ELSEVIER

Ca^{2+} signaling in dendritic spines

Brenda L Bloodgood and Bernardo L Sabatini

Recent studies have revealed that Ca^{2+} signals evoked by action potentials or by synaptic activity within individual dendritic spines are regulated at multiple levels. Ca^{2+} influx through glutamate receptors and voltage-sensitive Ca^{2+} channels located on spines depends on the channel subunit composition, the activity of kinases and phosphatases, the local membrane potential and past patterns of activity. Furthermore, sources of spine Ca^{2+} interact nonlinearly such that activation of one Ca^{2+} channel can enhance or depress the activity of others. These studies have revealed that each spine is a complex and partitioned Ca^{2+} signaling domain capable of autonomously regulating the electrical and biochemical consequences of synaptic activity.

Addresses
Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115, USA

Corresponding author: Sabatini, Bernardo L (bsabatini@hms.harvard.edu)

Current Opinion in Neurobiology 2007, **17**:345–351

This review comes from a themed issue on
Signalling mechanisms
Edited by Stuart Cull-Candy and Ruediger Klein

Available online 23rd April 2007

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2007.04.003

Introduction

In most principal neurons in the mammalian brain, each dendritic spine is typically associated with a single glutamatergic synapse and houses the postsynaptic density [1]. Ca^{2+} accumulation within the spine activates signaling cascades that act locally to regulate the associated synapse, the morphology of the spine and the trafficking of proteins and organelles (reviewed recently in [2–4]). Here, we summarize progress made over the past few years in identifying the factors that govern the kinetics, amplitude and spread of spine Ca^{2+} transients.

The amplitude and time course of Ca^{2+} signals within the spine depend on many factors, including the activation and inactivation kinetics of Ca^{2+} sources, the Ca^{2+} permeability of ion channels, the concentration and affinity of endogenous Ca^{2+}-binding proteins, the efficiency of Ca^{2+} extrusion, and the morphology of the spine. Recent studies have shown that nearly all of these factors are under dynamic regulation and vary from spine to spine. Much of the recent progress in understanding these factors has come from the use of two-photon laser-scanning microscopy combined with two-photon laser photoactivation of caged glutamate (Box 1, Figure 1); this approach enables direct stimulation of the postsynaptic terminal on a visualized dendritic spine and simultaneous monitoring of intracellular Ca^{2+} levels.

Mechanisms of Ca^{2+} influx

NMDA-type glutamate receptors

Ca^{2+} influx through NMDA receptors contributes a large fraction of synaptic Ca^{2+} signals in the spines of hippocampal pyramidal neurons [5,6••,7,8], cortical pyramidal neurons [9,10••,11], spiny stellate neurons [12], olfactory granule cells [13] and striatal medium spiny neurons (MSN) [14].

In rat CA1 pyramidal neurons, the NMDA receptors found in spines and at synapses consist of heteromers of NR1 and typically the NR2A or NR2B subunits. Immunogold electron microscopy demonstrates that NR2A-containing receptors and NR2B-containing receptors are segregated, such that many spines express either NR2A or NR2B but not both [15]. Similarly, studies using glutamate uncaging to stimulate individual spines have shown that the contributions of NR2A-containing receptors and NR2B-containing receptors to NMDA-receptor-dependent currents and spine head Ca^{2+} transients vary greatly from spine to spine [16•]. This suggests that, in the hippocampus, the subunit composition of NMDA receptors is regulated not only during development but also, for a given cell, on a synapse-by-synapse basis. Because NR2B-containing NMDA receptors deactivate more slowly [17], contribute relatively more Ca^{2+} to synaptically-evoked Ca^{2+} transients [16•] and are coupled to different downstream signaling systems from NR2A-containing receptors [18•,19••], this heterogeneity might enable NMDA receptor opening to have different functional implications for plasticity induction [20–22] (but see [18•,23,24]).

The Ca^{2+} permeability of NMDA receptors also depends on the phosphorylation state of the receptor and its subunit composition. NR2B-containing receptors contribute relatively more Ca^{2+} to the spine than NR2A-containing receptors but the Ca^{2+} permeability of both is enhanced by protein kinase A (PKA) activity [16•,19••,25••]. Furthermore, Ca^{2+} signaling through NMDA receptors is controlled by a negative feedback loop such that repetitive activation of NR2B-containing NMDA receptors activates a serine/threonine phosphatase that decreases Ca^{2+} permeability of NMDA receptors

Box 1 New approaches to the study of spine Ca^{2+} signaling

Analysis of spine Ca^{2+} signaling has benefited greatly from the use of microscopes that combine two-photon laser-scanning microscopy (2PLSM) and two-photon laser photoactivation (2PLP) of caged glutamate [6••,14,16•,19••,30•,72•,73]. One laser is used to trigger photolysis of caged glutamate while a second is used to monitor fluorescence from an intracellular Ca^{2+}-sensitive fluorophore (Figure 1). MNI-glutamate is the preferred choice of caged glutamate because its two-photon absorption cross-section is sufficient to enable photolysis in response to submillisecond pulses of light [74]. Using this approach, the postsynaptic terminal on a spine can be stimulated while the evoked signals are monitored. Furthermore, combined 2PLSM and 2PLP enables postsynaptic responses to be studied in conditions that perturb release of neurotransmitter from the presynaptic terminal.

