, stubby spines from their analysis. In fact, it is likely that stubby spines are diffusers, due to their large neck radius, and an increased dependence on spine extrusion mechanisms has been demonstrated in spines located further from the soma (Holthoff et al., 2002).

Using a low-affinity Ca^{2+} indicator to minimize the indicator's effect on intrinsic Ca^{2+} dynamics, Noguchi et al. (2005) confirm that efflux pathway heterogeneity does exist among spines (Figure 1). They find that pumpers are predominantly small spines that tend to have small neck conductances, increasing their reliance on Ca^{2+} pumps for the clearance of Ca^{2+} from the spine cytoplasm. Because clearance is slow, integrated Ca^{2+} signals in these spines are large. On the other hand, the large neck conductance of large spines makes them predominantly diffusers. Due to the combined action of diffusion and extrusion, peak Ca^{2+} amplitudes are lower in these spines despite the larger I_{NMDA}, suggesting that the specific geometry of the

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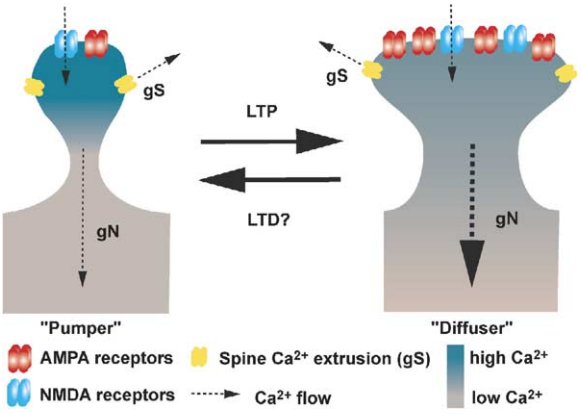

Figure 1. Ca^{2+} Compartmentalization and Dendritic Spine Geometry during Synaptic Activity

Small dendritic spines (left) have relatively larger NMDA currents than AMPA currents, which may be electrophysiologically recorded as "silent" synapses. Ca^{2+} efflux from the spine is accomplished primarily through Ca^{2+} extrusion pumps (spine head conductance [gS]) located in the spine head. These spines are predominantly "pumpers," as the spine neck is narrow and precludes Ca^{2+} diffusion into the dendrite (spine neck conductance [gN] is low). This results in large, prolonged Ca^{2+} signals in the spine and little Ca^{2+} increase in the dendritic shaft. Large dendritic spines (right), such as those observed after potentiation, have proportionally larger AMPA currents than NMDA currents. Ca^{2+} efflux from the spine head happens through two pathways: Ca^{2+} extrusion in the spine head (gS) and, due to the large radius of the spine neck, through Ca^{2+} diffusion in the dendritic shaft (gN). In these spines, Ca^{2+} increases are more moderate and transient, while dendritic Ca^{2+} concentrations are observed to change at the spine base.

