Calcium Signaling in Dendritic Spines

Michael J. Higley[1] and Bernardo L. Sabatini[2]

[1]Department of Neurobiology, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale School of Medicine, New Haven, Connecticut 06520

[2]Howard Hughes Medical Institute, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

Correspondence: bsabatini@hms.harvard.edu

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Calcium (Ca^{2+}) is a ubiquitous signaling molecule that accumulates in the cytoplasm in response to diverse classes of stimuli and, in turn, regulates many aspects of cell function. In neurons, Ca^{2+} influx in response to action potentials or synaptic stimulation triggers neurotransmitter release, modulates ion channels, induces synaptic plasticity, and activates transcription. In this article, we discuss the factors that regulate Ca^{2+} signaling in mammalian neurons with a particular focus on Ca^{2+} signaling within dendritic spines. This includes consideration of the routes of entry and exit of Ca^{2+}, the cellular mechanisms that establish the temporal and spatial profile of Ca^{2+} signaling, and the biophysical criteria that determine which downstream signals are activated when Ca^{2+} accumulates in a spine. Furthermore, we also briefly discuss the technical advances that made possible the quantitative study of Ca^{2+} signaling in dendritic spines.

For many neurons in the mammalian brain, the postsynaptic terminal of an excitatory synapse is found in a specialized structure protruding from the dendritic shaft, known as a dendritic spine (Fig. 1). The structure of the classic mushroom-shaped dendritic spine, in which a bulbous head (diameter ~0.5 μm) is separated from the parent dendritic shaft by a thin neck (length ~0.5 μm, diameter ~0.1 μm), suggests that it creates an isolated signaling compartment in which the machinery necessary to read out and regulate the activity of one synapse can operate independently of that associated with a neighboring synapse. Indeed, many studies have shown that the spine neck provides a significant barrier to diffusion that allows compartmentalization of biochemical and electrical signals in the spine head (Yuste and Denk 1995; Svoboda et al. 1996; Sabatini et al. 2002; Grunditz et al. 2008; Bloodgood et al. 2009). The generalizability of these conclusions to non-mushroom spines (see Harris and Weinberg 2012), such as those with no discernible neck (stubby spines) or those with a long neck and small head (thin spines), is still unclear.

The best-studied, synaptically evoked biochemical signal that accumulates in active spines is intracellular calcium (Ca^{2+}) (for review, see Sabatini et al. 2001; Bloodgood and Sabatini 2007a; Higley and Sabatini 2008). In pyramidal neurons of the neocortex and hippocampus, synaptic activation of NMDA-type glutamate

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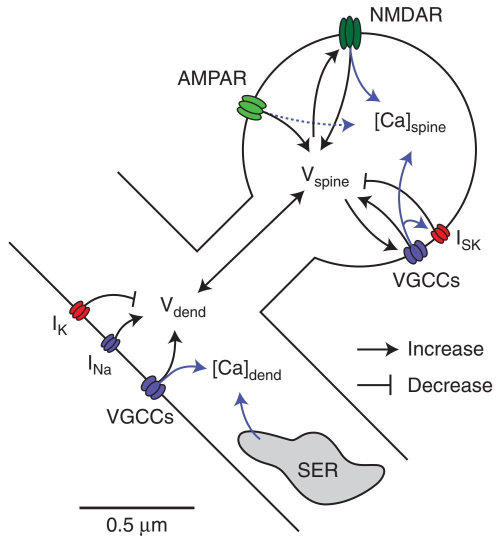

Figure 1. Sources and regulation of Ca^{2+} in dendritic spines. Synaptic stimulation produces postsynaptic depolarization via currents (black arrows) through AMPA- and NMDA-type glutamate receptors as well as various voltage-gated ion channels. Synaptic Ca^{2+} influx (blue arrows) is mediated by NMDARs and voltage-gated Ca^{2+} channels (VGCCs) and is possibly augmented by release from intracellular stores such as smooth endoplasmic reticulum (SER). The membrane potentials within the spine (V_{spine}) and dendritic shaft (V_{dend}) are electrically coupled by the spine neck. Similarly, the Ca^{2+} concentrations in the spine ($[Ca]_{spine}$ and dendritic shaft $[Ca]_{dend}$) are coupled by restricted diffusion across the neck. The presence of Ca-activated (SK) potassium channels in the spine head provides a negative-feedback loop regulating synaptic depolarization. Sodium (I_{Na}) and potassium (I_K) channels, as well as SER, are shown only in the dendritic shaft for simplicity but may also be present in the spine head. Note that for clarity the spine neck is not drawn to scale.

receptors (NMDARs) located in the postsynaptic density (PSD) leads to influx of Ca^{2+}, which accumulates in the head of the spine associated with the active synapse (Fig. 1). Additional Ca^{2+} enters through voltage-gated Ca^{2+} channels (VGCCs) or may be released from intracellular Ca^{2+} stores such as mitochondria and endoplasmic reticulum. Current carried by Ca^{2+} ions contributes to dendritic electrical signaling, producing postsynaptic depolarization. Additionally, Ca^{2+} ions in the spine head activate a wide variety of Ca-sensitive proteins—including calmodulin, Ca^{2+}/calmodulin-dependent kinase type II (CaMKII), small conductance Ca^{2+}-activated potassium channels, calcineurin, and calpain—that regulate many aspects of neuron and synapse function. Thus, the Ca^{2+} that accumulates in the spine is a central signaling molecule that regulates many aspects of synapse and cell function.

TOOLS TO STUDY Ca^{2+} SIGNALING IN DENDRITIC SPINES

The explosion in our quantitative understanding of Ca^{2+} signaling in dendritic spines has been made possible by three fundamental technical advances that are briefly discussed here (for review, see Yasuda et al. 2004). These are the development of bright, fast, and high-dynamic-range Ca^{2+}-sensitive fluorophores; the development and dissemination of two-photon laser-scanning microscopy; and the development of two-photon released caged neurotransmitters that allow direct stimulation of visualized spines (Tsien 1980; Minta et al. 1989; Denk et al. 1990; Matsuzaki et al. 2001; Carter and Sabatini 2004; Yasuda et al. 2004).