However, several limitations of this approach must be considered. First, although the volume in which glutamate is uncaged by two-photon processes using high numerical aperture objectives is small (<1 fL), it is far larger than that of the synaptic cleft, resulting in the possible stimulation of extrasynaptic receptors. This problem is exacerbated if the duration of the uncaging laser pulse is more than a few hundred microseconds. Second, the spatial resolution of 2PLSM is similar to the length of smaller dendritic spines, such that it is impossible to image fluorescence from stubby spines without contamination from dendritic fluorescence [75]. Similarly, the efficiency of glutamate receptor activation falls off with a length constant of ~1 μm; thus, receptors not on the targeted spine might be inadvertently activated [16•,74]. Lastly, adjusting the duration and power of the uncaging laser pulse enables the user to stimulate the postsynaptic terminal using any of a broad ranges of glutamate concentrations [72•,73]. Care must be taken to not use unnaturally strong stimuli that might uncover phenomena that have no physiological relevance. For this reason, it is necessary to develop methods to calibrate and standardize the amount of glutamate released onto spines that are located at different depths and in varying optical environments within the slice [6••].

[19••]. These data suggest that the Ca^{2+} permeability of NMDA receptors might be regulated by a PKA-anchoring protein (AKAP) complex (reviewed in [26]) that is associated with the NR2B subunit, although this has not been explicitly demonstrated.

The strongest and possibly most rapid modulator of NMDA receptor currents and Ca^{2+} influx is the membrane potential which, along with subunit composition, determines the efficacy of block of the receptor by extracellular Mg^{2+} [17,27–29]. Mg^{2+} block is incomplete at resting potentials and glutamate binding to NMDA receptors triggers Ca^{2+} influx even in the absence of depolarization. Because the spine volume is small and the driving force for Ca^{2+} influx is large, NMDA-receptor-mediated Ca^{2+} transients are substantial even in spines under good voltage clamp at resting potentials [8]. The effects of resting potential on NMDA-receptor-mediated currents have long been appreciated but recent studies indicate that the activation of a single synapse generates sufficient depolarization to partially relieve Mg^{2+} block and accentuate NMDA-receptor-mediated currents [30•]. Because the activity of a single synapse typically produces a submillivolt potential at the soma [31], this finding highlights the large attenuation of synaptic potentials that must occur along the dendrite. In pyramidal neurons in mouse hippocampus [30•] and rat amygdala [32•], spine depolarization is abbreviated by synaptic Ca^{2+} influx, which activates small-conductance Ca^{2+}-activated K^+ (SK) channels that repolarize the membrane, promote Mg^{2+} block of NMDA receptors, and terminate NMDA receptor signaling. In the case of mouse hippocampal pyramidal neurons, only Ca^{2+} that enters the spine through R-type voltage-sensitive Ca^{2+} channels (VSCCs) can activate SK channels; this indicates that, despite its small size, the spine is compartmentalized into functionally independent Ca^{2+} microdomains [6••].

NMDA-receptor-dependent Ca^{2+} influx in spines is also boosted by backpropagating action potentials (bAPs), which invade proximal dendrites and spines, transiently relieving Mg^{2+} block of NMDA receptors and enhancing synaptic Ca^{2+} signals. This effect has been demonstrated in multiple cell types [6••,7,9,10••,11,12,14] and might trigger the induction of spike-timing-dependent synaptic plasticity (STDP; see also the following section).