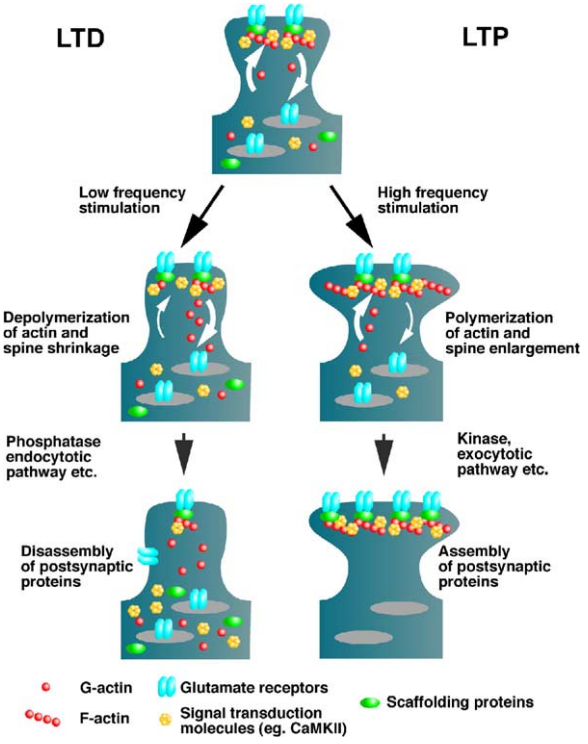

Figure 2. Rapid and Bidirectional Structural Plasticity of the Dendritic Spine

A short burst of stimulation, typically inducing LTP, shifts the equilibrium of F-actin/G-actin toward F-actin (right leg). The increased amount of postsynaptic F-actin enlarges the postsynaptic spine and provides a binding site for other proteins. For some proteins, this is sufficient as an activity-dependent delivery mechanism to the postsynapse. For other proteins, synergistic activation of other mechanisms, such as phosphorylation or other posttranslational modifications, are necessary for postsynaptic delivery. In contrast, prolonged low-frequency stimulation, typically inducing LTD, shifts the F-actin/G-actin equilibrium toward G-actin (left leg). This reduces postsynaptic F-actin and, hence, other F-actin binding proteins, resulting in disassembly of the postsynaptic protein complex and a shrinkage of the dendritic spine. This will eventually disrupt anchorage of surface glutamate receptors, leading to loss of synaptic receptors. Other mechanisms, such as dephosphorylation or endocytosis, are likely involved as well.

spine neck allows spines to tune electrical and Ca^{2+} handling properties independently. Although more experiments will be needed to determine the relative contributions of Ca^{2+} pumps and spine geometry to Ca^{2+} signaling in spines, these latest experiments go a long way toward resolving the current controversy, showing that different spines have different strategies for regulating Ca^{2+} efflux from the synapse.

Rapid Structural Plasticity of Dendritic Spine Geometry

Time-lapse observation of dendritic spines reveals constant modification of spine morphology on various time scales. Given such dynamic nature of dendritic spines, it is of particular interest to understand how dendritic spines are regulated by synaptic plasticity (Figure 2). Earlier studies showed the generation of new dendritic spines by local tetanic stimulation. In addition to this, another site of plasticity could be the geometry of existing dendritic spines. A pioneering electron microscopic study by Fifková et al. found swelling of spine heads and shortening of stalks following tetanic stimulation (Fifková and Morales, 1992). This view was further elaborated by several recent imaging studies that reported rapid structural plasticity of dendritic spines.

Using glutamate uncaging to induce LTP in single, identified dendritic spines, Matsuzaki et al. (2004) observed a rapid expansion of dendritic spines, beginning within 20 s of initiation of stimulation and peaking at around 60 s. A similar observation was made using synaptically stimulated spines following tetanic stimulation, although at a lower observed efficiency likely reflecting the difficulty in slice preparation of stimulating a presynaptic fiber innervating a given dendritic spine (Matsuzaki et al., 2004; Okamoto et al., 2004). The change in spine size was persistent, with enlargement lasting for at least an hour. Pharmacological tests indicated that CaMKII activation by Ca^{2+} influx through the NMDA receptor was necessary for the persistent phase (>5 min) of enlargement, but not for the rapid phase (<5 min). Neither of these groups saw the generation of new spines. One possible reason why the Kasai group did not see new spine generation may be that they focused their stimulation on single, existing dendritic spines, which may not be sufficient to induce the large Ca^{2+} transients that lead to spine formation.

In contrast to these two studies, performed in orga-

notypic slice culture prepared from relatively young animals (P6–P8), a study performed in adult hippocampal slice has shown that tetanic stimulation can induce only transient enlargement of spines (Lang et al., 2004). This may represent a different type of structural plasticity underlying LTP that predominantly takes place in mature neurons. The three types of structural plasticity—(1) generation of a new protrusion; (2) persistent expansion of existing spines; and (3) transient expansion of existing spines may take place at different stages of development. The spatial and temporal pattern of inputs, as well as the geometry, local density, and biochemical composition, of dendritic spines may determine which type of plasticity the spine can undergo. In fact, Matsuzaki et al. (2004) point out that spine geometry is at least one determinant. They found that small spines are persistently enlarged by stimulation, while large spines enlarged immediately after stimulation, but soon returned to their original size.

