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Calcium regulation of actin dynamics in dendritic spines

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Abstract

Most excitatory synapses in the brain are made on spines, small protrusions from dendrites that exist in many different shapes and sizes. Spines are highly motile, a process that reflects rapid rearrangements of the actin cytoskeleton inside the spine, and can also change shape and size over longer timescales. These different forms of morphological plasticity are regulated in an activity-dependent way, involving calcium influx through glutamate receptors and voltage-gated calcium channels. Many proteins regulating the turnover of filamentous actin (F-actin) are calcium-dependent and might transduce intracellular calcium levels into spine shape changes. On the other hand, the morphology of a spine might affect the function of the synapse residing on it. In particular, the induction of synaptic plasticity is known to require large elevations in the postsynaptic calcium concentration, which depend on the ability of the spine to compartmentalize calcium. Since the actin cytoskeleton is also known to anchor postsynaptic glutamate receptors, changes in the actin polymerization state have the potential to influence synaptic function in a number of ways. Here we review the most prominent types of changes in spine morphology in hippocampal pyramidal cells with regard to their calcium-dependence and discuss their potential impact on synaptic function.

Keywords: Calcium; AMPA; Dendritic spine; Synaptic plasticity; Motility

1. Introduction

1.1. Calcium and dendritic spine morphology—a two-way relationship?

Dendritic spines, which form the postsynaptic element at the majority of excitatory synapses in the brain, have a specialized cytoskeleton of dynamic actin filaments capable of producing rapid changes in their shape [1–3]. Spines also function as biochemical compartments that regulate the duration and spread of postsynaptic Ca^{2+} fluxes produced by glutamatergic neurotransmission at these synapses [4,5]. Live cell imaging studies have suggested a reciprocal influence of actin filaments and Ca^{2+} on one another's function, in which spine shape influences the course of postsynaptic Ca^{2+} fluxes [6] and, conversely, changes in Ca^{2+} concentration in the spine cytoplasm ($[Ca^{2+}]_i$) alter the arrangement and dynamics of the spine actin cytoskeleton leading to changes in spine morphology and stability [7,8].

Despite these advances, important questions remain regarding the character and the physiological relevance of both phenomena. Methodological constraints complicate the interpretation of imaging studies in which high-affinity dyes are used to track Ca^{2+} distribution [9]. Similarly, it is becoming increasingly clear that Ca^{2+} fluxes from different sources can induce diverse changes in spine morphology that in some cases are difficult to reconcile or even appear contradictory. Here we discuss these phenomena and consider their likely relationships in regulating synaptic function.

1.2. Calcium regulates various forms of actin-based spine motility

Under resting conditions dendritic spines on cultured hippocampal neurons undergo rapid, fine-scale shape changes. These are readily visible in time-lapse recordings of cells expressing either GFP-actin to label the spine cytoskeleton [7], or membrane-tagged GFP to label the cell surface [8,10], or soluble GFP as a volume marker [11]. This rapid motility has been referred to as "dancing" or "morphing" [2,12] but in character it most closely resembles the actin-based ruffling