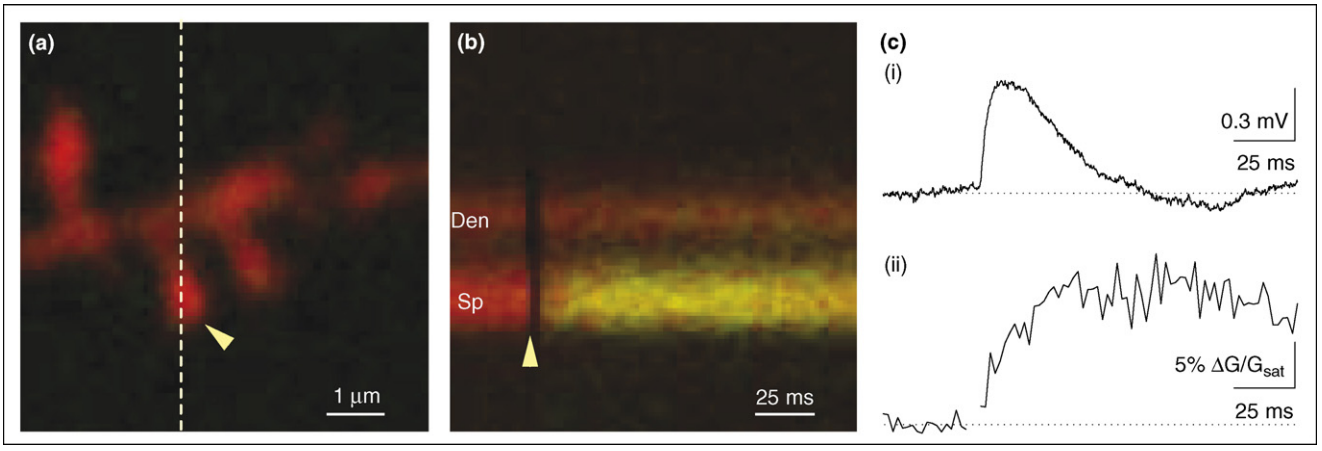
AMPA-type glutamate receptors

Ca^{2+}-permeable AMPA receptors are expressed in many GABAergic neuron classes (reviewed in [33]) and the contribution of Ca^{2+} influx through these channels to synaptic Ca^{2+} signals in spines of striatal medium spiny neurons has been directly demonstrated [14]. Ca^{2+}-permeable AMPA receptors were not thought to be present in synapses of excitatory neurons, most of which express the GluR2 AMPA receptor subunit: when incorporated into an AMPA receptor, this subunit renders the channel impermeable to Ca^{2+} [34]. Two recent studies used pharmacological and electrophysiological assays to infer the expression of synaptic Ca^{2+}-permeable AMPA receptors in hippocampal pyramidal neurons and to demonstrate a role for these receptors in homeostatic and Hebbian synaptic plasticity [35,36]; however, a direct contribution of Ca^{2+}-permeable AMPA receptors to synaptic Ca^{2+} signals has not been measured in these cells. AMPA receptors might also indirectly influence synaptic Ca^{2+} signals [5,12] by providing the depolarization that activates VSCCs [6••,37,38] and relieves Mg^{2+} block of NMDA receptors.

Metabotropic glutamate receptors

Strong or repeated activation of metabotropic glutamate (mGlu) receptors triggers widespread Ca^{2+} signals in dendrites [39–41] and induces forms of long-term depression (LTD) in several neuron classes [10••,42,43••,44]. However, whether mGlu-receptor-dependent release of Ca^{2+} from internal stores can be triggered by the activity of a single synapse and whether it contributes to synaptically evoked spine Ca^{2+} transients are unknown (although see [40,45]). Given the importance of mGlu receptor signaling to synaptic plasticity, the contribution

Figure 1



Monitoring of spine head Ca^{2+} levels and somatic potentials evoked by two-photon laser photoactivation of MNI-glutamate at individual spines. **(a)** Two-photon laser-scanning microscopy image of a spiny region of an apical dendrite of a CA1 hippocampal pyramidal neuron filled through a whole-cell recording electrode with the Ca^{2+}-insensitive fluorophore Alexa-594 (red fluorescence), to highlight the morphology of the cell, and with the Ca^{2+}-sensitive fluorophore Fluo-5F (green fluorescence). **(b)** Fluorescence collected in a line scan intersecting the spine (Sp) and dendrite (Den) shown by the dashed line in (a). The arrowhead indicates the timing of a 500 ms pulse of 725 nm laser light used to uncage MNI-glutamate at the position indicated in (a). The increase in green fluorescence indicates an increase in intracellular Ca^{2+}. **(c)** The uncaging-evoked EPSP measured at the soma (i) and quantification of the green fluorescence transient in the spine head (ii) for stimulation of the spine shown in (a). The green fluorescence transient is expressed as the increase from baseline (ΔG) relative to the maximal green fluorescence measured at saturating Ca^{2+} concentrations in a cuvette (G_{sat}). Adapted from [6••].

of these receptors to spine Ca^{2+} and unitary synaptic responses will hopefully be addressed in forthcoming years.

Voltage-sensitive Ca^{2+} channels

Activation of the VSCCs in dendritic spines mediates bAP-evoked Ca^{2+} signals [46,47] and shapes synaptic potentials and Ca^{2+} transients [6••,37]. The classes of VSCCs found in spines vary across cell types, recent studies have described what are probably T-type VSCCs in olfactory granule cells [13], T-type and P/Q-type VSCCs in Purkinje cells [48,49], L-type, R-type and T-type VSCCs in striatal MSNs [14], L-type P/Q-type and low-voltage-activated VSCCs in layer 2/3 cortical neurons [50], L-type and R-type VSCCs in rat hippocampal CA1 pyramidal neurons [47,51,52] and L-type, N-type, R-type and T-type VSCCs in the same neurons in mice [6••].

In spines of hippocampal pyramidal neurons, subclasses of VSCCs are independently regulated and activate different intracellular signaling cascades. During trains of bAPs, L-type VSCCs activate a Ca^{2+}/calmodulin protein kinase II (CaMKII)-dependent and PKA-dependent cascade that, in spines of apical dendrites, inhibits opening of R-type VSCCs [51]. Opening of somatic R-type VSCCs in these cells is enhanced by activation of muscarinic acetylcholine receptors and protein kinase C [53] but it is unknown whether this modulation also occurs in spines. Furthermore, L-type Ca^{2+} influx mediated by VSCCs in spines of basal dendrites is enhanced by β-adrenoceptors in a PKA-dependent manner [52]. Modulation of R-type and L-type channels occurs in spines but not in dendritic shafts, highlighting the spatial compartmentalization of VSCC signaling. The factors that permit the differential targeting and regulation of VSCCs in distinct dendritic compartments are unknown.

