Persistent calcium microdomains in dendritic spines

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Abstract

Calcium is a ubiquitous signaling molecule in the nervous system. Selective induction of a particular pathway may depend on activation of a sensor that reads highly local Ca$^{2+}$ levels. However, if Ca$^{2+}$ equilibration is rapid compared to the kinetics of the sensor the latter strategy would be precluded. To test whether Ca$^{2+}$ gradients persist for sufficiently long to differentially activate calmodulin (CaM) we developed a Monte Carlo model of intracellular Ca$^{2+}$ dynamics in a dendritic spine. The model quantitatively reproduced experimental measures of fluorescent Ca$^{2+}$ transients, and suggests a large, rapid spike in free intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) not predicted by the fluorescent signal. Because of the differential distribution of different Ca$^{2+}$ sources, excitatory postsynaptic potentials (EPSPs), but not action potentials, induced large gradients across the spine. This differential distribution of Ca$^{2+}$ produced location-specific activation of CaM following an EPSP, but a largely location-insensitive activation of CaM following an action potential. These simulations suggest functional Ca$^{2+}$ microdomains can exist in dendritic spines, that Ca$^{2+}$ sensors in spines can be sensitive to the mode of Ca$^{2+}$ entry, and that these can lead to specificity in activation of Ca$^{2+}$-dependent signaling pathways.
Introduction

Calcium is responsible for the induction of a large number of intracellular signaling processes in neurons (Kennedy, 1989), including induction of both long-term potentiation and depression at CA1 pyramidal cells (Malenka, 1994). The reliable activation of one, but not another \( \text{Ca}^{2+} \)-dependent signaling cascade may therefore depend on a sensor that reads the spatio-temporal average of intracellular \( \text{Ca}^{2+} \) in the entire spine ([\( \text{Ca}^{2+} \)])]. Alternatively, very local \( \text{Ca}^{2+} \) levels can be detected by spatially distinct \( \text{Ca}^{2+} \) sensors. In dendrites, but not necessarily spines, specific activation of different protein effectors downstream of \( \text{Ca}^{2+} \) have been shown to depend on the source of \( \text{Ca}^{2+} \) entering the cell, including N-methyl-D-aspartate receptor (NMDAR)-specific (Hardingham et al., 2001; Takasu et al., 2002) and voltage-dependent calcium channels (VDCC) subtype-specific (Deisseroth et al., 1996; Dolmetsch et al., 2001) activation pathways. In the last decade, analysis of \( \text{Ca}^{2+} \) dynamics in single spines of living tissue has been possible by the development of fluorescent \( \text{Ca}^{2+} \)-dependent indicators and the use of multi-photon imaging. Some studies have shown rapid, uniform increases in spine fluorescence following \( \text{Ca}^{2+} \) entry leading to the suggestion that \( \text{Ca}^{2+} \) equilibrates across the spine within a single millisecond, and thus that spines should be considered a well-mixed compartments, with respect to \( \text{Ca}^{2+} \) (Yuste et al., 2000), thus precluding a local \( \text{Ca}^{2+} \) signal detection in dendritic spines.

In hippocampal and neocortical pyramidal cells, the two main sources of spine calcium are VDCCs during a backpropagating action potential, and NMDARs in response to subthreshold synaptic stimulation (Yuste and Denk, 1995; Koester and Sakmann, 1998; Yuste et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002). Immunolabeling of VDCCs in dendritic spines of rat hippocampus have shown these channels to be anywhere on the surface of the spine at asymmetric synapses (Franks et al., 2001). In contrast, NMDARs are located solely at the synapse (Kharazia
et al., 1996; Takumi et al., 1999). The different location of \( \text{Ca}^{2+} \) sources must necessarily result in different spatial \( \text{Ca}^{2+} \) profiles in the spine immediately after influx through the two different sources. However, these profiles will only be physiologically relevant if they can be detected as distinct by calcium’s downstream targets. Calmodulin (CaM), a calcium-dependent protein required for the initiation of many different signaling pathways including LTP and LTD, requires the binding of multiple \( \text{Ca}^{2+} \) ions, and has a relatively low \( \text{Ca}^{2+} \) affinity with slow kinetics (Cohen and Klee, 1988). Thus, if equilibration of \( \text{Ca}^{2+} \) within the spine is extremely rapid, activation of CaM should be independent of the its spatial distribution in the spine or the source of calcium entry.