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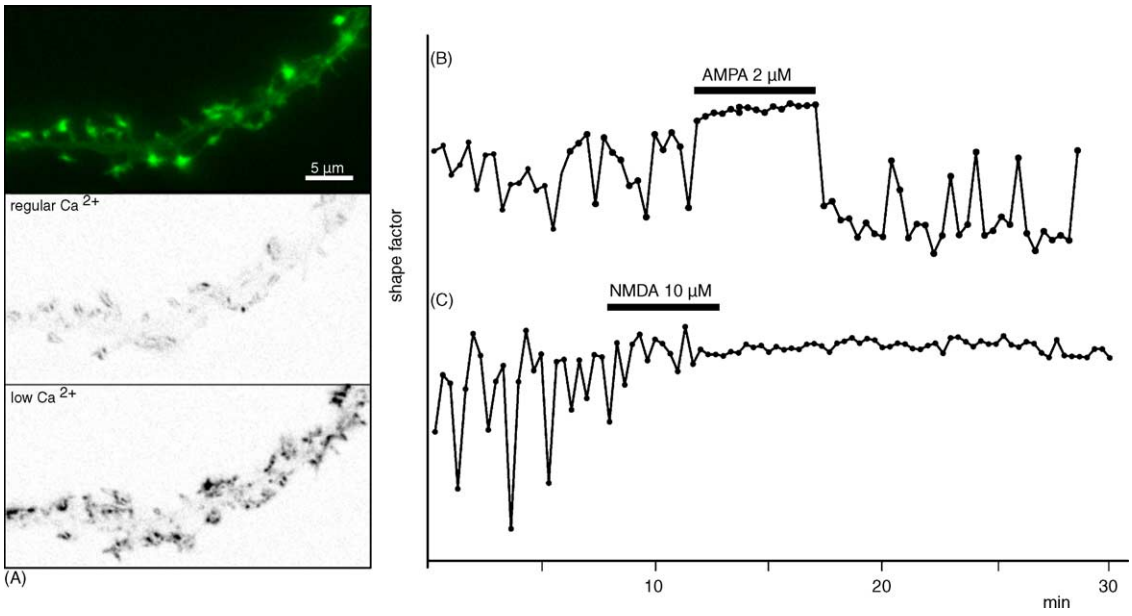

Fig. 1. Calcium influences on actin dynamics in dendritic spines. (A) When $[Ca^{2+}]_o$ is removed from the extracellular medium actin motility increases. The top panel shows a single frame from a time-lapse recording of GFP-actin in a dendritic segment of a hippocampal neuron in cell culture. Actin dynamics were assessed by summing pixel intensity differences between frames (lower panels). Dark patches show that actin dynamics were highest in dendritic spines and increased when Ca^{2+} was removed from the medium. (B and C) Shape factor analysis shows differing effects of glutamate receptor subtypes on actin dynamics. Activating AMPA receptors produces rapid and reversible blockade of spine motility (B) whereas stimulating NMDA receptors leads to long-lasting stabilization of spine morphology (C). For details of methods see [18].

motion of lamellipodia typical of actin-based motility in all cell types [10,13]. This interpretation is consistent with evidence that spine shape is regulated by the small GTPase Rac, which is the major regulator of lamellipodial activity in motile cells [14,15]. When Ca^{2+} is removed from the medium, spine motility increases (Fig. 1A) implicating Ca^{2+} in the regulation of spine motility.

The involvement of dynamic actin filaments in this ruffling activity has been visualized directly in time-lapse recordings of cultured neurons expressing GFP–actin [7]. The presence of dynamic actin filaments in dendritic spines has also been demonstrated by fluorescence recovery after photobleaching (FRAP) of GFP-actin in the spine cytoplasm, revealing that up to 85% of spine actin turns over rapidly under resting conditions [16]. The dependence of spine motility on actin filament dynamics is confirmed by the effects of drugs such as cytochalasin D and latrunculin that interfere with actin polymerization and also block ruffling activity on spine heads [7,11].

1.3. Suppression of spine motility by glutamate receptor activation

This rapid form of spine motility is modulated by two mechanisms operating through different types of receptor for glutamate, the excitatory neurotransmitter at spine synapses. Stimulating receptors of the (*S*)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtype, produced by exposing cultured neurons to glutamate or AMPA itself, produces a rapid blockade of spine motility so that in time-lapse recordings spines appear to "freeze" immediately [8,17] (Fig. 1B). This is associated with a rounding up of spine shape that appears to result from the retraction of actin-rich protrusions from the spine head [17]. This effect is transient so that motility immediately resumes when AMPA or glutamate is washed out (Fig. 1B).

These events require Ca^{2+} in the external medium and depend on low-voltage activated calcium channels [8,17,18]. Consistent with a voltage-activated channel mechanism, this AMPA receptor-induced suppression of spine motility can be replicated by K^+-induced depolarization of the neuronal membrane. Rapid contraction of spines in response to action potentials back-propagating into the dendrite may represent a similar phenomenon [19]. Thus, depolarization of the postsynaptic membrane can modulate spine actin dynamics with accompanying transient suppression of motility and microstructural changes in spine shape.