Ca^{2+} accumulation within cells and dendritic spines can be visualized through the use of Ca^{2+}-sensitive fluorescent molecules that are commonly referred to as "Ca^{2+} indicators." The current generation of these molecules typically consists of a Ca^{2+}-binding molecule, such as the common Ca^{2+} buffer 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N′*,*N′*-tetraacetic acid (BAPTA), that has been attached to a fluorophore such as fluorescein (Tsien 1980; Minta et al. 1989). Ca^{2+} binding to the buffer moiety triggers a change in the electronic properties of the fluorophore that alters its fluorescence, typically by altering its quantum yield or absorption cross section (Kao 1994; Wokosin et al. 2004). In this case, intracellular Ca^{2+} accumulation alters the emission of green fluorescence, which can be detected by CCD cameras or photomultiplier tubes (Fig. 2). Ca^{2+} indicators are now available that differ widely in their properties. For example, although indicators most often used for the study of dendritic spines fluoresce

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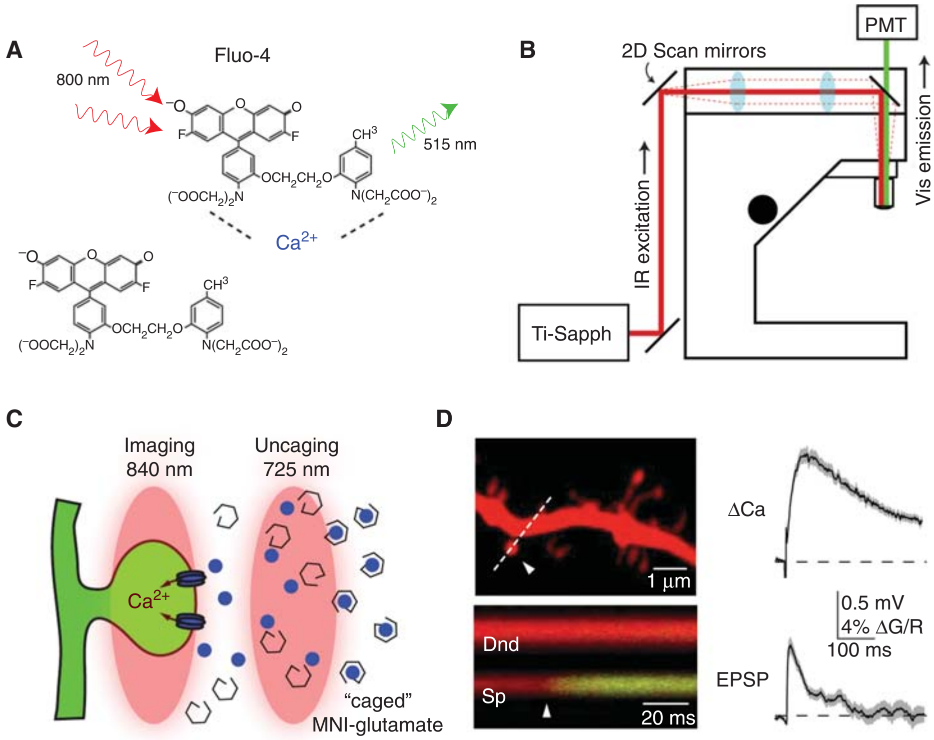

Figure 2. Ca^{2+}-sensitive fluorophores and two-photon excitation. (*A*) The structure of the Ca^{2+}-sensitive fluorophore Fluo-4 is shown (*lower left*). This molecule consists of aromatic moiety, based on fluorescein, connected to a negatively charged Ca^{2+}-binding molecule, based on BAPTA. When Ca^{2+} binds to the buffer via coordination of the negative charges (*upper* molecule), the quantum yield, and thus fluorescence, of Fluo-4 increases dramatically. In the Ca^{2+}-bound state, Fluo-4 can be excited by a single blue photon (not shown) or via near-simultaneous absorption of two low-energy red photons (i.e., two-photon stimulation). In either case, the emission consists of a single green photon. (*B*) Simple schematic of a two-photon scanning microscope. Infrared (~800 nm) light (red line) is generated by a high-energy titanium sapphire laser and directed into the microscope objective via a light path consisting of a scanning galvanometer mirror system that scans the laser in two dimensions and a telescope. The mirrors are used to scan the excitation beam through the sample. Visible fluorescence (green line) produced at the sample is collected and directed into a photomultiplier tube for image formation. (*C*) Schematic illustrating two-photon imaging of Ca^{2+} transients evoked by simultaneous two-photon glutamate uncaging. A neuron is filled with a Ca^{2+} indicator, such as Fluo-4, via the recording pipette and bathed in the inactive but photolabile compound MNI-glutamate. One laser (840 nm) is scanned across the tissue to image fluorescence. A second laser (725 nm) is directed next to the spine head, where a brief pulse breaks the covalent bond between glutamate and the blocking moiety. The released glutamate molecules bind synaptic NMDARs and lead to Ca^{2+} influx. (*D*) Example of uncaging-evoked synaptic Ca^{2+} transient in a dendritic spine. The neuron has been filled with the Ca^{2+}-insensitive red fluorophore Alexa Fluor-594 to reveal spine morphology and the Ca^{2+}-sensitive green fluorophore Fluo-4. Two-photon glutamate uncaging at the indicated location (arrowhead) evokes a brief Ca^{2+} transient (ΔCa) and corresponding excitatory postsynaptic potential (EPSP).

green light (e.g., Fura-2, Oregon Green-BAPTA, Fluo-4), some indicators emit blue or red light (e.g., X-Rhod). In addition, the affinities of available indicators for Ca^{2+} vary over several orders of magnitude, from $\sim 10^{-7}$ to 10^{-3} M. Last, although the ease of use, fast Ca^{2+} binding, and brightness of synthetic Ca^{2+} indicators have made them the gold standard, the progress in developing genetically encoded Ca^{2+} indicators may soon yield designer proteins suitable for the study of synaptic Ca^{2+} signaling (Miyawaki et al. 1997; Nagai et al. 2001; Mank et al.

2006; Tian et al. 2009). By careful consideration of the properties of the Ca^{2+} transient under study, it is typically possible to choose a Ca^{2+} indicator and the concentration at which it is used such that the magnitude of fluorescence change is directly and nearly linearly related to the change in intracellular Ca^{2+} concentration.

However, despite the relative ease of use of Ca^{2+} indicators, great care must be taken in the analysis of Ca^{2+}-dependent changes in the fluorescence emitted by a Ca^{2+} indicator. Because Ca^{2+} indicators bind Ca^{2+} and are often present at high concentrations, from a biochemical signaling point of view, they are functionally equivalent to a Ca^{2+} buffer such as BATPA or EGTA. Hence, their presence fundamentally perturbs the Ca^{2+} transient to be analyzed, typically greatly reducing its amplitude, increasing its duration, and accelerating its spatial spread (Box 1). Furthermore, the blunting effect they have on the amplitude of evoked Ca^{2+} transients typically prevents or dampens the activation of downstream Ca^{2+}-dependent processes. Thus, it is generally difficult to study both a Ca^{2+}-dependent process and the triggering Ca^{2+} transient at the same time. Several studies have discussed quantitative descriptions of the perturbing properties of Ca^{2+} buffers and practical considerations in selecting a Ca^{2+} indicator (Neher and Augustine 1992; Tank et al. 1995; Neher 1998; Sabatini et al. 2001; Yasuda et al. 2004; Higley and Sabatini 2008).