To test this hypothesis we developed a biophysically realistic Monte Carlo model (Stiles et al., 2001; Stiles and Bartol, 2001) of a dendritic spine that combined the processes of \( \text{Ca}^{2+} \) influx, extrusion and buffering. This enabled simulation of intracellular \( \text{Ca}^{2+} \) dynamics at high temporal and spatial resolution, and modeling of \( \text{Ca}^{2+} \)-dependent signal transduction cascades retaining both the three-dimensional spatial location of individual \( \text{Ca}^{2+} \) ions and its downstream protein targets, and the stochasticity inherent in signaling with small number of molecules. Our model quantitatively reproduced experimental measures of postsynaptic \( \text{Ca}^{2+} \) dynamics following both an action potential and EPSP. We then examined \( \text{Ca}^{2+} \) dynamics at high levels of spatial and temporal resolution and investigated the sensitivity of CaM to its spatial localization and the mode of \( \text{Ca}^{2+} \) entry.
Methods

We used MCell to perform the simulations (Bartol et al., 1991; Stiles et al., 2001; Stiles and Bartol, 2001). The activation of postsynaptic NMDA receptors by glutamate released in an idealized, 3D neuropil have been described elsewhere (Franks et al., 2002). Here we describe the methods used to model the voltage-dependent gating of Ca\(^{2+}\) channels and Ca\(^{2+}\) influx, the binding of Ca\(^{2+}\) to various intracellular binding partners, and establishing intracellular Ca\(^{2+}\) homeostasis.

Scheme and geometry of MCell model

The model consisted of a 4 \(\mu\)m \(\times\) 4 \(\mu\)m \(\times\) 4 \(\mu\)m volume of simplified neuropil composed of cuboidal elements, as described elsewhere (Franks et al., 2002). Within this simplified neuropil matrix, we embedded a segment of dendritic shaft containing a single synaptic spine, consisting of a spine neck 0.5 \(\mu\)m long and The presynaptic bouton consisted of a cuboid 0.5 \(\mu\)m on a side adjacent to the spine head, creating a 20 nm synaptic cleft (Fig. 1A, Table 1).

Voltage-dependent calcium influx

The NEURON Simulation Environment (Hines and Carnevale, 1997) was used to compute the time-series of voltage-dependent open probabilities (\(P_{\text{open}}\)) for each channel-type, which was then converted to kinetic rate constants (Bartol et al., 1991; Stiles and Bartol, 2001) and imported to MCell. By linking MCell with NEURON, it was therefore possible to simulate the entry of Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels.

NEURON Simulations

A compartmental model was simulated to determine dendritic voltage and gating of voltage-dependent parameters using NEURON. The mechanisms underlying initiation of an action po-
potential and its backpropagation into the dendrite are described in detail elsewhere (Mainen et al., 1995; Mainen and Sejnowski, 1996). EPSPs were simulated as synaptic current injections;

\[ I_{\text{syn}}(t) = g_{\text{syn}}(t) (V_m - E_{\text{syn}}) \]  

where \( I_{\text{syn}}(t) \) is the synaptic current and \( E_{\text{syn}} \) is the synaptic reversal potential (0 mV). Synaptic conductance, \( g_{\text{syn}}(t) \), was modeled as an alpha function described by

\[ g_{\text{syn}} = \bar{g}_{\text{syn}} \frac{t - t_{\text{onset}}}{\tau} e^{-(t-t_{\text{onset}})/\tau} \]  

where \( \bar{g}_{\text{syn}} \) is the maximal synaptic conductance, \( \tau \) is the decay constant and \( t_{\text{onset}} \) is the time of the beginning of the EPSP. The whole MCell model was assumed to be isopotential.