Influx of Ca^{2+} via postsynaptic *N*-methy-D-aspartic acid (NMDA) type glutamate receptors represents another mechanism for blocking dendritic spine motility [20] (Fig. 1C). This stabilization of spine morphology is long lasting: even 12 h after withdrawal of the stimulus spines are still immotile [20]. These effects are mediated by the influx of Ca^{2+} through the NMDA receptor and show a dose-dependent delay so that the time required for blockade of motility decreases with increasing concentration of agonist [18]. These observations suggest that suppression of actin dynamics in dendritic spines requires that $[Ca^{2+}]_i$ increases beyond a particular threshold

and that, depending on other factors such as the source of Ca^{2+}, the result may be transient or long-lasting stabilization of spine morphology (Fig. 1B and C).

Another form of structural plasticity in dendritic spines is characterized by a stimulation-induced increase in spine volume [21]. A recent study using repetitive stimulation of individual spines by local uncaging of glutamate reports that this swelling depends on the activation of NMDA receptors and calmodulin and is antagonized by the actin polymerization blocker lactrunculin A, implicating a Ca^{2+}-triggered calmodulin-dependent actin rearrangement [22]. The most parsimonious explanation for these events is a stimulus-dependent increase in the rate of actin polymerization, however other explanations, such as detachment of the membrane from the actin cytoskeleton coupled with insertion of new membrane by exocytosis, are also possible. Interestingly, a subset of stimulated spines shows long-duration volume increase (>1 h) that requires activation of Ca^{2+}/calmodulin-dependent protein kinase II. This long-lasting volume increase is correlated with an increase in AMPA currents, providing direct evidence for rapid morphological changes in existing spines following long-term potentiation (LTP) induction.

1.4. Calcium-induced changes in spine number

Over longer time scales both growth and atrophy of spines has been observed under different experimental circumstances. NMDA receptor-dependent processes associated with long-term potentiation, in which postsynaptic Ca^{2+} fluxes play an essential role [23], have been reported to induce the growth of new spines or filopodial-like spine precursors [24,25]. Alternatively, LTP-inducing stimuli have been associated with the formation of “multiple spine boutons”, synapses in which one presynaptic terminal is opposed to more than one spine [26]. Evidence was presented indicating that this phenomenon is accompanied by the accumulation of Ca^{2+} in the spine cytoplasm.

Regressive changes in spine structure involving atrophy and eventual spine loss have been observed to occur over a period of days following blockade of synaptic transmission in hippocampal slice cultures [27]. These events could be induced by blocking postsynaptic AMPA receptors but not by blocking neuronal activity with TTX, suggesting a crucial role for miniature excitatory postsynaptic currents (mEPSCs) in the maintenance of spine structure. This phenomenon may be related to the stabilization of spine morphology by AMPA receptor-induced influx of Ca^{2+} through low voltage activated channels [17]. Thus, occasional release of glutamate, signaling the presence of a presynaptic terminal, may raise postsynaptic Ca^{2+} levels sufficiently to stabilize the actin cytoskeleton and hence spine structure.

sApplication of NMDA to cultured neurons at concentrations known to induce excitotoxic effects has been shown to produce extensive spine loss, with the disappearance of filamentous actin (F-actin), in as little as 5 min [28]. The

