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Imaging calcium dynamics in dendritic spines

[Review article]

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Outline

- > [Abstract](#)
- > [Abbreviations](#)
- > [Introduction](#)
- > [Optical measurement technology](#)
- > [Ca²⁺ dynamics in dendritic spines](#)
 - > [Synaptically induced Ca²⁺ accumulation](#)
 - > [Spike-induced Ca²⁺ accumulation](#)
 - > [Non-linear Ca²⁺ dynamics](#)
- > [Mechanisms affecting Ca²⁺ dynamics in dendritic spines](#)
 - > [Voltage-dependent Ca²⁺ channels](#)
 - > [Ca²⁺ entry through non-NMDA-type glutamate receptors](#)
 - > [NMDA receptors on spines](#)
 - > [Ca²⁺ buffering, extrusion, and intracellular release](#)



- [Spines as electrical compartments](#)
- [Conclusions and perspective](#)
- [Acknowledgements](#)
- [References and recommended reading](#)
- [Copyright](#)

Abstract



Recent advances in optical imaging technology have enabled the measurement of Ca^{2+} dynamics in individual synaptic spines with high time resolution. Results from work using this new technology have confirmed the view that individual synaptic spines can act as functional chemical compartments with independent dynamics of second-messenger concentration. In particular, the ability of Ca^{2+} to directly mediate Hebbian coincidence detection has been confirmed.