Voltage-dependent gating of calcium channels

Low- (LVA) and high voltage-activated (HVA) VDCCs were modeled having two activation \( (m) \) and one inactivation \( (h) \) gate, where the probability of any single channel being open was the product of the state values of each gate,

\[ p_{\text{open}} = m^2 h. \]  

The gating of VDCCs in MCell simulations was represented as a two-state Markov process where channels can be either open or closed. State transitions were governed by opening (\( \beta \)) and closing (\( \alpha \)) rate constants,

\[ \text{Closed} \xrightarrow{\alpha} \text{Open}. \]  

The probability of a channel being open was

\[ P_{\text{open}} = \frac{\beta}{\alpha + \beta}. \]  

\( P_{\text{open}} \) for any channel was calculated for each time point. Holding \( \alpha \) constant at 1000 s\(^{-1} \) (arbitrary) allowed iterative determination of \( \beta \), which was then imported into MCell as a time series
governing the opening rate of VDCCs.

An previous MCell model of ligand-gating of NMDA receptors under zero-Mg\(^{2+}\) conditions (Franks et al., 2002) was modified to take into account for the voltage dependence of the Mg\(^{2+}\) block: The different ligand-gated states of NMDA receptors were supplemented with a voltage-dependent dimension, such that each ligand-dependent state could either be blocked or unblocked. Assuming a constant extracellular Mg\(^{2+}\) concentration of 1 mM, the standard relation (Jahr and Stevens, 1990) was used

\[
U(V_m) = \frac{1}{1 + e^{-\left(0.062 \times V_m\right)}} \cdot \frac{[\text{Mg}^{2+}]_o}{3.57}
\]

where \(U(V_m)\) is the unblocked probability of a single channel. Holding \(\alpha\) at 2000 s\(^{-1}\) (Nowak et al., 1984), \(\beta\) for each time point was determined from Eqn. 5, and imported into MCell as a time series governing the unblocking rate of NMDA channels. We assumed the rates describing ligand-dependent state transitions are identical whether the channel was in a blocked or an unblocked state.

**Calcium currents through open channels**

The large difference between intra- and extracellular Ca\(^{2+}\) concentrations produces rectifying currents through Ca\(^{2+}\) channels best described by the Goldman-Hodgkin-Katz equation. However, due to low K\(^+\) permeabilities these currents often have almost linear I-V relationships at physiological potentials. Therefore, although the actual reversal potential for Ca\(^{2+}\) is independent of channel type, different K\(^+\) permeabilities result in different apparent Ca\(^{2+}\) reversal potentials for different channel types (Hille, 2001). To simulate the passage of ions through an open channel, we therefore assumed ohmic conductances and a constant, non-depleting extracellular Ca\(^{2+}\) concentration. Thus, the rate at which Ca\(^{2+}\) ions entered the cell through a single channel (\(R_{\text{channel}}\))
was

\[ R_{\text{channel}}(V_m) = \gamma_{\text{channel}} \frac{V_m(t) - E_{\text{channel}}}{z \text{ion } e_c} \]  

(7)

where \( \gamma_{\text{channel}} \) is the single channel conductance, \( E_{\text{channel}} \) is the apparent reversal potential, \( z \) is the valence of the ion (\( z = 2 \) for \( \text{Ca}^{2+} \)) and \( e_c \) is the elementary charge, \( 1.6 \times 10^{-19} \text{ C} \). Thus, the average number of ions that entered the cell through a single channel per time-step, \( N_{\text{channel}} \), was

\[ N_{\text{channel}}(V_m) = R_{\text{channel}} \Delta t, \]  

(8)

where \( \Delta t \) is the time interval. The probability that exactly \( n \) ions will enter through an open channel, \( p(n) \), on any single time step was therefore given by

\[ p(n) = \left( \frac{N_{\text{channel}}}{n!} \right)^n e^{-N_{\text{channel}}}. \]  

(9)

For each iteration and channel, the value of a single random number was used to choose \( n \) according to the probability distribution, \( p(n) \). The entry of \( \text{Ca}^{2+} \) ions was modeled by generating the \( n \) ions at the cytoplasmic side of the channel. As voltage changed, \( R_{\text{channel}} \) and \( N_{\text{channel}} \) were updated accordingly.