A second complication that arises in the study of synaptic Ca^{2+} signaling is that dendritic spines are small (Harris and Stevens 1988, 1989; Südhof and Rizo 2011) Thus, many features, including the spine neck and PSD, cannot be accurately imaged with visible light-based microscopy. In addition, mammalian brain tissue is highly scattering and absorbent to visible photons, rendering the direct visualization of dendritic spines difficult. For this reason, much of the recent study of Ca^{2+} signaling in dendritic spines has taken advantage of two-photon laser-scanning fluorescence microscopy. This approach uses the nonlinear near-infrared light-based excitation of fluorophores to allow imaging of dendritic spines deep within brain tissue

BOX 1. Ca^{2+} BUFFERING BY ENDOGENOUS AND EXOGENOUS MOLECULES

Intracellular calcium (Ca) reversibly binds to endogenous molecules such as the Ca^{2+}-binding proteins calbindin, parvalbumin, and calmodulin. In addition, Ca^{2+} binds to experimentally introduced molecules including buffers such as EGTA and BAPTA, synthetic Ca^{2+} indicators such as Fura-2 and Fluo-4, and the genetically encoded Ca^{2+} indicators GCAMP and TN-XL. Although exogenously applied Ca^{2+} buffers have been used extensively as tools to study Ca^{2+} function, particularly when used as fluorescent indicators of intracellular Ca^{2+} concentration, all buffers (both endogenous and exogenous) alter the dynamics of Ca^{2+} signaling. Understanding the nature of this perturbation is critical to the proper interpretation of data derived with these tools.

The effects of buffering on the dynamics of intracellular Ca^{2+} concentration have been quantitatively described (Neher and Augustine 1992; Zhou and Neher 1993). The equilibrium between free Ca^{2+}, molecules of unbound buffer (B), and Ca^{2+}-bound buffer (BCa) is described by the laws of mass action as:

$$[\mathrm{Ca}] + [\mathrm{B}] \leftrightarrow [\mathrm{BCa}]$$

with equilibrium dissociation constant:

$$K_D = \frac{[\mathrm{Ca}][\mathrm{B}]}{[\mathrm{BCa}]}$$

The specific relationship between a change in the concentration of free Ca^{2+} concentration and the corresponding change in the concentration of Ca^{2+}-bound buffer is described quantitatively by

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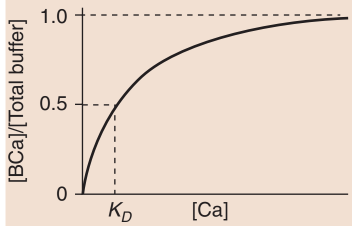

Figure 3. Curve showing the relationship between the concentrations of free Ca^{2+} and Ca^{2+}-bound buffer.

the buffer capacity, κ_B, defined as the incremental change in BCa^{2+} for an incremental change in free Ca^{2+}:

$$\kappa_B = \frac{\delta[\mathrm{BCa}]}{\delta[\mathrm{Ca}]}$$

This relationship defines the slope of the familiar saturating curve relating the concentrations of free Ca^{2+} and Ca^{2+}-bound buffer (Fig. 3). The nonlinearity of the curve indicates that κ_B is not constant but decreases as Ca^{2+} increases. Intuitively, at higher Ca^{2+} concentrations, a greater fraction of the total buffer is bound, and less is available to sequester further increases in Ca^{2+}. The buffer capacity κ_B is directly proportional to Ca^{2+} affinity (or inversely proportional to K_D) and the concentration of the buffer. Thus, high-affinity buffers strongly sequester free Ca^{2+}, but are also saturated more readily (Higley and Sabatini 2008).

In response to a brief increase in total cytosolic Ca^{2+} (such as occurs during a synaptic event or action potential), the presence of exogenous buffer produces several perturbations in the dynamics of free Ca^{2+} concentration compared with what would occur in the absence of added buffers. The degree of perturbation is directly proportional to the fractional buffer capacity that comes from exogenous buffers.

First, the added buffer temporarily sequesters Ca^{2+} ions, preventing their interaction with other intracellular molecules. Quantitatively, the change in free Ca^{2+} is equal to the change in total Ca^{2+} scaled by the sum of the capacities of each buffer in the cell. Or, considering simply one exogenous buffer with capacity κ_E and total native buffer capacity κ_N:

$$\Delta[\mathrm{Ca}] = \frac{\Delta \mathrm{Ca}_T}{1 + \kappa_E + \kappa_N}$$

Thus, relative to the situation without added buffers (i.e., $\kappa_E = 0$), the amplitudes of evoked Ca^{2+} transients are reduced in proportion to the relative increases in buffer capacity. Because 100 μM of EGTA or BAPTA provides a buffer capacity of ~250 and spines have native buffer capacities of ~20 (Sabatini et al. 2002), the exogenous buffers can very quickly dominate and reduce the amplitude of free Ca^{2+} transients by more than a factor of 10. The functional consequences of this reduction are well known to investigators of Ca^{2+}-dependent long-term synaptic plasticity, as the experimental introduction of EGTA or BAPTA can reduce or prevent plasticity induction as well as many other Ca^{2+}-dependent processes.

Second, somewhat counter-intuitively, the presence of buffer prolongs the time that Ca^{2+} is present in the cytosol by slowing the time course of Ca^{2+} clearance. Free Ca^{2+} is primarily cleared by extrusion through the cell membrane or by pumping into intracellular storage sites such as mitochondria and endoplasmic reticulum. By preventing Ca^{2+} ions from binding to pumps, buffers increase the total time necessary to remove Ca^{2+} from the cytosol. Quantitatively, the time constant (τ) of the exponential time course of decay of free Ca^{2+} is inversely proportional

Continued

to the rate of clearance (γ) and directly proportional to the total buffer capacity:

$$\tau = \frac{1 + \kappa_E + \kappa_N}{\gamma}$$

Thus, the fractional slowing of Ca^{2+} clearance is proportional to the relative change in buffer capacity caused by the addition of an exogenous buffer. Furthermore, cells with higher native buffer capacity will, given a fixed membrane concentration of Ca^{2+} pumps, clear Ca^{2+} more slowly.

Third, the presence of buffer expands the spatial extent of Ca^{2+} signaling in the cytosol. Intracellular Ca^{2+} signaling is spatially limited because the spread of Ca^{2+} ions is limited by binding to endogenous large Ca^{2+}-binding proteins (Soler-Llavina and Sabatini 2006; Schmidt et al. 2007). In contrast, exogenous Ca^{2+} buffers are typically small (~500 Da), charged molecules and are highly mobile in the intracellular space. Thus, Ca^{2+} "catches a ride" while bound on the mobile buffer, bypassing the Ca^{2+}-binding proteins and ultimately unbinding at a more distal location. Quantitatively, the effective diffusion coefficient for Ca^{2+} (D_{eff}) in the presence of buffers is the average of the diffusion coefficients of free and buffer-bound Ca^{2+}, weighted by the buffer capacities:

$$D_{\text{eff}} = \frac{D_{\text{Ca}} + D_E\kappa_E + D_N\kappa_N}{1 + \kappa_E + \kappa_N}$$

Here, D_{Ca}, D_E, and D_N are the diffusion coefficients of free Ca^{2+}, endogenous, and native buffers in the cytoplasm. Thus, the presence of added buffer may break down normal diffusional barriers in small cellular compartments that are critical for physiological Ca^{2+} signaling.

In summary, in response to a brief rise in intracellular Ca^{2+} following synaptic activity or a back-propagating action potential, the presence of Ca^{2+} buffers reduces the magnitude of the increase in free Ca^{2+}, prolongs the kinetics of decay of the Ca^{2+} transient, and increases the diffusional spread of Ca^{2+}.