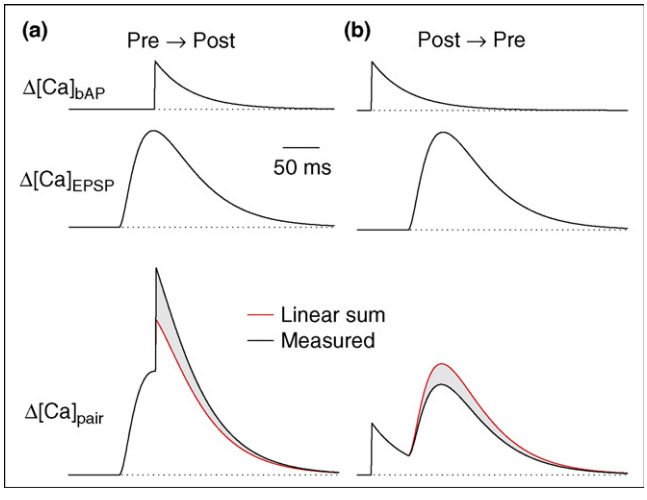
Linear and nonlinear modes of spine Ca^{2+} signaling

Under certain conditions, the factors that govern Ca^{2+} levels in the spine head are linear, such that knowing the impulse response for the spine — that is, the time course of Ca^{2+} clearance from the spine in response to a small and instantaneous increase in Ca^{2+} — is sufficient to predict the time course of Ca^{2+} signals in the spine for any Ca^{2+} current [8,19••]. The impulse response is typically estimated from the decay of spine Ca^{2+} transients generated by bAPs or step depolarizations. In this linear regime, total Ca^{2+} influx is given by the sum of the fluxes from each Ca^{2+} source. Similarly, the time course of a Ca^{2+} transient in the spine is given by the convolution of the impulse response and the time course of Ca^{2+} influx into the spine. This approach accurately predicts the time course of NMDA-receptor-mediated Ca^{2+} transients in the spine head in voltage-clamped CA1 hippocampal pyramidal neurons [8,19••].

However, linearity of spine head Ca^{2+} signaling breaks down in these cells in at least three cases. First, the high levels of Ca^{2+} reached in spines during bAP trains inhibit Ca^{2+} extrusion, making the time course of Ca^{2+} clearance following bAP trains much slower than

following a single bAP [54••]. Second, R-type VSCCs open during the excitatory postsynaptic potential (EPSP) and activate SK channels, which repolarize the spine and reduce Ca^{2+} influx through NMDA receptors [6••]. Therefore, Ca^{2+} currents through the R-type VSCCs and NMDA receptors are not independent and sum sublinearly. Third, pairing of bAPs and EPSP nonlinearly affects spine Ca^{2+} transients, generating supralinear Ca^{2+} signals when the EPSP precedes the bAP and sublinear signals when the timing is reversed (Figure 2) [9,12]. When a bAP closely follows an EPSP, Ca^{2+} influx in active spines is enhanced owing to relief of Mg^{2+} block of NMDA receptors by the bAP. Because bAPs are brief and Mg^{2+} reblock of NMDA receptors is rapid [55,56], this provides a short-lived and precisely timed enhancement of Ca^{2+} influx through NMDA receptors that is thought to trigger the LTP induced by similar pairing protocols. Less well understood is the small depression of spine Ca^{2+} influx that has been reported when a bAP arrives before an EPSP. The mechanism of this decrease in Ca^{2+} influx, and how it relates to the endocannabinoid-dependent and mGlu-receptor-dependent LTD [10••,43••,57] that is induced by this pairing protocol, are unknown. It is possible that prolonged pairing, as required for induction of STDP, leads to changes in Ca^{2+} signaling that are not revealed in the conditions used to study spine Ca^{2+}. Alternatively, the induction of STDP might require integration of signals downstream of these nonlinearities in Ca^{2+} signaling.

Figure 2



Spike-timing dependent nonlinearities in spine Ca^{2+} signals. Schematic of spine Ca^{2+} transients evoked by a bAP ($\Delta[Ca]_{bAP}$, top), an EPSP ($\Delta[Ca]_{EPSP}$, middle) and a paired EPSP and bAP ($\Delta[Ca]_{pair}$, bottom). **(a)** When an EPSP is followed shortly (50 ms delay) by a bAP, a supralinear Ca^{2+} transient is observed (black trace in the bottom panel) that is larger than that the sum of $\Delta[Ca]_{bAP}$ and $\Delta[Ca]_{EPSP}$ measured in isolation (red trace). **(b)** When the order of the EPSP and bAP is reversed, a sublinear Ca^{2+} transient is observed (black trace in the bottom panel) that is smaller than that the sum of $\Delta[Ca]_{bAP}$ and $\Delta[Ca]_{EPSP}$ measured in isolation (red trace).

Impact of spine morphology on Ca^{2+} signaling

The morphology of dendritic spines is dynamically regulated and can be rapidly altered in response to changes in activity (reviewed in [4,58]). In theory, many aspects of the spine morphology can affect the amplitude and time course of Ca^{2+} signals within the spine head. For example, the surface-to-volume ratio of a spine decreases with spine size. Therefore, if the density of Ca^{2+} sources and Ca^{2+} pumps in the spine head membrane is independent of spine size, one would expect smaller and more prolonged Ca^{2+} transients in spines that have large heads. Similarly, because diffusional equilibration occurs more slowly across long and thin spine necks [59,60,61••,62], if diffusion of Ca^{2+} across the spine neck is significant in clearing Ca^{2+} from the spine head, one would expect Ca^{2+} transients in spines that have long and thin necks to be larger and more prolonged than in those that have short and thick necks. These theoretical constraints on Ca^{2+} signaling are predicted from basic physical principals and have been reviewed many times [1,63,64].