**Voltage-Dependent Calcium Channels**

Through the range of voltages in the spine (-65 mV to -3 mV; from NEURON simulations), the I-V relationship of HVA channels is linear, with an apparent reversal potential of 25 mV (Magee and Johnston, 1995a). Magee and Johnston (1995) report 2 inactivating HVA and 1 non-inactivating HVA channel per dendritic patch. Their distinction between inactivating and non-inactivating HVA channels was based on differing responses to prolonged step depolarizations. Here, the activating depolarization was the brief action potential, and we assumed deactivation rates far greater than inactivation rates. Consequently, these channels were grouped together as a single type of HVA \( \text{Ca}^{2+} \) channel. Assuming an average patch size of 1.5 \( \mu \text{m}^2 \) (Sakmann and Neher, 1995), we
inserted HVA channels at a density of 3 \( \mu m^{-2} \), corresponding to, on average, \( \sim 4 \) and 48 HVA channels in the spine and dendritic shaft respectively (Fig 1B). These values are consistent with recently published estimates of the number of \( Ca^{2+} \) channels in spines (Sabatini and Svoboda, 2000). We assumed a single-channel conductance of 2.5 pS (Church and Stanley, 1996).

**NMDA Receptors**

NMDA and AMPA receptors were placed on a disk (350 nm diameter) at a density of 210 and 830 /\( \mu m^2 \), respectively, corresponding to approximately 20 NMDA and 80 AMPA receptors per synapse (Fig 1B), consistent with physiological and anatomical estimates (Spruston et al., 1995; Nusser et al., 1998; Takumi et al., 1999). AMPA receptors were assumed to be \( Ca^{2+} \)-impermeable and were therefore only considered as competitors with NMDA receptors for transmitter. Through a range of negative membrane potentials, approximately 10\% of the current through NMDA channels is carried by \( Ca^{2+} \) which, in the absence of the \( Mg^{2+} \) block, has a linear I-V-relationship through the range of physiological voltages (Schneggenburger et al., 1993; Garaschuk et al., 1996; Schneggenburger, 1996). We therefore modeled \( Ca^{2+} \) entry through NMDA channels according to Eqn. 7 with \( g_{NMDA} \) of 4.5 pS, or 10\% of the total 45 pS total conductance (Spruston et al., 1995) and \( E_{NMDA} \) extrapolated to 40 mV (Schneggenburger et al., 1993; Spruston et al., 1995).

**Calcium Leak and Extrusion**

\( Ca^{2+} \) was extruded from the cytoplasm by pumps distributed across membrane surfaces (Fig. 1C). Pumps were modeled with Michaelis-Menten kinetics (\( K_M \) 0.2 \( \mu M \); Carafoli, 1992) according to

\[
Ca^{2+} + Pump_U \xrightleftharpoons[k_3]{k_1} Pump_B \cdot Ca^{2+} \xrightarrow{k_2} Pump_U
\]

(10)

where \( k_3 \) is the turnover rate (100 s\(^{-1}\)), in which a \( Ca^{2+} \)-bound pump returns to the unbound state with the destruction of a \( Ca^{2+} \) ion. \( V_{max} \) was defined as the product of the turnover rate
and pump density, rather than pump concentration, and was defined per unit area. Pumps were placed on the surface of the dendrite endoplasmic reticulum and spine apparatus (int. pumps) at a density of 4,500 µm$^{-2}$ and on the cell membrane (ex. pumps) at a density of 1,200 µm$^{-2}$. Resting Ca$^{2+}$ levels were maintained by balancing extrusion with a nonspecific leak current, assuming a constant density of leak channels on plasma and intracellular membranes. Fixing the rate of each leak channel to 100 ions s$^{-1}$, or $3.2 \times 10^{-17}$ A, a density of 360µm$^{-2}$ channels obtained a dendritic [Ca$^{2+}$]$_{i}$ of 50 nM (Helmchen et al., 1996; Maravall et al., 2000; Majewska et al., 2000a).