(Denk et al. 1990; Denk and Svoboda 1997). However, it does so at the cost of reduced imaging resolution (owing to the longer wavelengths used), further obscuring many features of dendritic spines (however, for a practical consideration of this point, see Cox and Sheppard 2004). Importantly, the advent of laser-scanning supra-resolution microscopy, in which imaging resolution is better than the limit imposed by the diffraction of light, may soon overcome this challenge.

Last, the study of synaptic Ca^{2+} signaling in dendritic spines is made difficult by the complexity of the mammalian brain. The high packing density of synapses and the dense intercrossing of axons within the neuropil make it difficult to stimulate a single synapse in isolation. It is even more challenging to visualize a dendritic spine while selectively stimulating the apposed presynaptic axon (although, see Yuste and Denk 1995; Emptage et al. 1999; Oertner et al. 2002). Thus, the study of postsynaptic signaling in dendritic spines was greatly helped by the development of photosensitive derivatives of neurotransmitters that are inert in their parent form but release a fully functional neurotransmitter following exposure to light of the appropriate wavelength. The best characterized of these is MNI-glutamate, which can be readily photolyzed via two-photon excitation to selectively activate glutamate receptors on individual visualized spines within complex brain tissue (Canepari et al. 2001; Matsuzaki et al. 2001). This approach has been coupled with two-photon fluorescence imaging to allow the direct probing of synaptic Ca^{2+} signaling in an individual dendritic spine without the need to electrically activate axons.

MECHANISMS OF Ca^{2+} ENTRY

Synaptically coupled Ca^{2+} signals within dendritic spines originate from several sources that vary in the magnitude of Ca^{2+} transient produced, the time course of Ca^{2+} rise and decay, and the coupling to downstream biochemical pathways. Here, we consider the three most well-characterized sources of synaptic Ca^{2+}: ionotropic glutamate receptors, voltage-gated calcium channels, and internal calcium stores.

Glutamate Receptors

Ionotropic glutamate receptors comprise a heterogeneous group of ligand-gated ion channels, which bind the amino acid glutamate and are permeable to monovalent (and, in some cases, divalent) cations. They show reversal potentials near 0 mV and mediate the vast majority of excitatory synaptic transmission in the nervous system. Glutamate receptors were initially grouped by their sensitivity to exogenous agonists, including *N*-methyl-D-aspartate (NMDA), 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA), and kainic acid. This broad classification—into NMDA-, AMPA-, and kainate-type glutamate receptors—has remained useful and is now known to reflect different subunit compositions. As discussed below, glutamate receptors are found throughout the central nervous system on dendritic shafts and spines of multiple cell types and contribute to cytosolic Ca^{2+} elevation both directly and indirectly.

NMDA-Type Glutamate Receptors

NMDA-type glutamate receptors (NMDARs) show the highest fractional Ca^{2+} permeability of all glutamate receptors. Approximately 15% of the current through NMDARs is mediated by Ca^{2+} influx under physiological conditions (Schneggenburger et al. 1993; Jane et al. 2009). Thus, NMDARs are the predominant source of synaptic Ca^{2+} signals in a variety of cells, including pyramidal neurons in both the CA1 (Müller and Connor 1991; Regehr and Tank 1992; Mainen et al. 1999; Yuste et al. 1999; Kovalchuk et al. 2000; Sobczyk et al. 2005; Bloodgood and Sabatini 2007b) and CA3 (Reid et al. 2001) regions of the hippocampus, spiny stellate (Nevian and Sakmann 2004), and pyramidal neurons of the neocortex (Koester and Sakmann 1998; Schiller et al. 1998), striatal medium spiny neurons (Carter and Sabatini 2004; Higley and Sabatini 2010), and olfactory granule cells (Egger et al. 2005). Because NMDARs are usually located on dendritic spines, their activation by glutamate produces a highly compartmentalized Ca^{2+} transient that is largely limited to the activated spine, and blocking NMDAR activation with pharmacological antagonists such as APV or CPP typically reduces or eliminates synaptic Ca^{2+} signals in spines (Sabatini et al. 2002; Sobczyk et al. 2005). This rise in Ca^{2+} concentration regulates diverse processes including local biochemical signaling, protein/membrane trafficking, synaptic plasticity, and cell growth.

The number of NMDARs activated in a single spine during a synaptic event was estimated using two-photon fluorescent imaging (Nimchinsky et al. 2004). Results suggested that less than five and, in some cases, only a single receptor opens under physiological conditions. In a resting neuron, this opening produces a total Ca^{2+} influx of about 6000 ions into a spine head with a volume of 1 fL, corresponding to a concentration of $\sim$10 μM (Sabatini et al. 2002). Of course, 90–95% of these ions are rapidly bound by various internal buffering molecules, leading to a change in free Ca^{2+} concentration of $\sim$1 μM (Sabatini et al. 2002; Higley and Sabatini 2008).

Structurally, NMDARs are heteromeric tetramers typically consisting of two obligatory GluN1 subunits and two accessory GluN2 subunits (see Smart and Paoletti 2012). Some subunits also have multiple isoforms and, in some cases, multiple splice variants (Nakanishi et al. 1992; Sugihara et al. 1992). The structural diversity of NMDAR subunits contributes to functional heterogeneity of the channel. For example, in comparison with GluN2A-containing receptors, NMDARs with GluN2B subunits show higher affinity for glutamate binding, slower channel kinetics, and higher fractional Ca^{2+} permeability (Monyer et al. 1994; Vicini 1998; Sobczyk et al. 2005).

The influence of subunit composition on Ca^{2+} signaling suggests that activation of receptors composed of distinct subunit combinations may trigger different biological pathways.

This hypothesis is supported by immunogold electron microscopy studies showing that GluN2A- and GluN2B-containing receptors may be differentially expressed across synapses (He et al. 1998). Similarly, stimulation of single postsynaptic contacts with two-photon glutamate uncaging has shown that the contributions of GluN2A- and GluN2B-containing receptors to NMDAR-dependent currents and Ca^{2+} transients vary widely from spine to spine (Sobczyk et al. 2005). Antagonism of GluN2B-mediated Ca^{2+} transients with the selective blocker ifenprodil reduces both the amplitude and interspine variability of NMDAR-mediated Ca^{2+} transients, consistent with heterogeneous expression of GluN2B subunits at subsets of synapses (Sobczyk et al. 2005).

Distinct subunits may also play a critical role in the plasticity of synaptic strength. In many brain areas, there is a developmental switch in synapses from GluN2B- to GluN2A-containing NMDARs that coincides with the maturation of neuronal circuits (Monyer et al. 1994; Sheng et al. 1994). Additionally, subunit composition may be rapidly regulated in response to plasticity-inducing stimuli. Thus, induction of long-term potentiation at CA3 to CA1 synapses in the hippocampus of young rats is accompanied by a subunit switch from GluN2B to GluN2A (Bellone and Nicoll 2007). Finally, differential coupling to downstream Ca^{2+}-dependent signaling pathways may allow GluN2A- versus GluN2B-containing receptors to have different functional implications for plasticity induction (Liu et al. 2004; Massey et al. 2004).