In contrast, studies in which neurons were given an LTD-inducing stimulus show a persistent reduction in dendritic spine size (Okamoto et al., 2004; Zhou et al., 2004). In some cases, LTD led to a complete loss of the spine, a result confirmed by an independent report that showed a reduction in the number of dendritic spines after LTD induction (Nagerl et al., 2004). Interestingly, Zhou et al. could see the reversal of spine shrinkage with the application of high-frequency stimulation. Okamoto et al. showed that the expansion/shrinkage is dependent on input frequency. A 10 Hz stimulation, which typically shows neither potentiation nor depotentiation in synaptic current, also did not change the spine size, except for transient expansion. These results indicate that dendritic spine size reliably follows synaptic potentiation and depotentiation and furthermore that spine size may represent the temporal summation of the synapse's plasticity history.

Experience-Dependent Plasticity and Spines In Vivo

Several research groups investigated whether structural plasticity of dendritic spines is inducible in vivo. The Svoboda group found that in the somatosensory cortex the proportion of filopodial structures devoid of a typical head is relatively high during the critical period, but decreases rapidly as the animal passes this period (Lendvai et al., 2000). The structure of the dendritic protrusions, both filopodia and mature spines, is motile and undergoes constant remodeling. Importantly, this motility also decreases during development. Sensory deprivation decreased the motility while the overall structural classification of spines/filopodia did not change (Lendvai et al., 2000). Majewska and Sur (2003) found in the binocular region of visual cortex during and after the critical period that spine motility is high during the critical period and is then downregulated toward the end of the period, while the structural classification of protrusions remains unchanged. Unlike in the somatosensory cortex, visual deprivation increased spine motility specifically during the critical period, but not before or after. The reason for this discrepancy is unclear, but may be due to differences in cortical regions or cell type involved in these studies. However, both studies agreed that the motility of dendritic spines can be regulated by sensory input.

Observation of the same dendritic segments in adult animals over days or months revealed that the majority of dendritic spines can last throughout the entire observation period, while others may appear or disappear (Grutzendler et al., 2002; Holtmaat et al., 2005; Trachtenberg et al., 2002; Zuo et al., 2005). In general, the fraction of filopodial structures and mature spines decrease and increase, respectively, with age, and concomitantly the elimination/addition of spines decreases. There is, however, an important discrepancy between these studies again. While the Svoboda group observed a relatively high number of transient spines in somatosensory cortex (~40% turnover per week), the Gan group reported a much lower number (~4% turnover per month) in visual cortex (Grutzendler et al., 2002; Trachtenberg et al., 2002). Regional differences between cortical areas seem to be one source of variability (Holtmaat et al., 2005). Although some discrepancies still exist, a majority of dendritic spines in different adult cortical regions appear to last over many months, while others are generated or eliminated. This is important in view of the different types of structural plasticity observed in vitro; the change in number of dendritic spines is one of the brain's rewiring mechanisms. Do changes in the geometry of existing spines, such as expansion or shrinkage, then mediate plasticity in vivo? It is noteworthy that Zuo et al. (2005) described the fluctuation of dendritic spine size, which may represent the type of plasticity mediated by the modulation of the geometry of existing dendritic spines.

Structural Elements Involved in Rapid Dendritic Spine Plasticity

The primary cytoskeletal component in the dendritic spine is actin, which exists in equilibrium between filamentous (F)-actin and globular (G)-actin. Okamoto et al. (2004) developed a fluorescent-resonance energy transfer (FRET)-based technique to visualize F-actin/G-actin equilibrium and showed that local tetanic stimulation induced a rapid shift of the equilibrium toward F-actin, concomitant to spine head expansion. The analysis of faster time-lapse images revealed the following sequence of events. (1) Shift of the actin equilibrium toward F-actin. (2) Accumulation of actin itself in the spine head. (3) Expansion of dendritic spines. This sequence of events indicates that a shift of the actin equilibrium triggers actin accumulation and the subsequent expansion of the dendritic spine. On the other hand, this FRET system revealed that LTD induction led to a persistent shift of the actin equilibrium toward G-actin. This result is consistent with the finding of Zhou et al. (2004), showing that cofilin, an actin depolymerization factor, mediates the decrease in spine size.