**Intracellular Calcium Binding and Calcium Fluorescence**

Endogenous calcium binding proteins (CBPs) were uniformly distributed throughout the cytoplasm of the dendrite, spine and neck (Fig 1D), and were immobile (Helmchen et al., 1996; Lee et al., 2000; Murthy et al., 2000). These bound single, free Ca$^{2+}$ ions reversibly, according to

$$CBP_{\theta} + Ca^{2+} \overset{k_{on}}{\underset{k_{off}}{\rightleftharpoons}} CBP_{\theta} \cdot Ca^{2+} \quad (11)$$

where CBP$_{\theta}$ represents the $\theta$ species of calcium binding protein. The CBPs all had the same affinity but differed in their kinetics (see Table 2). A CBP with calmodulin-like kinetics (CaM) was concentrated in PSD the volume extending 50 nm below the top of the spine, with lower levels in the rest of the spine (see Table 2). CaM bound calcium according to the following scheme:

$$4Ca^{2+} + CaM_{0} \overset{k_{for1}}{\underset{k_{back1}}{\rightleftharpoons}} 3Ca^{2+} + CaM_{1} \overset{k_{for2}}{\underset{k_{back2}}{\rightleftharpoons}} 2Ca^{2+} + CaM_{2} \overset{k_{for3}}{\underset{k_{back3}}{\rightleftharpoons}} Ca^{2+} + CaM_{3} \overset{k_{for4}}{\underset{k_{back4}}{\rightleftharpoons}} CaM_{4} \quad (12)$$

with rate constants listed in Table 2.

In some simulations a CBP with Calcium Green-like kinetics (hereafter Calcium Green) was also included. Fluorescence, F(t), was measured as

$$F(t) = Rf \cdot IB + IU \quad (13)$$
where $I_B$ and $I_U$ are the number of indicator molecules that are $\text{Ca}^{2+}$-bound and $\text{Ca}^{2+}$-free, respectively, and $R_f$ is the difference in fluorescence intensity of the $\text{Ca}^{2+}$-bound versus the $\text{Ca}^{2+}$-free species of indicator; in this case, 9 (Haugland, 1996). Fluorescent transients, $(\Delta F/F)$, were measured as

$$\Delta F(t) = \frac{F(t) - F_0}{F_0}$$

where $F_0$ is the fluorescence averaged over a pre-stimulus interval (Neher and Augustine, 1992). $[\text{Ca}^{2+}]_i$ was measured by counting the number of free $\text{Ca}^{2+}$ ions in a compartment and dividing by its volume. The fluorescence-predicted $\text{Ca}^{2+}$ concentration ($[\text{Ca}^{2+}]_{\text{pred}}$) however, was calculated using the standard equation for determining concentration from fluorescence;

$$[\text{Ca}^{2+}]_{\text{pred}} = K_{D(I)} \frac{F(t) - F_{\text{min}}}{F_{\text{max}} - F(t)}$$

where $K_{D(I)}$ is the dissociation constant of the indicator, 233 nM (Eberhard and Erne, 1991), $F_{\text{min}}$ equals $I_T$, the total number of indicator molecules and $F_{\text{max}}$ equals the product of $I_T$ and $R_f$ (Tsien, 1989).

Fluorescence-predicted $[\text{Ca}^{2+}]_i$ allows the measurement of buffering capacities (Neher and Augustine, 1992; Helmchen et al., 1996; Maravall et al., 2000; Sabatini et al., 2002). The change in $[\text{Ca}^{2+}]_{\text{pred}}$ in a compartment immediately after a brief $\text{Ca}^{2+}$ current, such as an action potential, can be described by

$$\Delta[\text{Ca}^{2+}] = \frac{\Delta[\text{Ca}^{2+}]_T}{1 + \kappa_B + \kappa_S}$$

where $[\text{Ca}^{2+}]_T$ is the total amount of calcium entering the compartment and $\kappa_B$ and $\kappa_S$ are the added and endogenous $\text{Ca}^{2+}$-binding ratios, respectively. $\kappa_B$ is defined as

$$\kappa_B = \frac{\Delta[I \cdot \text{Ca}]}{\Delta[\text{Ca}^{2+}]_i} = \frac{[I]_T K_{D(I)}}{([\text{Ca}^{2+}]_{\text{rest}} + K_{D(I)}) ([\text{Ca}^{2+}]_{\text{peak}} + K_{D(I)})}$$