One of the most notable features of the NMDAR is that the conductance of cations, including Ca^{2+}, is strongly regulated by membrane potential due to pore blockade by extracellular magnesium ions. Depolarization of the membrane potential by 20 mV decreases the affinity of Mg^{2+} for the NMDAR by nearly 10-fold (Jahr and Stevens 1990b). This property allows NMDARs to serve as coincidence detectors, in that synaptic current and local Ca^{2+} influx are strongly augmented by near-simultaneous membrane depolarization and glutamate binding. Postsynaptic membrane depolarization that relieves Mg^{2+} block occurs following strong synaptic activation. In addition, transient depolarization occurs when action potentials propagate antidromically through the dendritic arbor, potentiating Ca^{2+} influx through NMDARs (Yuste and Denk 1995; Magee and Johnston 1997; Koester and Sakmann 1998; Carter and Sabatini 2004; Nevian and Sakmann 2004). This enhancement may contribute to Ca^{2+}-dependent synaptic plasticity that is mediated by precisely timed presynaptic and postsynaptic activity (Bender et al. 2006; Nevian and Sakmann 2006). Nevertheless, even at the resting potentials of most cells, Mg^{2+} block is incomplete, and glutamate binding to NMDARs can evoke Ca^{2+} influx in the absence of additional depolarization (Jahr and Stevens 1990a; Sabatini et al. 2002).

Ca^{2+} influx through NMDARs is also regulated by receptor phosphorylation, providing a biochemical means to alter synaptic Ca^{2+} signals. Protein kinase A (PKA) regulates the Ca^{2+} permeability of both GluN2A- and GluN2B-containing receptors (Skeberdis et al. 2006; Higley and Sabatini 2008; Chalifoux and Carter 2010). This mechanism underlies the modulation of NMDAR Ca^{2+} signaling by various G-protein-coupled receptors (see Box 2). Ca^{2+} permeability of NMDARs is also controlled by a negative-feedback loop such that repetitive activation of GluN2B-containing receptors activates a serine-threonine phosphatase that decreases Ca^{2+} permeability (Sobczyk and Svoboda 2007).

Non-NMDA-Type Glutamate Receptors

AMPA-type glutamate receptors (AMPARs) are heteromeric tetramers, typically comprising dimer pairs of GluA2 and either GluA1, GluA3, or GluA4 (Greger et al. 2007; Nakagawa 2010). The presence of a GluA2 subunit renders AMPARs minimally permeable to Ca^{2+} ions. However, a subset of AMPARs lacking GluA2 subunits is expressed in populations of inhibitory neurons, including cortical and hippocampal interneurons (Burnashev et al. 1992; Cull-Candy et al. 2006), striatal medium spiny neurons (Carter and Sabatini 2004), and cerebellar Purkinje cells (Denk et al. 1995). Ca^{2+}-permeable AMPARs have also been found in excitatory neurons of

BOX 2. REGULATION OF NMDA RECEPTOR-DEPENDENT Ca^{2+} INFLUX BY PKA

Ca^{2+} influx through NMDA-type glutamate receptors (NMDARs) is an essential step in the linkage between synaptic transmission and a variety of cellular processes including synaptogenesis, long-term changes in synaptic efficacy, membrane protein trafficking, gene transcription and translation, and cell death and survival pathways (Kennedy et al. 2005). Dysregulation of NMDAR-mediated Ca^{2+} influx is implicated in schizophrenia and in excitotoxic cell death associated with stroke, epilepsy, head trauma, and neurodegenerative disease (Lau and Zukin 2007; Lau and Tymianski 2010). Intriguingly, several recent studies have shown that the Ca^{2+} permeability of NMDARs is controlled by distinct neuromodulatory systems in the brain, opening up new avenues in our understanding of this signaling pathway.

NMDARs are regulated by the activity of protein kinase A (PKA), which, along with protein phosphatase-1, is coupled to NMDARs via an A-kinase anchoring protein (AKAP) (Westphal et al. 1999). Furthermore, NMDARs are known molecular targets of PKA phosphorylation. Skeberdis et al. (2006) showed that PKA selectively augmented the permeation of Ca^{2+} ions through NMDARs in both cultured hippocampal neurons and acutely prepared hippocampal slices. This modulation was further seen as an enhancement of NMDAR-mediated Ca^{2+} transients in synaptically activated dendritic spines with no concomitant change in total synaptic current. Blocking PKA activity reduced the early phase of NMDAR-dependent long-term potentiation of hippocampal synapses, suggesting that NMDAR Ca^{2+} permeability is a key target for the modulation of synaptic plasticity.

Higley and Sabatini (2010) showed that the activation of type 2 dopamine receptors (D2Rs) inhibited NMDAR-mediated synaptic Ca^{2+} influx into the dendritic spines of striatal medium spiny neurons. D2Rs are negatively coupled to cyclic AMP generation and PKA activity via their associated $G_{\alpha i}$ subunit, and the inhibition was both mimicked and occluded by blockers of PKA. Moreover, the actions of D2Rs were opposed by activating type A2 adenosine receptors (A2ARs), which are positively coupled to PKA via $G_{\alpha s}$. These results suggest a likely cellular mechanism underlying the analogous actions of D2Rs and A2ARs on NMDAR-dependent long-term plasticity of corticostriatal synapses (Shen et al. 2008). In addition, Chalifoux and Carter (2010) found that activation of $G_{\alpha i}$-coupled $GABA_B$ receptors also inhibits synaptic NMDAR-mediated Ca^{2+} influx in pyramidal neurons of the neocortex, further indicating the generalization of this mechanism.

These studies provide strong links between the activity of PKA and the functional regulation of synaptic Ca^{2+} signaling. Moreover, they provide a mechanism for coupling neuromodulatory pathways to myriad cellular phenomenon such as long-term synaptic plasticity. Given the connection between NMDAR-mediated Ca^{2+} signals and neuropsychiatric disease, they suggest new avenues for potential therapeutic interventions.

the hippocampus (Thiagarajan et al. 2005; Plant et al. 2006) and amygdala (Clem and Huganir 2010). The presence of GluA2-lacking AMPARs is often identified by their characteristic electrophysiological signature of strong inward rectification at depolarized membrane potentials. Because of their fast kinetics, Ca^{2+} influx through these receptors is significantly briefer and of lower magnitude than that occurring through NMDARs. Furthermore, the functional consequences of Ca^{2+} influx through GluA2-lacking AMPARs is less clear, although they may contribute to synaptic plasticity in some cells (Thiagarajan et al. 2005; Plant et al. 2006; Clem and Huganir 2010).

Importantly, AMPARs indirectly contribute to Ca^{2+} signaling by providing membrane depolarization, relieving Mg block from NMDARs, and activating voltage-gated Ca^{2+} channels (see below). Similarly, kainate-type glutamate receptors are minimally permeable to Ca^{2+}. However, they may also contribute indirectly to Ca^{2+} influx via membrane depolarization in some cells (Jane et al. 2009).