Abbreviations

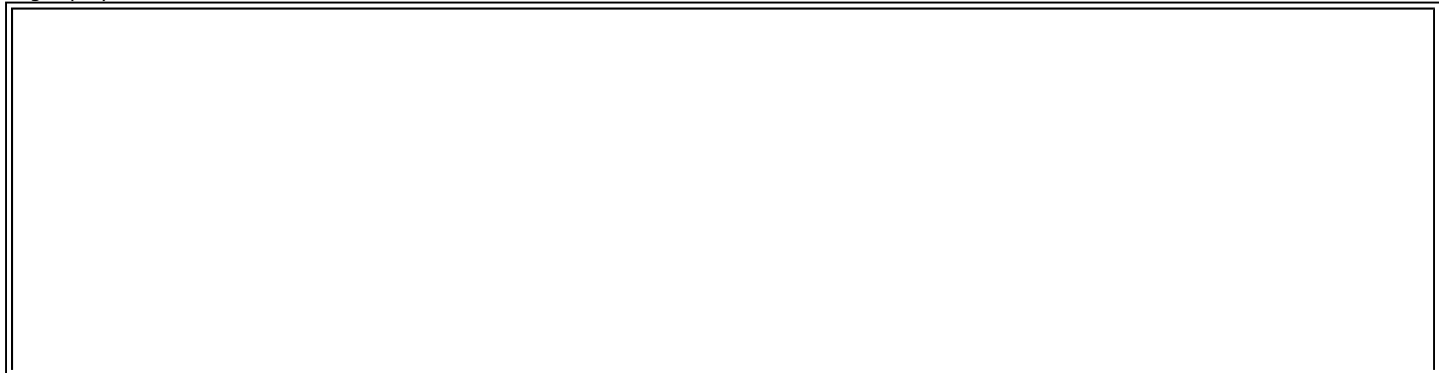


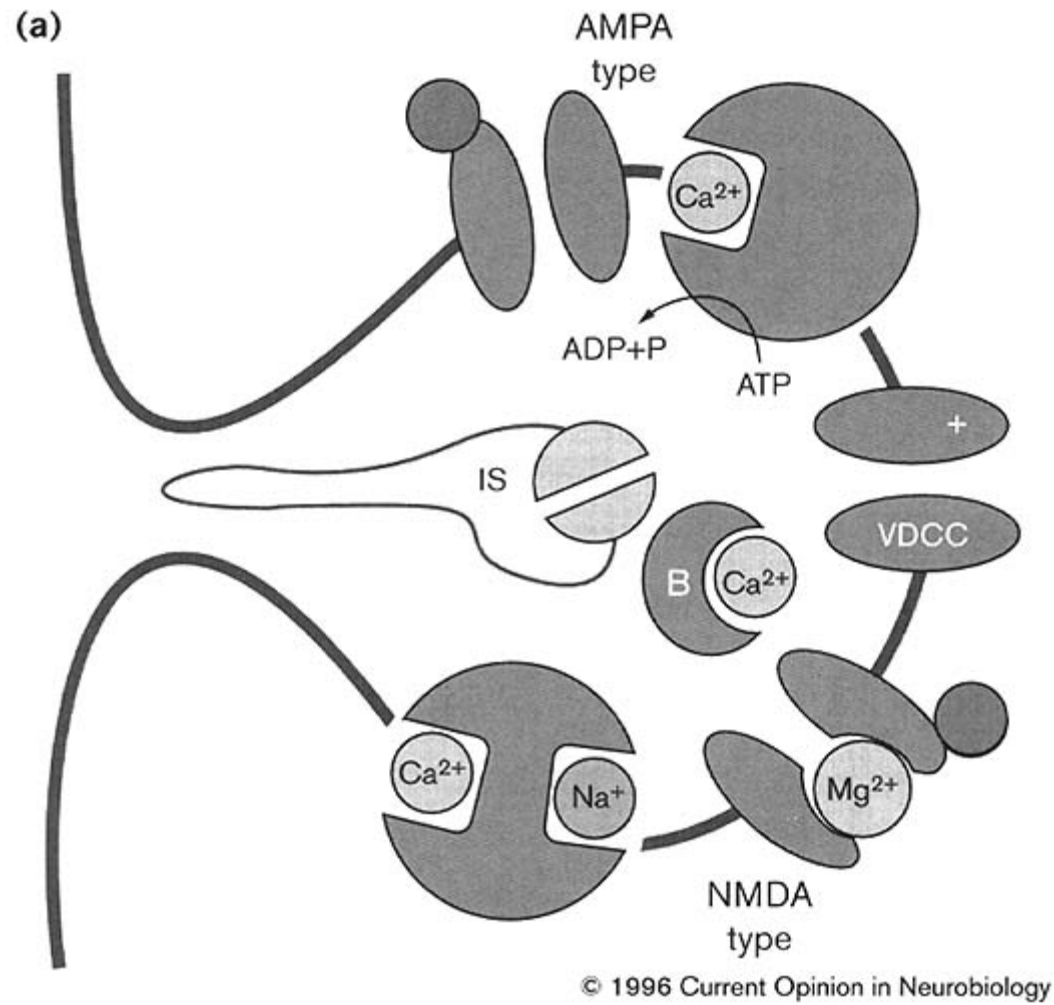
AMPA— α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid;
CCD—charge-coupled device;
EPSC—excitatory postsynaptic current;
LTD—long-term depression;
LTP—long-term potentiation;
NMDA—*N*-methyl-**D**-aspartate;
VDCC—voltage-dependent Ca^{2+} channel.