where $[\text{Ca}^{2+}]_{\text{rest}}$ and $[\text{Ca}^{2+}]_{\text{peak}}$ are resting and peak values of $[\text{Ca}^{2+}]_{\text{pred}}$ and $\Delta[I \cdot \text{Ca}]$ is the increase in the amount of $\text{Ca}^{2+}$-bound indicator. $\kappa_S$ can then be predicted when plotting the inverse
of the change in predicted Ca$^{2+}$ concentration (1/$\Delta$[Ca$^{2+}$]) against $\kappa_B$ (Neher and Augustine, 1992). This value estimates the ratio of Ca$^{2+}$ ions that enter a compartment that are bound by endogenous CBPs to those that remain free. A central assumption in the determination of $\kappa_S$ is that extrusion is very slow compared to buffering. For determination of $\kappa_S$ we therefore measured the change in $[\text{Ca}^{2+}]_{\text{pred}}$ with different concentrations of Calcium Green by running simulations in which the pump and leak densities were set to zero.

A diffusion coefficient of 220 $\mu$m$^2$s$^{-1}$ (Allbritton et al., 1992) was assumed for intracellular calcium. Parameters were either selected or adjusted to simulate experiments conducted between 30-32$^\circ$ C. All MCell simulations were run with a time-step ($\Delta t$) of 100 ns. Because NEURON simulations were run with an integration time step of 25 $\mu$s, all MCell values imported from NEURON were updated only when they changed. The parameter values specified above represent the mean values used. At initialization of each simulation, the number and positions of receptors, channels, pumps and binding proteins were randomly assigned on specified surfaces (Stiles and Bartol, 2001). Simulations were run on a cluster of 933 MHz PC workstations running FreeBSD 4.0. It took approximately 30 hours of computer time to simulate 1 s of real time. Unless stated otherwise, data are given as mean $\pm$ SD.
Results

NEURON Simulations

A layer 5 neocortical pyramidal cell was reconstructed and membrane voltages and currents were simulated using NEURON (Fig 2A). A brief somatic current injection (3 nA for 1.5 msec) induced an action potential that propagated back into the dendritic arbor (Fig 2C). A detailed analysis of the properties of the backpropagating action potential in this model has been reported (Mainen et al., 1995). Peak $P_{\text{open}}$ for HVA channels was 0.54 (Fig 2E), whereas LVA channels were nearly all inactivated at -65 mV (Fig 2G). The unblocked probability of NMDA receptors increased from 0.050 at rest to 0.75 at the peak of the action potential. These data are shown here as unblocking rates (Fig 2I). EPSPs were simulated using an alpha function to approximate the synaptic current.

Paired neocortical pyramidal cells have, on average, between 5 and 6 synaptic connections, and induction of an action potential in one cell results in an EPSP of approximately 2 mV peak amplitude and 30 msec half-width, as measured in the soma of the other (Markram et al, 1997). Five synapses were placed on the cell with a distribution roughly corresponding to those reported in the literature ((Markram et al., 1997) (Fig 2B). Assuming each synapse is reliable and equal in strength, the size and width of each was adjusted equally until an EPSP similar to that of Markram et al (1997) was recorded in the soma. This yielded synapses with $\tau = 2.5$ ms and $g_{\text{max}} = 0.75$ nS (see Eqn. 2). A single EPSP had an amplitude of 5.8 mV when measured locally, but only produced 0.4 mV voltage in the soma (Fig 2D), and corresponds to a 13 nA synaptic current in a neuron with an input resistance of 30 M$\Omega$, consistent with experimental estimates (Otmakhov et al., 1993; Dobrunz and Stevens, 1997). The relatively small increase in membrane voltage associated with an EPSP had almost no effect on voltage-gated channels. The $P_{\text{open}}$ for HVA (Fig 2F) and LVA (Fig 2H) were minimally affected by a single EPSP. EPSPs also only had a small effect on the gating
of NMDA receptors, increasing their peak $P_{open}$ to 0.08 (Fig 2J). Because neither action potentials nor EPSPs activated LVA VDCCs, they were henceforth excluded from the model.