The challenge is to determine which, if any, of these morphological features influence spine Ca^{2+} signaling under physiological conditions — that is, at 37 °C and without perturbations of Ca^{2+} buffering. Both of these conditions are hard to meet. First, at physiological temperatures, brain slices deteriorate quickly and the osmolarity of the rapidly-evaporating bathing solution is difficult to control. As a result, many studies are done at 'near-physiological' temperatures (32–35 °C) or at room temperature (typically 20–24 °C). Data gathered at room temperature is of limited utility in predicting the Ca^{2+} handling properties of spines at 37 °C because of the large and variable effects that temperature has on ion channels, Ca^{2+} buffers, Ca^{2+} pumps and many enzymatic processes (e.g. [65–68]). Second, measurement of intracellular Ca^{2+} requires introduction of a Ca^{2+}-sensitive fluorophore which, by necessity, acts as a Ca^{2+} buffer and consequently perturbs the amplitude, kinetics and spatial spread of Ca^{2+} transients (reviewed in [63]). Although a theoretical framework has been developed that describes the effects of exogenous Ca^{2+} buffers on Ca^{2+} signaling [69–71], it is difficult to apply to Ca^{2+} signals in small, heterogeneous and morphologically complex structures such as spines and dendrites.

Owing to these complications, few studies have measured spine Ca^{2+} under the conditions necessary to test the effects of spine morphology on Ca^{2+} handling. Studies performed at near-physiological temperatures that consider the effects of the Ca^{2+} indicator find that Ca^{2+} is cleared much more rapidly from the spine head by active extrusion than by diffusion across the neck [8]. Therefore, variability in size of the neck is expected to have little impact on spine head Ca^{2+} signals. Furthermore, the lack of significant Ca^{2+} diffusion across the neck is supported by studies that have examined the linearity of Ca^{2+}

handling in the spine. These report that the impulse response estimated from bAP-evoked Ca^{2+} influx can predict the time course of NMDA-receptor-mediated Ca^{2+} transients [8,19••]. The impulse response measured following a spatially uniform (bAP-mediated) Ca^{2+} increase can describe synaptically evoked Ca^{2+} transients in the presence of large gradients across the neck. Therefore, the clearance of Ca^{2+} by movement across the neck must be insignificant in determining the time course of Ca^{2+} signals in the spine. An exception might be short stubby spines [72•] which, by definition, have nearly no neck; however, these spines are also difficult to analyze given the resolution limits of two-photon laser-scanning microscopy (Box 1).

Conclusions

Spines are small membranous protrusions that typically contain a single postsynaptic density. The activation of glutamate receptors and VSCCs located on the spine generates Ca^{2+} transients that, under physiological conditions, are restricted to the stimulated spine. Furthermore, evoked Ca^{2+} transients are modulated on a spine-by-spine basis by regulation of ion channel subunit composition, the activity of kinases and phosphatases, the local membrane potential and the rate of Ca^{2+} extrusion. Lastly, spine Ca^{2+} signaling is nonlinear because Ca^{2+}-dependent processes regulate Ca^{2+} sources and Ca^{2+} extrusion. This multi-level and spatially delimited regulation of Ca^{2+} signals enables each spine to operate as a complex and autonomous cellular compartment that controls many aspects of postsynaptic signals.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Nimchinsky EA, Sabatini BL, Svoboda K: **Structure and function of dendritic spines**. *Annu Rev Physiol* 2002, **64**:313-353.

2. Kennedy MJ, Ehlers MD: **Organelles and trafficking machinery for postsynaptic plasticity**. *Annu Rev Neurosci* 2006, **29**:325-362.

3. Tada T, Sheng M: **Molecular mechanisms of dendritic spine morphogenesis**. *Curr Opin Neurobiol* 2006, **16**:95-101.

4. Alvarez VA, Sabatini BL: **Anatomical and physiological plasticity of dendritic spines**. *Annu Rev Neurosci* 2007 doi: 10.1146/annurev.neuro.30.051606.094222 http://arjournals.annualreviews.org/loi/neuro.

5. Kovalchuk Y, Eilers J, Lisman J, Konnerth A: **NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons**. *J Neurosci* 2000, **20**:1791-1799.

6. •• Bloodgood BL, Sabatini BL: **Nonlinear regulation of unitary synaptic signals by CaV2.3 voltage-sensitive calcium channels located in dendritic spines**. *Neuron* 2007, **53**:249-260.
In this study, two-photon uncaging of glutamate and Ca^{2+} imaging was used to investigate the role of voltage-gated Na^+ and Ca^{2+} channels in shaping synaptic signals at individual spines of CA1 pyramidal neurons in acute hippocampal slices. Synaptic activation of $CaV_{2.3}$ was enhanced by opening of Na^+ channels and generated a Ca^{2+} microdomain within the spine capable of activating SK channels. SK channels repolarized the spine and terminated synaptic signaling through NMDA receptors.