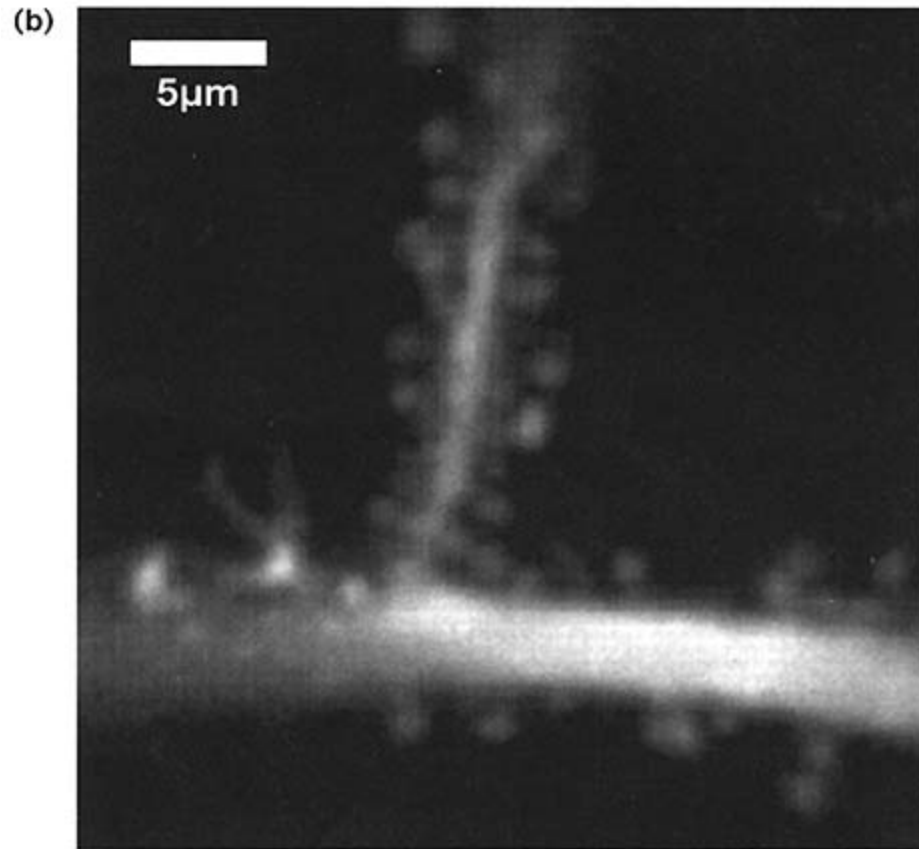
Introduction



Although their existence has been known for over 100 years [1], the function of dendritic spines and the reasons for their peculiar morphology are still not completely understood. Speculation about the function of dendritic spines (for a review see [2]) has usually focused on their resistance to electrical current [3] or to the exchange of molecules [4] through the narrow neck connecting spines to their parent shaft. Recently, an explosion of morphological (Fig. 1; for a review, see [5•]) and functional imaging (for a review, see [6]) studies have narrowed down the number of possible roles that spines might play.







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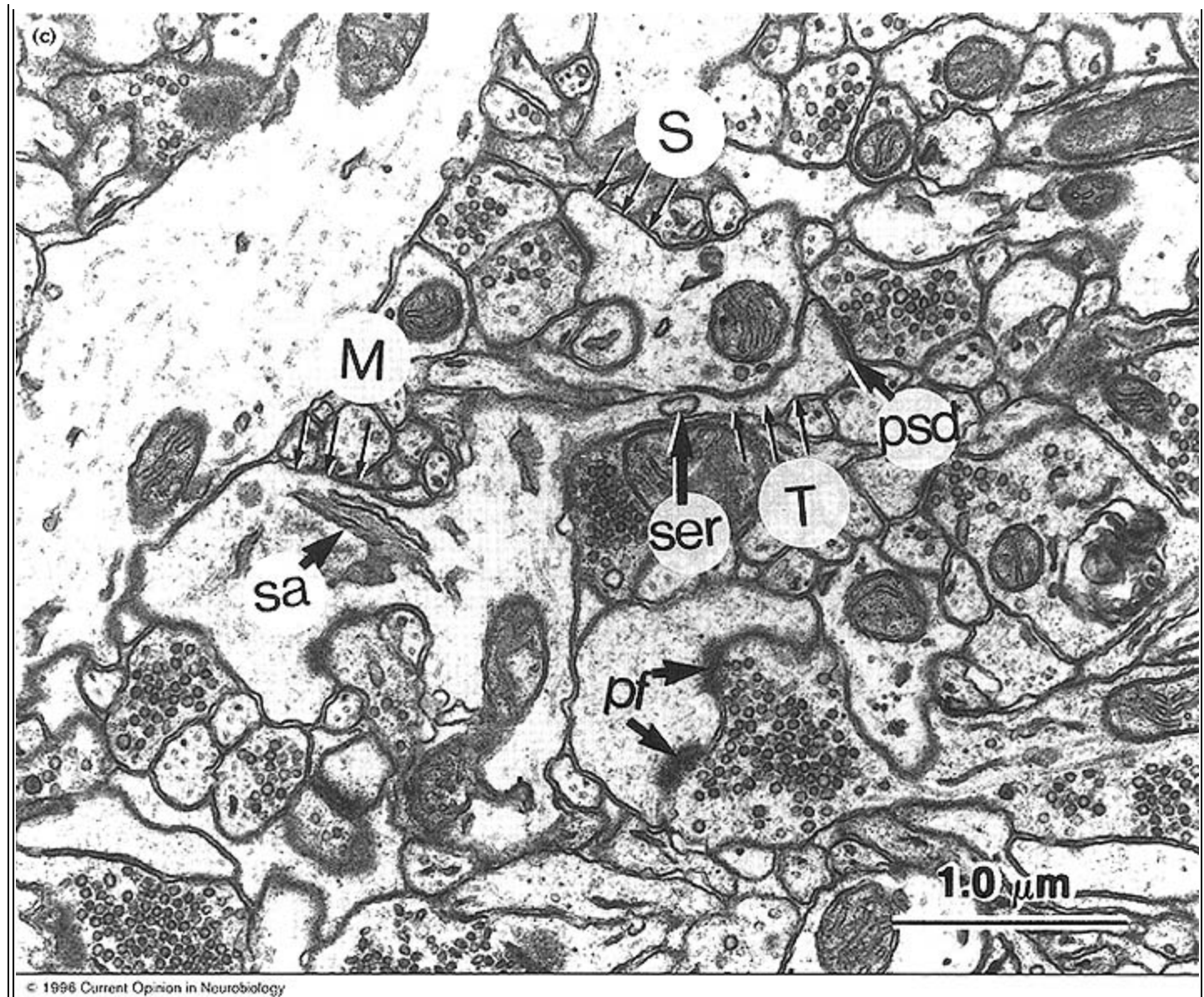