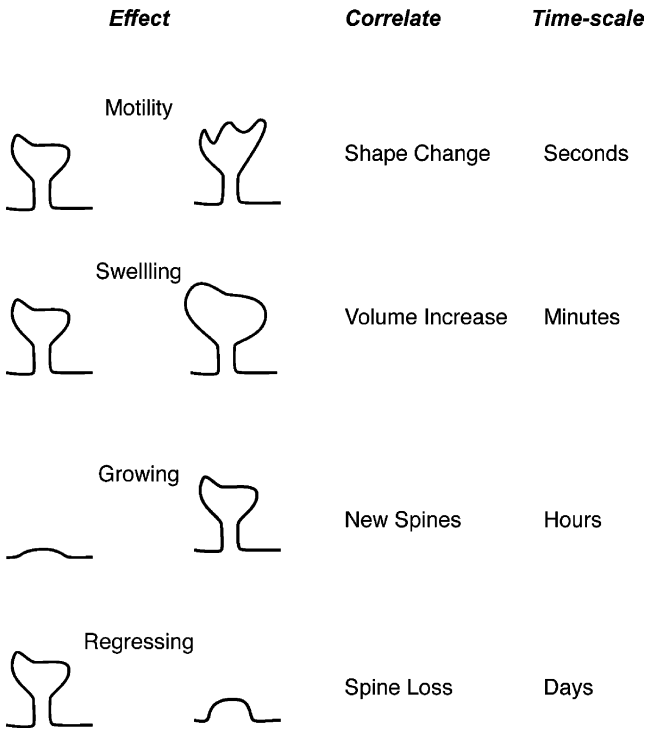

Fig. 2. Schematic representation of the various Ca^{2+}-regulated dendritic spine plasticity discussed in the text.

continued presence of other markers of synaptic structure such as synapsin and PSD-95 indicates that synapses remain intact. This sudden and dramatic spine loss depends on Ca^{2+} influx through NMDA receptors and is attenuated by drugs that stabilize filamentous actin. Antagonists of calcineurin also block the effect, suggesting an alternative mechanism by which Ca^{2+} can influence the organization of the actin cytoskeleton.

1.5. Spine motility and synaptic function

The examples listed above illustrate the effects of glutamate receptor activation on spine motility and morphology (Fig. 2). Should we view these effects as epiphenomena that accompany the induction of functional plasticity, or do they directly affect synaptic function? The high degree of motility in the absence of any synaptic activity seems to reflect a high level of actin turnover inside spines with local fluctuations in the equilibrium between polymerization and depolymerization. This view is supported by the photobleaching experiments of Star et al. [16], which suggest that under resting conditions virtually all actin in a spine turns over within 2 min. How is it possible that a highly dynamic actin cytoskeleton ‘anchors’ neurotransmitter receptors in the postsynaptic membrane? One clue comes from experiments showing that depolymerizing the actin cytoskeleton with drugs such as latrunculin A causes loss of AMPA receptors and reduces synaptic currents [29]. This suggests that even without providing a permanent anchor,

polymerized actin close to the membrane might affect the dwell time of receptors at a synapse.

Time-lapse studies following individual AMPA receptors by single-particle tracking revealed that AMPA receptors constantly diffuse laterally in and out of the synapse (reviewed in ref. [30]). In a recent study, membrane-bound GFP was used to assess the correlation between spine motility and diffusion rates of proteins in the plasma membrane [8]. In immobile spines, membrane-bound GFP diffuses much faster into the spine, demonstrating the ability of filamentous actin to provide a diffusion barrier for membrane proteins. This new data suggests that appearances may be deceptive in the case of dendritic spines: round, static looking spines could be the ones undergoing major rearrangements of their postsynaptic glutamate receptors. This interpretation is consistent with experiments showing that if actin polymerization is inhibited pharmacologically, no stable LTP can be induced in hippocampal slices [31,32].

1.6. Changes in spine volume and neck geometry

Although both morphing of the spine head and functional changes in synaptic transmission are both highly dependent on spine actin dynamics they might yet not be causally related to each other. Volume changes of the spine head, on the other hand, will directly affect amplitude and duration of subsequent calcium transients. A two-fold increase in spine volume, for example, would dilute subsequent calcium transients by the same factor if influx and extrusion of calcium were constant. If influx and extrusion increase proportional to the membrane area, spine calcium concentration would still be reduced to ~80% due to the decreased surface/volume ratio of the enlarged spine. Generally speaking, small spines experience faster and larger amplitude calcium transients, which might facilitate the induction of plasticity.