Voltage-Gated Ca^{2+} Channels

Voltage-gated Ca^{2+} channels (VGCCs) make up a second class of contributors to Ca^{2+} influx. VGCCs are heteromeric complexes comprising a primary $\alpha 1$ pore-forming subunit and four additional associated subunits ($\alpha 2$, β, or γ). The molecular identity of the $\alpha 1$ subunit determines the functional classification of neuronal channels as L-type (α1C and D or $Ca_V1.2$ and 1.3), P/Q-type (α1A or $Ca_V2.1$), N-type (α1B or $Ca_V2.2$), R-type (α1E or $Ca_V2.3$), or T-type (α1G, H, and I or $Ca_V3.1$, 3.2, and 3.3) (Hille 2001).

Within dendrites and spines, VGCCs open following synaptically evoked depolarization (Miyakawa et al. 1992; Christie et al. 1995; Denk et al. 1995; Eilers et al. 1995; Magee et al. 1995; Markram et al. 1995; Finch and Augustine 1998; Schiller et al. 1998; Reid et al. 2001). Sufficient depolarization for VGCC activation can also be provided by the back-propagation of somatically generated action potentials that spread antidromically through at least the proximal portions of the dendritic arbor (Callaway and Ross 1995; Schiller et al. 1995; Yuste and Denk 1995; Svoboda et al. 1997; Helmchen et al. 1999; Koester and Sakmann 2000; Waters et al. 2003; Carter and Sabatini 2004; Nevian and Sakmann 2004; Bloodgood and Sabatini 2007b). Opening of VGCCs by action potential-evoked depolarization was used, in combination with fluctuation analysis, to estimate the number of VGCCs in a single dendritic spine at about one to 20, with the number correlating with spine volume (Sabatini and Svoboda 2000).

Numerous electrophysiological and imaging studies have revealed considerable heterogeneity in the expression of VGCC classes between different dendritic regions and across different cell types and species. In CA1 neurons of the hippocampus, Ca^{2+} influx into the dendritic shaft occurs via L-, R-, and T-type channels (Christie et al. 1995; Magee et al. 1995; Sabatini and Svoboda 2000), whereas influx into individual spine heads appears to be primarily limited to R- and T-type channels with a small contribution from L-type channels (Sabatini and Svoboda 2000; Yasuda et al. 2003; Hoogland and Saggau 2004; Bloodgood and Sabatini 2007b). In cortical pyramidal neurons, dendritic VGCCs include L-, N-, P/Q-, and R-type channels (Markram et al. 1995), whereas L-, P/Q-, and T-type channels are found in spines (Koester and Sakmann 2000). T-type VGCCs also contribute to dendritic Ca^{2+} signals in both olfactory granule cells (Egger et al. 2005) and cerebellar Purkinje cells (Isope and Murphy 2005). In the lateral nucleus of the amygdala, cortical and thalamic synapses are associated with spines of different sizes and voltage-gated Ca^{2+} channel content such that thalamic inputs are preferentially found on large spines that contain R-type channels, which may underlie differences in the amplitude of evoked Ca^{2+} transients and the ability to express spike-timing dependent plasticity at these two classes of synapses (Humeau et al. 2005). Within striatal medium spiny neurons, Ca^{2+} influx occurs primarily through R- and T-type channels in both dendritic shafts and spines (Higley and Sabatini 2010). Nevertheless, interpretation of many of these studies is limited by imprecise mapping between pharmacological sensitivity and VGCC α-subunit expression, making the molecular composition of the channels mediating dendritic and spine Ca^{2+} influx difficult to establish conclusively. This is particular true for L-type channels (see Box 3).

Internal Stores

The contributions of Ca^{2+} release from internal stores to dendritic and spine Ca^{2+} transients following synaptic activation are more controversial and likely depend critically on the cell type under consideration as well as the experimental protocols used. Strong activation of glutamatergic inputs to hippocampal CA1 pyramidal neurons can lead to activation of group I metabotropic glutamate receptors (mGluRs), triggering a phospholipase C (PLC)– and inositol triphosphate (IP3)–dependent Ca^{2+} release from internal stores, and contributing to long-term heterosynaptic plasticity (Watanabe et al. 2006; Dudman et al. 2007; Hong and Ross 2007). This may lead to Ca^{2+} waves throughout large regions of the apical dendrite, regulating

BOX 3. MYSTERIES OF R- AND L-TYPE VOLTAGE-GATED Ca^{2+} CHANNELS—Ca^{2+} MICRODOMAIN ORGANIZATION OF THE SPINE

Among the many sources of Ca^{2+} that are active in dendritic spines, the R- and L-type voltage-gated Ca^{2+} channels appear to have special functions that reveal an intricate subdivision of the spine into smaller Ca^{2+} signaling microdomains. L-type channels are often defined pharmacologically by their sensitivity to blockade by dihydropyridines such as nifedipine and nimodipine. However, neuronal L-types comprise two distinct molecular subclasses, $Ca_V1.2$ and $Ca_V1.3$, based on the identity of the pore-forming subunit, encoded by the α_{1C} or α_{1D} gene, respectively. One major challenge to analyzing the function of L-type channels is that $Ca_V1.3/\alpha_{1D}$-encoded channels have diminished sensitivity to dihydropyridines and require substantial depolarization to display significant block by these agents (Lipscombe et al. 2004).

These properties present difficulties in detecting the activation of L-type channels during brief physiological stimuli. For example, action potential-evoked Ca^{2+} influx in dendritic spines of striatal medium spiny neurons has little or no sensitivity to concentrations of dihydropyridines that are selective for blockade of L-type channels. However, prolonged step depolarizations show that dihydropyridine-sensitive Ca^{2+} channels are, in fact, present in the spine (Higley and Sabatini 2010). A similarly perplexing result was found by the study of Yasuda et al. (2003), which described that Ca^{2+} influx through L-type Ca^{2+} channels activated a kinase cascade producing inhibition of other spine Ca^{2+} channels. However, no L-type-mediated Ca^{2+} influx could be detected in either dendrites or spines via fluorescence imaging. The resolution of this paradox is likely that the total Ca^{2+} influx carried by L-type channels is too small to detect but that this Ca^{2+} has a privileged capacity to activate downstream signaling cascades. A close apposition of Ca-sensitive proteins to the mouth of the L-type channel would allow brief and large Ca^{2+} accumulation within a "microdomain." Tethering of CaMKII to the carboxy-terminal domain of the α-subunit is one such example (Hudmon et al. 2005).