Figure 1 Synaptic spines: morphology and mechanisms. **(a)** Schematic of the mechanisms known or strongly suspected to play a significant role in Ca^{2+} dynamics in dendritic spines: including different types of glutamate-gated ion channels (AMPA type and NMDA type), voltage-gated channels selective for Ca^{2+} (VDCCs), extrusion via $\text{Na}^+/\text{Ca}^{2+}$ exchange and Ca^{2+} -ATPase-driven pumps, buffering by Ca^{2+} -binding molecules (B) and intracellular stores (IS). **(b)** Light microscope and **(c)** electron microscope images of spines show the

respective resolution of these techniques. The image in (b) was taken using a two-photon-excitation laser scanning fluorescence microscope, described in [16] [17] [19•], and shows spines around a dendritic branchpoint in a CA1 pyramidal cell (R Yuste, W Denk, unpublished data). In (c), the transmission electron micrograph (kindly provided by Dr Kristin Harris [5•]) clearly shows the presence of intracellular organelles (sa, spine apparatus; ser, smooth endoplasmic reticulum) in CA1 spines. M, mushroom-type spine; pf, perforated postsynaptic density; psd, postsynaptic density; S, stubby spine; T, thin spine.

[Return to text reference \[1\]](#)

Among the studies that have provided insight into the physiology of individual spines, the microspectrofluorimetric measurement of the intracellular free Ca^{2+} ion concentration ($[\text{Ca}^{2+}]_i$) dynamics has taken a prominent place both because of the importance of $[\text{Ca}^{2+}]_i$ in controlling intracellular biochemical processes and because $[\text{Ca}^{2+}]_i$ dynamics can be an indicator of local synaptic and electrical activation. In this review, we focus on recent advances in functional optical imaging of dendritic spines in two types of widely studied neurons, pyramidal cells in the CA1 area of hippocampus and cerebellar Purkinje cells, and we also discuss the implications of those findings for understanding the function of synaptic spines.

Optical measurement technology



The emergence of new optical measurement technology has been crucial for enabling measurement of $[\text{Ca}^{2+}]_i$ gradients and differences between structures such as dendritic spines and shafts that are separated in space by barely more than the optical resolving power. The development of fluorescent probes of Ca^{2+} activity with increased sensitivity and more suitable excitation and emission spectra (for a review, see [7]) has gone hand in hand with the development of suitable instrumentation for the excitation and sensitive quantitative detection of fluorescence. Imaging with cooled charge-coupled devices (CCDs) [8] [9] allows the spatially resolved detection of low levels of fluorescence. This is particularly important for small structures such as dendritic spines, which contain only a relatively small number of fluorescence indicator molecules (600 to 60 000, at a concentration of 100 μM , assuming volumes ranging from 0.01 to 1 femtoliters [5•]).