Recent studies suggest that the extracellular matrix (ECM) may play an important role in mediating spine dynamics and synaptic plasticity and that developmental changes in the ECM may be a contributing factor to the decline of plasticity in adult animals. Two recent papers investigated whether the extracellular matrix could be a site of regulation of dendritic spines by neuronal activity (Mataga et al., 2004; Oray et al., 2004). It has been reported that pharmacological blockade or knockout of tissue plasminogen activator (tPA), an extracellular protease that degrades a wide spectrum of extracellular matrix proteins, blocks visual cortical plasticity. These studies show that tPA activity in

fact modulates the geometry, dynamics, and number of dendritic spines in young animals. Such extracellular and intracellular mechanisms are likely to work in concert to orchestrate the dynamic regulation of spine geometry.

Functional Significance of the Structural Plasticity

It is still an open question whether this structural plasticity is in fact necessary for functional synaptic plasticity. As a spine is potentiated, it is likely to accommodate stronger electrical signaling and a larger complement of postsynaptic proteins, including synaptic glutamate receptors (Hayashi et al., 2000). However, there is only limited evidence to directly connect structural and functional plasticity. The Kasai group found that spine expansion is accompanied by enhanced glutamate sensitivity, while sensitivity in adjacent spines is unchanged (Matsuzaki et al., 2004). A spine-by-spine plot of expansion and enhancement of synaptic transmission shows a positive correlation.

One might still argue that these observations do not rule out that these are simply two independent, mechanistically unrelated phenomena that take place in the same spine. A more mechanistic correlation, but one less specific to plasticity, is obtained from pharmacological work using reagents that target the actin cytoskeleton. A depolymerization of actin causes shrinkage of the spine and a concomitant reduction of synaptic transmission. Under such conditions, both LTP and LTD are blocked, indicating that a functional actin equilibrium is important for both types of synaptic plasticity. However, although actin-polymerizing reagents can enlarge dendritic spines and even deliver some postsynaptic proteins to the spine head (Okamoto et al., 2004), they cannot alone enhance synaptic transmission. Therefore, actin-mediated expansion is not sufficient to induce LTP by itself. An increased amount of F-actin and postsynaptic surface area may provide additional scaffolding capacity at the postsynapse, but in order to potentiate synaptic transmission, other mechanisms, such as persistent activation of kinases and delivery of receptor molecules, need to work together.

Given that spine geometry influences spine calcium dynamics, it is also possible that spine morphology is coregulated with synaptic transmission to maintain an appropriate level of calcium signaling. Noguchi et al. (2005) show that, following potentiation, not only does the spine head volume increase, but morphological changes in the spine neck allow faster diffusion between the spine and dendrite, therefore limiting the peak Ca^{2+} accumulation reached following NMDA stimulation. These structural changes, and the resultant change in Ca^{2+} handling at the single spine level, could have a profound effect on synaptic function. In fact, changes in Ca^{2+} efflux pathways have been shown to affect a dendritic spine's ability to undergo LTD (Holthoff et al., 2002). This effect is dependent on initial spine geometry, the spine's classification as a "pumper" or "diffuser," and its location within the dendritic tree. Therefore the coordinated changes in spine morphology following potentiation may act as an internal control mechanism that prohibits spines from getting infinitely larger (Figure 1). This logically explains the finding by Matsuzaki et al. (2004) that larger spines are relatively resistant to expansion. Thus, spine geometry itself acts as an internal feedback mechanism to prevent the spine's infinite growth.

In summary, recent experiments have shown that spine geometry is exquisitely tuned by synaptic activity and regulates the spine's function as a biochemical compartment. Although there are many details that still need to be explored, it is becoming increasingly clear that these tiny synaptic compartments are highly complex structures that are morphologically and biochemically dynamic.

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