How important is the spine neck geometry for the electrical function of a synapse? The spine neck is not expected to filter excitatory postsynaptic potentials (EPSPs) very strongly, because spine neck conductance is large compared to synaptic conductance [33]. On the other hand, modeling studies taking into account voltage-gated sodium channels in spines suggest the possibility of a 'spine spike', which would lead to a considerable boost in EPSP amplitude [34]. If these theoretical predictions are confirmed experimentally, our view of the spine as a weak passive filter would have to be revised and changes in spine neck impedance could turn a non-spiking spine into a spiking spine [35].

Numerous theoretical and experimental studies have shown that spine neck geometry influences diffusional coupling between spine and dendrite [4-6,36]. Whereas there is a general consensus that spine neck geometry has a strong impact on the diffusion of fluorescent reporter molecules, there is disagreement as to whether this means that equivalent differences in spine calcium handling occur. Dye molecules cannot leave the cell, whereas free calcium ions are rapidly extruded. Biophysical models of spine diffusion are widely used for the interpretation of calcium imaging data, but their conclusions are critically dependent on the assumed diffusion coefficient of endogenous calcium buffers, which range from small, highly diffusible molecules like ATP to essentially immobile large proteins (compare refs. [9,36]). In an unpertubed cell, the majority of calcium ions entering a spine bind to endogenous buffers and the remainder are removed very quickly (time constant ≈12 ms), leaving little time for diffusion into the dendrite [9]. It should be emphasized that even if free calcium ions are essentially confined to the spine head because of rapid extrusion, other second messengers like IP3 or calcium-activated proteins might well be able to diffuse from the active spine into the dendrite, a process that would be expected to depend strongly on spine geometry. Genetically encoded indicators reporting the activity of various second messenger systems will make these processes more accessible to experimental testing in the future.

1.7. From calcium to actin

Although it is clear that calcium influx, actin dynamics and changes in spine shape are closely linked, it is difficult to predict the morphological changes resulting from a specific stimulation. Differences in the concentration and duration of $[Ca^{2+}]_i$ produced by different patterns of stimulation is likely to be a major factor in this variation. Unfortunately technical issues make it very difficult to combine motility experiments with precise calcium measurements in single spines: the whole cell patch clamp technique commonly used to fill individual cells with calcium-sensitive dyes can suppress morphological changes [22]. This effect is presumably due to washout of soluble factors such as ATP that are required for cell motility.

Differences in down-stream molecular responses, which may vary between individual spines, are certain to play an important role. Experiments on growth cones have shown that interactions between local and global Ca^{2+} levels can profoundly influence actin-based motility, switching the direction of growth cone extension [37]. Actin regulatory proteins, many of which respond to Ca^{2+} signals [38,39] are likely to play a major role in mediating such effects. The relatively few detailed studies published to date demonstrate the potential impact of these proteins on synaptic structure and function. A striking example occurs in the FRAP study by Star et al. [16] which shows that in wild-type neurons NMDA receptor activation and Ca^{2+} influx strongly suppresses actin turnover. However, in mutant mice lacking gelsolin, a Ca^{2+}-activated protein that caps and severs actin filaments, this suppression is almost absent, implicating gelsolin as a major link between activity-dependent calcium influx and stabilization of spine morphology.

Ca^{2+} influx through NMDA receptors is also implicated in triggering the effects of another actin-binding protein, profilin, whose activation is correlated with suppression of actin dynamics and blockade of spine motility [20]. These effects could be eliminated by expressing in cells a peptide

that competes for the binding of profilin to cell surface-associated adaptor proteins of the VASP/MENA family. This suggests that a Ca^{2+}-dependent interaction between the actin cytoskeleton and surface-associated proteins, mediated by profilin, regulates spine morphology. Other actin-binding proteins associated with dendritic spines whose activity may be influenced by Ca^{2+} include spectrin [40], α-actinin [41], calponin [42].

It is likely that these various proteins may be activated by different levels and durations of intracellular Ca^{2+} signals, thus producing different responses in the spine actin cytoskeleton. Such variations may account for at least some of the different effects of Ca^{2+} on actin function in spines that currently appear so difficult to reconcile.

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