The confocal microscope ([10]; for an introduction, see [11]), with its optical sectioning properties, allows imaging of spines buried inside brain slices [12] [13] where light scattering prevents the acquisition of high resolution images with full field illumination and CCD detection. The confocal microscope's rejection of scattered light is a two-edged sword, however, since not only is light coming from fluorophores outside the focus rejected, but light that originates at the focus but is subsequently scattered is rejected as well. The increased number of molecular excitations required to ensure an adequate number of detected photons then leads to increased photodynamic damage and photobleaching. This has even led to the use of the laser scanning microscope in non-confocal mode, by opening up the pin-hole, in cases where signal-to-noise ratio and temporal resolution were more important than spatial resolution and optical sectioning [14].

Such a compromise can be avoided by using two-photon-excitation laser scanning microscopy [15] where resolution and optical sectioning are accomplished during excitation alone via the strongly non-linear (quadratic) dependence of the excitation rate on the local light intensity; this method eliminates out-of-focus photobleaching and photodamage, and permits the use of scattered fluorescence photons (for reviews, see [16] [17] [18]). The two-photon technique allows protracted imaging of $[\text{Ca}^{2+}]_i$ dynamics in dendritic spines and shafts deep inside brain slices, with high spatial and temporal resolution [19•] [20•].

Ca²⁺ dynamics in dendritic spines



Synaptically induced Ca²⁺ accumulation



It has been known for some time that synaptic activation leads to Ca²⁺ influx into dendrites of mammalian CA1 pyramidal cells [21]. Subsequently, it was shown that [Ca²⁺]_i increases in spines [22], that such [Ca²⁺]_i increases can be confined to individual spines [19•] [23], and that [Ca²⁺]_i can rise to more than 10 μM [24•]. This view of the spine as a chemical compartment was also supported by the observation of irradiation-induced [Ca²⁺]_i gradients [25]. Spontaneous Ca²⁺ accumulations observed in cultured cells seem to suggest, however, that whole sections of dendrite containing 10 or more spines act as the basic integrative unit, at least as far as the Ca²⁺ dynamics is concerned [26•]. More recently, a correlation between these spontaneous Ca²⁺ responses and somatically detected excitatory postsynaptic currents (EPSCs) was demonstrated [27•].

During synaptic activation, [Ca²⁺]_i rises quickly (< 2 ms) in spine heads and dendritic shafts of CA1 cells [19•], which implies that Ca²⁺ influx occurs directly into the spine, presumably through ion channels on the spine head. Parallel fiber synaptic input produces a hierarchy of activation in Purkinje cells, from single spines [20•], to branchlets [28•], to substantial sections of the dendritic tree [29].

The measurement of [Ca²⁺]_i in individual spines allows the observation of what very likely is the activation of a single synaptic contact. The frequency of instances during which [Ca²⁺]_i fails to rise in response to single presynaptic shocks [19•] is in agreement with estimates of synaptic reliability made using minimal stimulation of presynaptic fibers combined with detection of EPSCs using a patch-clamp electrode at the soma (for reviews, see [30] [31]; see also [32] [33]).

The ability to unambiguously detect activity in a single synaptic contact by optical means might become a way to examine whether or not the modulation of release probabilities underlies the changes of synaptic strength that occur during long-term potentiation (LTP) and long-term depression (LTD), and thereby resolve a long-standing controversy [32] [33] [34] [35] [36].

Spike-induced Ca²⁺ accumulation



A number of experiments over the past decade have shown that somatically triggered Na⁺ action potentials lead to Ca²⁺ accumulation in CA1 dendrites [21] [37]: under these conditions, Ca²⁺ has been shown to rise in shafts as well as in spines [12] [13] [19•] [38•], and it has been suggested that the Ca²⁺ concentrations in spines exceed those in the shaft [13] [38•]. Experiments with higher time resolution show that [Ca²⁺]_i in spines and shafts rises within 2 ms of action potential initiation [19•], making a strong case that Ca²⁺ influx does occur through the spine-head membrane.