7. Yuste R, Majewska A, Cash SS, Denk W: **Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis**. *J Neurosci* 1999, **19**:1976-1987.

8. Sabatini BL, Oertner TG, Svoboda K: **The life cycle of Ca(2+) ions in dendritic spines**. *Neuron* 2002, **33**:439-452.

9. Koester HJ, Sakmann B: **Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials**. *Proc Natl Acad Sci USA* 1998, **95**:9596-9601.

10. •• Nevian T, Sakmann B: **Spine Ca^{2+} signaling in spike-timing-dependent plasticity**. *J Neurosci* 2006, **26**:11001-11013.
The authors examined the correlation between STDP and spine Ca^{2+} signals evoked by paired synaptic stimulation and bAPs. They conclude that the induction of spike-timing-dependent depression requires the activation of VSCCs, mGlu receptors and phospholipase C, and ultimately endocannabinoid production. This study also showed that the induction of spike-timing-dependent LTP requires a large postsynaptic NMDA-receptor-mediated Ca^{2+} influx.

11. Schiller J, Schiller Y, Clapham DE: **NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation**. *Nat Neurosci* 1998, **1**:114-118.

12. Nevian T, Sakmann B: **Single spine Ca^{2+} signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex**. *J Neurosci* 2004, **24**:1689-1699.

13. Egger V, Svoboda K, Mainen ZF: **Dendrodendritic synaptic signals in olfactory bulb granule cells: local spine boost and global low-threshold spike**. *J Neurosci* 2005, **25**:3521-3530.

14. Carter AG, Sabatini BL: **State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons**. *Neuron* 2004, **44**:483-493.

15. Janssen WG, Vissavajjhala P, Andrews G, Moran T, Hof PR, Morrison JH: **Cellular and synaptic distribution of NR2A and NR2B in macaque monkey and rat hippocampus as visualized with subunit-specific monoclonal antibodies**. *Exp Neurol* 2005, **191(Suppl 1)**:S28-S44.

16. • Sobczyk A, Scheuss V, Svoboda K: **NMDA receptor subunit-dependent [Ca2+] signaling in individual hippocampal dendritic spines**. *J Neurosci* 2005, **25**:6037-6046.
Two-photon glutamate uncaging at single spines was used to demonstrate that NMDA receptor currents and Ca^{2+} transients are highly variable among apical spines of CA1 pyramidal neurons. Pharmacological blockade of NR2B-containing receptors reduced this heterogeneity.

17. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH: **Developmental and regional expression in the rat brain and functional properties of four NMDA receptors**. *Neuron* 1994, **12**:529-540.

18. • Barria A, Malinow R: **NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII**. *Neuron* 2005, **48**:289-301.
The authors examined LTP induction in CA1 pyramidal neurons in organotypic cultures. Cells that expressed forms of NR2A or NR2B that were unable to interact with CaMKII failed to potentiate, but LTP was induced in neurons that expressed a form of NR2A that interacted more strongly with CaMKII. The authors speculate that differences in CAMKII affinity might account for the reduced plasticity observed after the developmental switch from NR2B-containing to NR2A-containing NMDA receptors.

19. •• Sobczyk A, Svoboda K: **Activity-dependent plasticity of the NMDA-receptor fractional Ca^{2+} current**. *Neuron* 2007, **53**:17-24.
The authors showed that in apical spines of CA1 pyramidal neurons from acute hippocampal slices, repetitive activation of NR2B-containing NMDA receptors resulted in decreased NMDA-receptor-mediated current and Ca^{2+} levels. These decreases involved the action of a serine/threonine phosphatase.

20. Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI: **Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression**. *J Neurosci* 2004, **24**:7821-7828.

21. Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT: **Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity**. *Science* 2004, **304**:1021-1024.

22. Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, Massey PV, Bortolotto ZA, Fitzjohn SM, Bashir ZI, Collingridge GL, Lodge D: **Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus**. *Neuropharmacology* 2007, **52**:60-70.

23. Morishita W, Lu W, Smith GB, Nicoll RA, Bear MF, Malenka RC: **Activation of NR2B-containing NMDA receptors is not required for NMDA receptor-dependent long-term depression**. *Neuropharmacology* 2007, **52**:71-76.

24. Berberich S, Punnakkal P, Jensen V, Pawlak V, Seeburg PH, Hvalby O, Kohr G: **Lack of NMDA receptor subtype selectivity for hippocampal long-term potentiation**. *J Neurosci* 2005, **25**:6907-6910.

25. •• Skeberdis VA, Chevaleyre V, Lau CG, Goldberg JH, Pettit DL, Suadicani SO, Lin Y, Bennett MV, Yuste R, Castillo PE *et al.*: **Protein kinase A regulates calcium permeability of NMDA receptors**. *Nat Neurosci* 2006, **9**:501-510.

Using Ca^{2+} imaging, electrophysiology and pharmacology in primary hippocampal neurons, the authors found that the Ca^{2+} permeability of NMDA receptor is regulated by PKA independently of the total channel current.