Another example of Ca^{2+} microdomain signaling involves R-type voltage-gated channels (α_{1E}/$Ca_V2.3$). These are probably the least well-characterized class of voltage-gated Ca^{2+} channel because of a historical lack of sensitivity to known pharmacological agents (R = resistant). However, a spider toxin (SNX-482) is available that blocks these channels with good specificity, allowing the study of their contribution to Ca^{2+} signaling (Newcomb et al. 1998). Early reports showed that R-type channels likely contributed the bulk of action potential-evoked Ca^{2+} influx in spines of hippocampal pyramidal neurons (Sabatini and Svoboda 2000; Yasuda et al. 2003). Interestingly, recent studies using SNX-482 showed that blocking Ca^{2+} influx through these channels increased the amplitude of synaptic potentials and, quite perplexingly, also *increased* the magnitude of synaptically evoked Ca^{2+} transients in active spines (Bloodgood and Sabatini 2007b). To explain this observation, a series of studies has now shown that Ca^{2+} entering through R-type channels has a privileged ability to activate small conductance type Ca-activated potassium channels known as SK channels. SK channels open quickly during synaptic potentials and repolarize the active spine, thereby reducing the magnitude and of synaptic potentials and associated NMDA receptor-dependent Ca^{2+} influx (Ngo-Anh et al. 2005; Bloodgood and Sabatini 2007b). Blocking Ca^{2+} influx through R-type channels prevents the activation of SK channels, essentially disinhibiting synaptic signals. Interestingly, this signaling cascade is modulated by muscarinic cholinergic receptors, and the inhibition of SK channels explains much of the ability of muscarinic agents to enhance synaptic potentials, contributing to the induction of synaptic plasticity and hippocampal-dependent learning (Giessel and Sabatini 2010).

In summary, both L-type and R-type voltage-gated Ca^{2+} channels appear to have privileged functions in dendritic spines. That is, Ca^{2+} entering through these channels activates signaling cascades that are not otherwise triggered by bulk elevation in cytoplasmic Ca^{2+} concentration. These results are undoubtedly a manifestation of a Ca^{2+} microdomain organization within the spine in which specific Ca^{2+} sources are physically associated with Ca^{2+}-sensitive proteins, producing important consequences for synaptic function.

cell-wide signaling pathways. Putative IP3- and Ca^{2+}-induced Ca^{2+} release from internal stores may also contribute to synaptically evoked Ca^{2+} transients in individual dendritic spines (Emptage et al. 1999). However, other studies have failed to find evidence for Ca^{2+} release from internal stores following more limited synaptic stimulation of hippocampal afferents (Mainen et al. 1999; Yuste et al. 1999; Kovalchuk et al. 2000). This disparity may be due to Ca^{2+} depletion of internal stores during whole-cell recordings (Hong and Ross 2007) as well as by the relatively few spines that contain endoplasmic reticulum (Holbro et al. 2009). More evidence for synaptically evoked Ca^{2+} release from internal stores exists for cerebellar Purkinje cells, where several groups have shown that activation of parallel fiber inputs can lead to Ca^{2+} release via a Group 1 mGluR-PLC-IP3-coupled pathway (Finch and Augustine 1998; Takechi et al. 1998; Miyata et al. 2000; Wang et al. 2000).

MECHANISMS OF Ca^{2+} HANDLING AND CLEARANCE

Following entry into the spine, free Ca^{2+} concentration increases and then decreases rapidly because of the action of Ca^{2+}-binding proteins and Ca^{2+} extrusion mechanisms. This rapid clearance is essential to maintaining spatially and temporally localized Ca^{2+} signals that can mediate synapse-specific (homosynaptic) forms of plasticity as well as the induction of spike timing-dependent plasticity. The timing and magnitude of synaptic Ca^{2+} transients in active dendritic spines are determined by an interplay between the kinetics of opening of Ca^{2+} sources, the on and off rates of Ca^{2+} binding to proteins and lipids, and the membrane densities and transport rates of Ca^{2+} transporters and exchangers.

Ca^{2+}-Binding Proteins

The human genome contains more than 200 proteins with EF-hands, prototypical Ca^{2+}-binding domains, suggesting a large family of Ca^{2+}-binding proteins. Some of these represent direct Ca^{2+}-activated enzymes, such as the phosphatase calcineurin or the protease calpain. Others, such as calmodulin, are present at high levels and, upon Ca^{2+} binding, undergo a conformational change that promotes interaction with and activation of enzymes such as CaMKII. Notably, CaMKII also binds Ca^{2+} directly, leading to a synergistic activation via Ca^{2+} and Ca^{2+}/calmodulin signaling. However, a subset of Ca^{2+}-binding proteins may simply bind Ca^{2+} in order to modulate the spatial and temporal profile of Ca^{2+} signaling. This group includes the prototypical Ca^{2+} buffering proteins parvalbumin, calretinin, and calbindin. These proteins are found in the dendrites and axons of many neuron classes, particularly inhibitory interneurons. However, the properties and expression patterns of Ca^{2+} buffering proteins vary greatly, and their functional significance is poorly understood.

The Ca^{2+} buffering capacity of a molecule is typically referred to as κ and is roughly defined as the ratio of the number of Ca^{2+} ions that are bound to proteins to the number of Ca^{2+} ions that remain free (see Box 1) (Neher and Augustine 1992):

$$\kappa = \frac{[\mathrm{Ca}]_{\mathrm{bound}}}{[\mathrm{Ca}]_{\mathrm{free}}}$$

The value of κ has profound implications for Ca^{2+} signaling because the amplitude of evoked Ca^{2+} transients is inversely proportional to κ, whereas their duration is directly proportional to κ (Box 1). For pyramidal neurons, κ is 100–200 near the soma and base of the apical dendrite, whereas $\kappa = 20$ in distal dendrites and dendritic spines. Thus, only ~1%–5% of the Ca^{2+} that enters the cell remains unbound (Helmchen et al. 1996; Lee et al. 2000a; Maravall et al. 2000; Sabatini et al. 2002). The relatively low κ of spines and dendrites allows for rapid Ca^{2+} signaling in which large transients are generated when a Ca^{2+} channel opens and are rapidly dissipated (with ~15 msec) after closure of the channel. For these reasons, action potential and synaptically evoked Ca^{2+} transients are large (~1 μM) and closely follow the kinetics of opening of Ca^{2+} sources (Sabatini et al. 2002).

In contrast, κ is significantly larger in cerebellar Purkinje cells and inhibitory interneurons that express parvalbumin (PV), a kinetically slow and high-affinity Ca^{2+}-binding protein (Lee et al. 2000a,b; Goldberg et al. 2003; Soler-Llavina and Sabatini 2006). The peculiar properties of PV shape Ca^{2+} signaling in these cells. Because PV binds Mg^{2+} and Ca^{2+} competitively, Ca^{2+} equilibrates slowly with PV such that the binding reaction does not reach equilibrium for tens of milliseconds. During this "non-equilibrium" phase of Ca^{2+} signaling, Ca^{2+} remains free and at elevated levels. However, the binding of Ca^{2+} to PV subsequently returns Ca^{2+} to near resting levels, and once Ca^{2+} reaches equilibrium with PV, the further decay of $[Ca^{2+}]$ to resting levels occurs slowly (Collin et al. 2005; Soler-Llavina and Sabatini 2006; Muller et al. 2007). For these reasons, in PV-expressing neurons, the decay of evoked Ca^{2+} transients is typically complex and displays multiple time constants. In contrast, in pyramidal neurons for which Ca^{2+} buffers are thought to rapidly bind Ca^{2+}, equilibrium is reached quickly and the time course of Ca^{2+} clearance is well described by a single exponential process corresponding to extrusion (Helmchen et al. 1996; Sabatini et al. 2002).