Non-linear Ca²⁺ dynamics



How various forms of neural activity interact via the Ca^{2+} dynamics in spines and dendrites is crucial for understanding the role Ca^{2+} plays during information processing in neurons. The properties of the NMDA receptor, which is blocked at hyperpolarized potentials by extracellular Mg^{2+} [39] and is highly permeable for Ca^{2+} [40], have led to the prediction that a particularly large Ca^{2+} influx should occur during simultaneous pre- and postsynaptic activity. Measurements of Ca^{2+} in spines have shown that the combination of postsynaptic action potentials with synaptic stimulation leads to Ca^{2+} accumulations that can exceed by far the sum of the influxes observed with either stimulation mode alone [19•]. Such supralinearity was restricted to the activated spine and could therefore contribute to 'Hebbian' input specificity of synaptic plasticity since it is well known that Ca^{2+} serves as a signal for the induction of LTP or LTD [41] [42] [43]. The dependence of the supralinearity on the exact stimulus conditions and its detailed pharmacology remains to be determined. Of particular interest is the issue of whether or not the $[\text{Ca}^{2+}]_i$ levels reached under particular stimulating conditions are sufficient to predict the type and extent of synaptic plasticity that is induced with such stimuli.

Mechanisms affecting Ca^{2+} dynamics in dendritic spines



Voltage-dependent Ca^{2+} channels



Initial evidence that voltage-dependent Ca^{2+} channels (VDCCs) are located on spine heads came from measurements showing that $[\text{Ca}^{2+}]_i$ levels seen in spines during depolarization often exceed the levels reached in the shafts bearing these spines [13]. The time resolution of those measurements did not, however, allow the definitive elimination of slower processes, such as Ca^{2+} release from intracellular stores, which has been suggested to contribute to Ca^{2+} accumulations in dendrites [12] [44]. The more recent results in CA1 pyramidal cells showing that $[\text{Ca}^{2+}]_i$ rises with a delay of less than 2 ms in both spines and shafts during retrograde action potentials [19•] leave little doubt that in these cells there are VDCCs on spine heads as well as on dendritic shafts.

Ca^{2+} entry through non-NMDA-type glutamate receptors



The Ca^{2+} permeability of ion channels controlled by non-NMDA-type glutamate receptors is dependent on the protein subunit composition and varies widely [45]. This has been shown, for example, by measuring changes in the reversal potential of glutamate-mediated EPSCs as a function of Ca^{2+} ion concentration [46] [47]. Alternatively, fluorescent indicators were used to measure Ca^{2+} flux through channels expressed in HEK 293 cells [48] or those natively present in forebrain neurons [49] or Purkinje cells [50•].

In Purkinje cells, a rather low Ca^{2+} permeability was estimated; however, this estimate was derived from measurements averaged over a large numbers of spines [50•]. Direct measurements of Ca^{2+} influx into individual spines of Purkinje neurons [20•] show two classes of parallel-fiber-innervated spines, one of which appears to possess Ca^{2+} -permeable, AMPA-type channels. Therefore, it is quite clear that Ca^{2+} permeability of AMPA-type receptors affects the Ca^{2+} dynamics in spines, but it remains to be seen whether the variations in Ca^{2+} permeabilities are functionally significant.

A related issue is whether these apparent heterogeneities in the distribution of ion channels are

actively generated and maintained by the cell [51], or whether the presence of a low concentration of highly Ca^{2+} permeable channels (less than 1 per spine on average) randomly endows a subset of spines with a single Ca^{2+} -permeable AMPA channel each. In hair cell stereocilia (which are of comparable volume to dendritic spines) [52], the influx of Ca^{2+} is sufficient to raise the $[\text{Ca}^{2+}]_i$ to almost micromolar levels [20•].

NMDA receptors on spines



The pathway of Ca^{2+} entry into hippocampal CA1 cell dendrites during synaptic stimulation has proven to be somewhat elusive since the NMDA receptor, in the presence of glutamate and extracellular Mg^{2+} , behaves quite similar to a VDCC. The most convincing evidence for Ca^{2+} influx through NMDA receptors on dendrites of CA1 cells comes from work showing that $[\text{Ca}^{2+}]_i$ rises in response to synaptic stimulation, even when the cell is depolarized beyond the reversal potential of the synaptic conductance [53] [54•]. Some evidence that Ca^{2+} influx occurs through NMDA receptors directly into spines is provided by the observation that NMDA application raises $[\text{Ca}^{2+}]_i$ in spines to levels higher than in the shaft [38•]. As in the case of VDCCs, more convincing evidence would be to observe Ca^{2+} into single spines with a temporal resolution fast enough to rule out the possibility of Ca^{2+} diffusion from the shaft and secondary release from intracellular stores.