26. Dell'Acqua ML, Smith KE, Gorski JA, Horne EA, Gibson ES, Gomez LL: **Regulation of neuronal PKA signaling through AKAP targeting dynamics**. *Eur J Cell Biol* 2006, **85**:627-633.

27. Monyer H, Seeburg PH, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B: **Heteromeric NMDA receptors: molecular and functional distinction of subtypes**. *Science* 1992, **256**:1217-1221.

28. Mayer ML, Westbrook GL, Guthrie PB: **Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones**. *Nature* 1984, **309**:261-263.

29. Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A: **Magnesium gates glutamate-activated channels in mouse central neurones**. *Nature* 1984, **307**:462-465.

30. • Ngo-Anh TJ, Bloodgood BL, Lin M, Sabatini BL, Maylie TJ, Adelman JP: **SK channels and NMDA receptors form a Ca^{2+}-mediated feedback loop in dendritic spines**. *Nat Neurosci* 2005, **8**:642-649.

Extracellular stimulation and two-photon glutamate uncaging were used to activate synapses from CA1 pyramidal neurons. Using electrophysiology, pharmacology and two-photon Ca^{2+} imaging, the authors demonstrated that synaptic activation of SK channels located on the spine repolarized the spine, terminated synaptic NMDA receptor signaling and reduced synaptic potentials.

31. Magee JC, Cook EP: **Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons**. *Nat Neurosci* 2000, **3**:895-903.

32. • Faber ESL, Delaney AJ, Sah P: **SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala**. *Nat Neurosci* 2005, **8**:635-641.

In this study, whole-cell patch-clamp recordings were made from pyramidal cells from the lateral amygdala. Synaptic stimulation was shown to activate SK channels that then terminated synaptic NMDA receptor signaling.

33. Cull-Candy S, Kelly L, Farrant M: **Regulation of Ca^{2+}-permeable AMPA receptors: synaptic plasticity and beyond**. *Curr Opin Neurobiol* 2006, **16**:288-297.

34. Burnashev N, Monyer H, Seeburg PH, Sakmann B: **Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit**. *Neuron* 1992, **8**:189-198.

35. Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, Collingridge GL, Isaac JT: **Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation**. *Nat Neurosci* 2006, **9**:602-604.

36. Thiagarajan TC, Lindskog M, Tsien RW: **Adaptation to synaptic inactivity in hippocampal neurons**. *Neuron* 2005, **47**:725-737.

37. Reid CA, Fabian-Fine R, Fine A: **Postsynaptic calcium transients evoked by activation of individual hippocampal mossy fiber synapses**. *J Neurosci* 2001, **21**:2206-2214.

38. Denk W, Sugimori M, Llinas R: **Two types of calcium response limited to single spines in cerebellar Purkinje cells**. *Proc Natl Acad Sci USA* 1995, **92**:8279-8282.

39. Finch EA, Augustine GJ: **Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites**. *Nature* 1998, **396**:753-756.

40. Wang SSH, Denk W, Hausser M: **Coincidence detection in single dendritic spines mediated by calcium release**. *Nat Neurosci* 2000, **3**:1266-1273.

41. Nakamura T, Barbara JG, Nakamura K, Ross WN: **Synergistic release of Ca^{2+} from IP3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials**. *Neuron* 1999, **24**:727-737.

42. Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, Zwingman TA, Tonegawa S: **Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice**. *Cell* 1994, **79**:377-388.

43. •• Bender VA, Bender KJ, Brasier DJ, Feldman DE: **Two coincidence detectors for spike timing-dependent plasticity in somatosensory cortex**. *J Neurosci* 2006, **26**:4166-4177.

In this study, whole-cell recordings were made from pyramidal neurons in layer 2/3 of rat somatosensory cortex and STDP was induced at layer 4 inputs to these cells. The authors showed that LTP was induced by classical NMDA-receptor-dependent mechanisms, whereas LTD induction required mGlu receptor activation and Ca^{2+} signals generated by VSCC activation and release from inositol 1,4,5-trisphosphate (IP_3)-receptor-gated stores. LTD expression was presynaptic and required retrograde endocannabinoid signaling.

44. Huber KM, Roder JC, Bear MF: **Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1**. *J Neurophysiol* 2001, **86**:321-325.

45. Emptage N, Bliss TVP, Fine A: **Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines**. *Neuron* 1999, **22**:115-124.

46. Yuste R, Denk W: **Dendritic spines as basic functional units of neuronal integration**. *Nature* 1995, **375**:682-684.

47. Sabatini BL, Svoboda K: **Analysis of calcium channels in single spines using optical fluctuation analysis**. *Nature* 2000, **408**:589-593.

48. Isope P, Murphy TH: **Low threshold calcium currents in rat cerebellar Purkinje cell dendritic spines are mediated by T-type calcium channels**. *J Physiol* 2005, **562**:257-269.

49. Kulik A *et al.*: **Immunocytochemical localization of the alpha1A subunit of the P/Q-type calcium channel in the rat cerebellum**. *Eur J Neurosci* 2004, **19**:2169-2178.

50. Koester HJ, Sakmann B: **Calcium dynamics associated with action potentials in single nerve terminals of pyramidal cells in layer 2/3 of the young rat neocortex**. *J Physiol* 2000, **529**:625-646.