In addition to regulating the time course of Ca^{2+} clearance, PV may play an important role in compartmentalizing Ca^{2+} in neurons that lack dendritic spines (Goldberg et al. 2003; Soler-Llavina and Sabatini 2006). This has been closely examined in cerebellar stellate cells, aspiny cells that express synapse-specific and Ca^{2+}-dependent forms of plasticity. If Ca^{2+} diffused rapidly away from the site of entry into the dendrite, it would be impossible to maintain the synapse specificity of Ca^{2+}-dependent processes. In contrast, if an aspiny neuron restricted diffusion by the expression of a high concentration of fast-acting Ca^{2+} buffer, then the amplitude of evoked Ca^{2+} transients might be too small to effectively activate Ca^{2+}-dependent processing. PV allows for large Ca^{2+} transients because of its slow kinetics, but because of its high affinity, effectively buffers Ca^{2+} after a short lag. This allows synaptic Ca^{2+} in smooth dendrites to induce synapse specific Ca^{2+}-dependent forms of plasticity (Soler-Llavina and Sabatini 2006).

The function of Ca^{2+}-binding proteins in pyramidal neurons is less clear. Because the spine head is a small volume that is separated from the dendrite by a thin neck, it naturally allows for large and compartmentalized Ca^{2+} transients such that Ca^{2+}-binding proteins are not needed for this purpose. Thus, Ca^{2+}-binding proteins may largely serve a signaling role. This is illustrated by calmodulin, which binds four Ca^{2+} ions and once fully occupied can bind to and activate a variety of proteins, being a major determinant of the Ca^{2+} buffering capacity of dendritic spines (Kakiuchi et al. 1982; Sabatini et al. 2002), at least for CA1 pyramidal neurons. On the other hand, it is difficult to draw conclusions that apply to all pyramidal neurons because there are differences in the Ca^{2+} buffering across these neurons that are of functional importance. For example, pyramidal neurons of the CA2 subfield of the hippocampus express more calcium-binding proteins than CA1 pyramidal neurons (Leranth and Ribak 1991; Seress et al. 1993; Lein et al. 2007), a difference the reduces the amplitude of spine Ca^{2+} transients (see below) (Simons et al. 2009). The relative low amplitude of synaptic Ca^{2+} transients in active spines of these cells may explain the difficulty of induction of long-term potentiation in CA2 compared with in CA1 as well as their resistance to seizure-induced death (Leranth and Ribak 1991; Simons et al. 2009). It is interesting to note that the high κ is reflected in the low amplitude of evoked transients but that a high Ca^{2+} extrusion rate (see below) compensates for the expected slowing of Ca^{2+} clearance, yielding Ca^{2+} transients whose kinetics do not differ significantly from those in CA1 (Simons et al. 2009).

Ca^{2+} Extrusion

Three main avenues exist to clear Ca^{2+} from the cytoplasm. First, ATP-dependent Ca^{2+} pumps, such as the plasmalemmal Ca^{2+} ATPase (PMCA), move Ca^{2+} across the cell membrane from the cytosol into the extracellular space. PMCA isoforms have been found both in the postsynaptic density and localized to spines, placing them appropriately to rapidly clear Ca^{2+} from the spine head (Garside et al. 2009; Burette et al.

2010; Kenyon et al. 2010). The high expression of PMCA in pyramidal neurons may explain the rapid Ca^{2+} clearance capacity of these cells (Jensen et al. 2004; Kip et al. 2006). The efficacy of PMCA is itself regulated by intracellular Ca^{2+} such that at high Ca^{2+} concentrations, the efficiency of PMCA-dependent Ca^{2+} extrusion is decreased, resulting in slowed Ca^{2+} clearance during prolonged excitation (Scheuss et al. 2006). The mechanism of this slowing may include Ca^{2+}-dependent activation of proteases that act on PMCA (Ferragamo et al. 2009), although this irreversible mechanism cannot account for the transient slowing of Ca^{2+} clearance seen following action potential trains (Scheuss et al. 2006). The proteins comprising the Na^+/Ca^{2+} exchangers (NCX1-3) have also been localized to dendrites and dendritic spines with the distribution varying by isoform (Lorincz et al. 2007; Minelli et al. 2007). Functional analysis suggests that these proteins play a major functional role in clearance of spine Ca^{2+} (Scheuss et al. 2006).

Second, Ca^{2+} can be actively pumped into the lumen of intracellular organelles. The sarcoendoplasmic reticulum Ca-ATPase (SERCA) moves Ca^{2+} across the endoplasmic reticulum (ER) membrane and sequesters it within this organelle. The activity of SERCA may account for up to 50% of the clearance of spine Ca^{2+} transients during single action or synaptic potentials (Majewska et al. 2000a; Sabatini et al. 2002). This same pump serves to constitutively load ER with Ca^{2+}, and its activity is necessary to support IP3- and Ca^{2+}-induced Ca^{2+} release from intracellular stores (see "Internal Stores" above). Although mitochondria can also rapidly uptake and release Ca^{2+}, the significance of mitochondrial Ca^{2+} handling to postsynaptic signaling is unclear. Pharmacological manipulation of mitochondrial Ca^{2+} signaling is typically accomplished by destroying the mitochondrial membrane potential and associated ATP production. Thus, it has been difficult to selectively examine the function of mitochondrial Ca^{2+} uptake without also perturbing the energetic state of the cell.

Third, in theory, Ca^{2+} can diffuse away from the site of entry. This mechanism of clearance can only be relevant in conditions in which gradients of Ca^{2+} exist within the cell, such as when a synapse is active and its neighbors are not. Within the spine head, Ca^{2+} is expected to quickly reach diffusional equilibration such that the concentration of free Ca^{2+} throughout the head is likely uniform within $\sim$1 msec of closure of the Ca^{2+} sources. Synaptically evoked Ca^{2+} signals do form a gradient between the spine head and dendritic shaft, suggesting that Ca^{2+} might dissipate by diffusion across the spine neck (Majewska et al. 2000a,b; Noguchi et al. 2005). However, other studies suggest that when the effects of Ca^{2+} indicators on Ca^{2+} signaling are properly accounted for, free Ca^{2+} has a very short lifetime in the spine and does not diffuse far (Sabatini et al. 2002; Sobczyk et al. 2005). Instead, Ca^{2+} is quickly removed from the spine cytoplasm before it has a chance to diffuse across the neck.

CONCLUSION

In summary, synaptically evoked Ca^{2+} transients in dendritic spines are shaped by a complex interplay between many Ca^{2+} sources, pumps, and binding proteins, as well as by the biophysics of Ca^{2+} diffusion and spine morphology. Many classes of intracellular proteins respond to changes in postsynaptic Ca^{2+} to transduce electrical signaling into the regulation of enzymatic cascades. Our understanding of the factors governing Ca^{2+} signaling has increased dramatically, but many basic questions, such as the importance of intracellular Ca^{2+} stores, remain unanswered. Furthermore, as more classes of neurons are studied biophysically, it is becoming clear that neurons have highly heterogeneous Ca^{2+} handling mechanisms that likely have significant functional implications.

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Michael J. Higley and Bernardo L. Sabatini

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