Ca^{2+} buffering, extrusion, and intracellular release



It has been recognized for quite some time ([4] [55]; for reviews, see [2] [56]) that in addition to the obvious importance of influx through membrane channels, the dynamics of $[\text{Ca}^{2+}]_i$ is strongly dependent on intracellular binding, sequestration into organelles, extrusion across the plasma membrane, and diffusion through the spine neck [57]. Since only a small fraction (between 10^{-2} and 10^{-3}) of the total Ca^{2+} exists as free ions [55] [58], the prevailing buffer can dominate Ca^{2+} transport properties [59] [60] and peak levels reached [49] [55]. If the endogenous buffer capacity in spines is comparable to that found in chromaffin cells [55], then a high-affinity indicator, such as Calcium-green-1 or Fura-2, if present at a concentration of 100 μM , could well dominate Ca^{2+} buffering and hence mobility at resting $[\text{Ca}^{2+}]_i$ levels.

Measurements using the low-affinity Ca^{2+} indicator fura-2, which has a much smaller effect on cytoplasmic Ca^{2+} buffering, suggest that $[\text{Ca}^{2+}]_i$ in CA1 dendritic spines can reach 50 μM during repeated synaptic stimulation combined with steady state depolarization, albeit under conditions where K^+ channels are blocked by intracellular Cs^+ . The uncertainty of concentration and mobility of endogenous Ca^{2+} buffers makes observed decay rates for Ca^{2+} -dependent fluorescence a very imprecise measure of the true value of $[\text{Ca}^{2+}]_i$ decay. The apparent decay might be sped up by the presence of the indicator, which comprises a dominant and mobile buffer, whereas the true decay time could be as slow as several seconds if endogenous buffers were largely immobile and removal occurred solely by diffusion through the spine neck (assuming unbuffered diffusional time constants of 20–100 ms [61•]). On the other hand, if Ca^{2+} removal is dependent on extrusion across the spine-head membrane, the presence of a Ca^{2+} indicator would slow down Ca^{2+} removal.

Spines as electrical compartments



To what degree spines can sustain electrical activation independent from the rest of the dendrite has long been in dispute. This issue bears on $[Ca^{2+}]_i$ dynamics insofar as a sufficiently large depolarization of a spine with respect to the dendritic shaft could selectively activate VDCCs on that spine. This, in turn, would lead to a Ca^{2+} accumulation localized to that spine. The degree of differential depolarization depends strongly on the electrical resistance of the spine neck, which cannot be measured directly. Estimates of spine neck resistance have been made, however, using the intracellular medium's resistivity [62] [63] in conjunction with the spine geometry [5•], and more recently by measuring diffusional exchange between spine and shaft using two-photon fluorescent photobleaching and photorelease [61•]. Both methods yield spine neck resistances between 5 and 150 $M\Omega$. Even the largest of these values is too small to modulate substantially the flow of synaptic current, but could be sufficient to limit activation of VDCCs to the spine receiving synaptic input. Direct evidence for electrogenic events limited to single spines is that some Purkinje cell spines show isolated Ca^{2+} accumulations [20•] that can be suppressed by somatic hyperpolarization.