51. Yasuda R, Sabatini BL, Svoboda K: **Plasticity of calcium channels in dendritic spines**. *Nat Neurosci* 2003, **6**:948-955.

52. Hoogland TM, Saggau P: **Facilitation of L-type Ca^{2+} channels in dendritic spines by activation of {beta}2 adrenergic receptors**. *J Neurosci* 2004, **24**:8416-8427.

53. Tai C, Kuzmiski JB, MacVicar BA: **Muscarinic enhancement of R-type calcium currents in hippocampal CA1 pyramidal neurons**. *J Neurosci* 2006, **26**:6249-6258.

54. •• Scheuss V, Yasuda R, Sobczyk A, Svoboda K: **Nonlinear $[Ca^{2+}]$ signaling in dendrites and spines caused by activity-dependent depression of Ca^{2+} extrusion**. *J Neurosci* 2006, **26**:8183-8194.

Ca^{2+} transients evoked by a single bAP or trains of bAPs were measured in apical spines of CA1 pyramidal neurons from an acute slice. Trains of bAPs evoked Ca^{2+} transients that decayed more slowly than those seen following a single bAP. This effect resulted from Ca^{2+}-dependent suppression of Ca^{2+} extrusion.

55. Jahr CE, Stevens CF: **A quantitative description of NMDA receptor-channel kinetic behavior**. *J Neurosci* 1990, **10**:1830-1837.

56. Lester RA, Clements JD, Westbrook GL, Jahr CE: **Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents**. *Nature* 1990, **346**:565-567.

57. Sjostrom PJ, Turrigiano GG, Nelson SB: **Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors**. *Neuron* 2003, **39**:641-654.

58. Yuste R, Bonhoeffer T: **Morphological changes in dendritic spines associated with long-term synaptic plasticity**. *Annu Rev Neurosci* 2001, **24**:1071-1089.

59. Svoboda K, Tank DW, Denk W: **Direct measurement of coupling between dendritic spines and shafts**. *Science* 1996, **272**:716-719.

60. Korkotian E, Holcman D, Segal M: **Dynamic regulation of spine–dendrite coupling in cultured hippocampal neurons**. *Eur J Neurosci* 2004, **20**:2649-2663.

61. •• Bloodgood BL, Sabatini BL: **Neuronal activity regulates diffusion across the neck of dendritic spines**. *Science* 2005, **310**:866-869.
Diffusional coupling between spines and dendrites of CA1 pyramidal neurons from organotypic culture was examined in this study. The authors observed that the diffusion across the spine neck varied from spine to spine and was regulated by pairing synaptic activation with bAPs. This might provide a mechanism for accumulating molecules within the spine head or determining the amplitude of synaptic potentials.

62. Majewska A, Tashiro A, Yuste R: **Regulation of spine calcium dynamics by rapid spine motility**. *J Neurosci* 2000, **20**:8262-8268.

63. Sabatini BL, Maravall M, Svoboda K: **Ca(2+) signaling in dendritic spines**. *Curr Opin Neurobiol* 2001, **11**:349-356.

64. Yuste R, Majewska A, Holthoff K: **From form to function: calcium compartmentalization in dendritic spines**. *Nat Neurosci* 2000, **3**:653-659.

65. Sabatini BL, Regehr WG: **Timing of neurotransmission at fast synapses in the mammalian brain**. *Nature* 1996, **384**:170-172.

66. Liu Z, Ren J, Murphy TH: **Decoding of synaptic voltage waveforms by specific classes of recombinant high-threshold Ca2+ channels**. *J Physiol* 2003, **553**:473-488.

67. Marshall C, Elias C, Xue XH, Le HD, Omelchenko A, Hryshko LV, Tibbits GF: **Temperature dependence of cardiac Na^+/Ca^{2+} exchanger**. *Ann N Y Acad Sci* 2002, **976**:109-112.

68. Asztely F, Erdemli G, Kullmann DM: **Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake**. *Neuron* 1997, **18**:281-293.

69. Neher E: **Usefulness and limitations of linear approximations to the understanding of Ca++ signals**. *Cell Calcium* 1998, **24**:345-357.

70. Tank DW, Regehr WG, Delaney KR: **A quantitative analysis of presynaptic calcium dynamics that contribute to short-term enhancement**. *J Neurosci* 1995, **15**:7940-7952.

71. Neher E, Augustine GJ: **Calcium gradients and buffers in bovine chromaffin cells**. *J Physiol* 1992, **450**:273-301.

72. • Noguchi J, Matsuzaki M, Ellis-Davies GC, Kasai H: **Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites**. *Neuron* 2005, **46**:609-622.
In this study, glutamate uncaging was used to measure NMDA-receptor-evoked Ca^{2+} signals in dendritic spines and dendrites of hippocampal pyramidal neurons from acute slices. The magnitude of Ca^{2+} accumulation in the dendrite at the base of the spine was found to depend on the morphology of the stimulated spine and was greatest when short, stubby spines were activated.

73. Losonczy A, Magee JC: **Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons**. *Neuron* 2006, **50**:291-307.

74. Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H: **Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons**. *Nat Neurosci* 2001, **4**:1086-1092.

75. Svoboda K, Yasuda R: **Principles of two-photon excitation microscopy and its applications to neuroscience**. *Neuron* 2006, **50**:823-839.