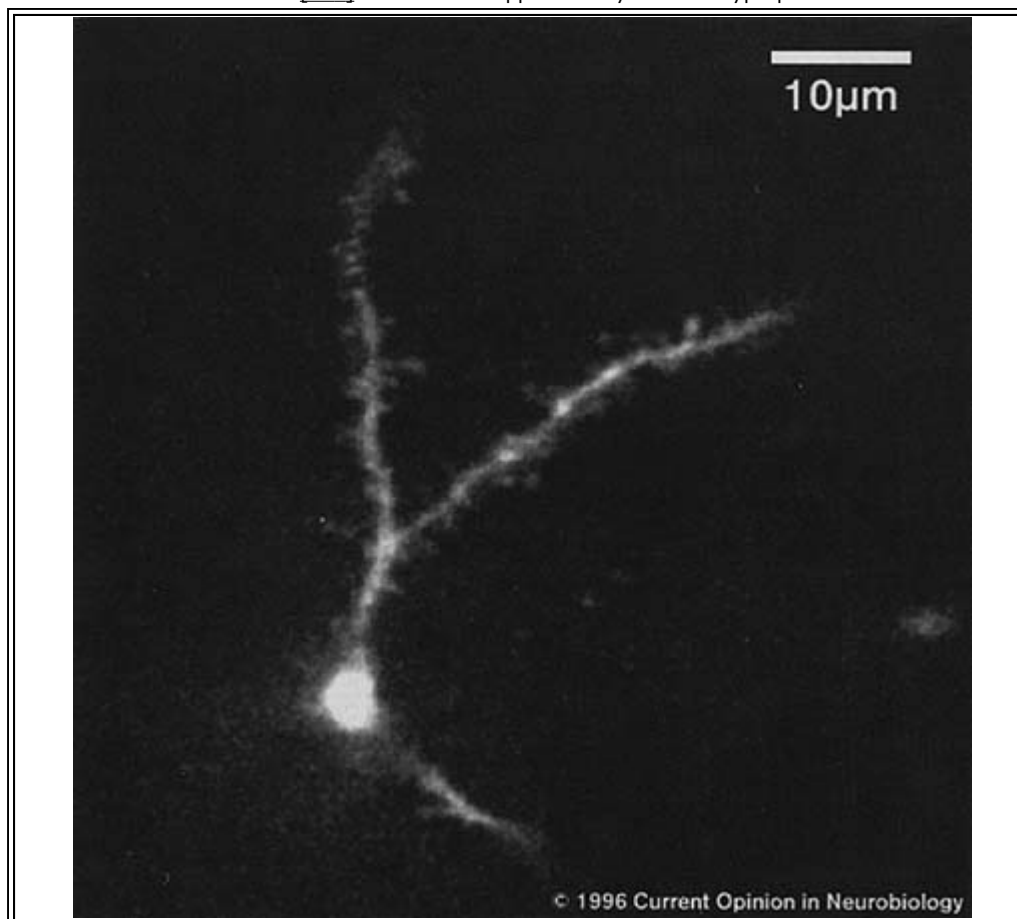


Figure 2 Two-photon-excitation fluorescence microscopy image of dendritic spines *in vivo*. A layer 2/3 pyramidal cell in rat somatosensory cortex was filled by iontophoresis with the fluorescent indicator Calcium-green-1. Methods for recording and imaging are as described in [16] [22]. The image shows a single focal slice, about 70 μ m above the soma, taken in a single sweep with 20 μ s pixel dwell time (W Denk, D Kleinfeld, DW Tank, unpublished data). The focal plane was located 250 μ m below the surface of the brain. The large white spot is a cross-section through the primary apical dendrite.

[Return to text reference \[1\]](#)

Conclusions and perspective

The view that synaptic spines serve mainly as chemical compartments is supported by an increasing number of direct measurements, consisting mostly of optical imaging studies performed with high spatial and temporal resolution. Existing imaging technology should be capable of providing a yet more quantitative description and detailed understanding of the specific mechanisms of Ca^{2+} ion flux, buffering, release, and extrusion, all of which contribute to dendritic spine Ca^{2+} dynamics. For example, quantitative measurements of $[\text{Ca}^{2+}]_i$ decay times as a function of exogenous Ca^{2+} buffer concentration should provide quantitative estimates of endogenous buffer characteristics and extrusion mechanisms, using methods previously demonstrated in cell bodies [55] and presynaptic nerve terminals [58]. Further development of optics-based failure analysis might well contribute significantly to the understanding of noise and plasticity in central nervous system synapses.

Our present understanding of spine Ca^{2+} dynamics is based upon experiments performed in brain slice and tissue culture preparations. It is likely that most of the work in the near future will continue to be performed in such preparations. An entirely new domain of experimentation may become possible, however, with the advent of techniques for high-resolution optical imaging in the intact brain. [Fig. 2](#) shows an image taken *in vivo* of the apical dendrite and associated dendritic spines of a layer 2/3 pyramidal cell, injected with Calcium-green-1, in rat somatosensory cortex. *In vivo* imaging techniques may eventually allow us to ask questions about the role that Ca^{2+} dynamics in dendritic spines plays in behaving animals.

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