**Voltage-gating of channels in MCell**

Voltage-gating and Ca$^{2+}$ flux through VDCCs and NMDARs were first described using a model of 1 $\mu$m$^2$ patch of membrane, at average densities of 3 $\mu$m$^{-2}$ (range: 0-10 channels) and 210 $\mu$m$^{-2}$ (range: 6-31 receptors), respectively. The peak number of open VDCCs during an action potential was $2.8 \pm 1.5$ ($n = 100$), with the ensemble average of open channels peaking at 1.8 (Fig 3A). NMDARs had a far lower $P_{open}$ during an EPSP, with an average of $1.2 \pm 0.6$ channels opening, and an ensemble average of only $\sim 0.1$ (Fig 3B). The relatively large change in membrane voltage caused a significant decrease in the Ca$^{2+}$ driving force through VDCCs during an action potential (Fig 3C), whereas the small change in membrane potential barely affected the rate of flux through open NMDARs (Fig 3D). The average flux of Ca$^{2+}$ ions through VDCCs peaked at $0.33$ pA·$\mu$m$^{-2}$ with a total flux of 5,200 ions crossing one square micron of membrane following an action potential (Fig. 3E). Release of glutamate resulted in a peak Ca$^{2+}$ current of $\sim 0.03$ pA through NMDARs (current density, 0.3 pA $\mu$m$^2$), with an influx of 7,000 ions within 1 s after transmitter release (Fig. 3F).

**Simulating Ca$^{2+}$ dynamics**

**Calcium dynamics after an action potential**

To compare the output of the model with experimental measures, we combined the mechanisms of Ca$^{2+}$ influx, buffering and extrusion, and included 100 $\mu$M Calcium Green. Figure 4 shows the volume-averaged simulated fluorescent transients measured in the dendritic shaft and spine. The average peak amplitude, $\Delta F/F_{max}$, of the transient was $0.63 \pm 0.084$ (Fig. 4A, $n = 20$) in the
shaft and 1.4 ± 0.62 in the spine (Fig. 4B). These results compare closely with experimental measures (see Discussion for a detailed comparison.) There was more variability in fluorescent signals measured in the spine (CV 0.44) than in the shaft (CV 0.14) due to the lower number of stochastic VDCCs, in agreement with experimental measures (Sabatini and Svoboda, 2000). The fluorescent transients were fitted with single exponential decay time constants of 56 ± 0.64 ms in the shaft and 42 ± 4.4 ms in the spine head.

Fluorescent indicators are used experimentally to indirectly estimate [Ca\(^{2+}\)]. Here, we used the fluorescent transient to adjust parameters to match measured Ca\(^{2+}\) dynamics: if experimentally measured fluorescent transients can be reproduced, the simulation should then make quantitative predictions about the levels of free cytoplasmic Ca\(^{2+}\). Following a single action potential, Ca\(^{2+}\) levels were briefly elevated in the dendrite shaft (0.59 ± 0.10 μM, Fig. 4C) and the spine (5.1 ± 4.4 μM, Fig. 4D). Calcium levels then dropped precipitously with fast and slow decay time constants of 5.5 ms (74 %) and 47 ms in the shaft, and 3.8 ms (83%) and 27 ms in the spine. The fast phase of the decay corresponded to the rapid buffering of free Ca\(^{2+}\) immediately after the VDCCs closed and the slow decay was a function of extrusion and the affinity of the different CBPs. Note that derivation of intracellular Ca\(^{2+}\) concentration from the volume-averaged fluorescence transient assumes spatial uniformity. The fluorescence transient predicted a peak Ca\(^{2+}\) concentration of only 0.12 μM and 0.42 μM in the shaft (Fig. 4C) and spine (Fig. 4D), respectively. This dramatically underestimates and prolongs the free Ca\(^{2+}\) transient. Furthermore, in the absence of indicator, peak Ca\(^{2+}\) levels were dramatically greater than with 100 μM Calcium-Green: shaft, 1.6 ± 0.37 μM; spine, 11 ± 9.4 μM (n = 20). The decay constants for free intracellular Ca\(^{2+}\) under these conditions were 5.4 ms (88%) and 20 ms in the shaft (Fig. 4C) and 3.9 ms (93%) and 18 ms in the spine (Fig. 4D). The model therefore reproduces experimental measures of action potential-induced fluorescent Ca\(^{2+}\) transients, revealing a very large, rapid Ca\(^{2+}\) spike not predicted by
Calcium dynamics after an EPSP

The membrane depolarization of a single, subthreshold EPSP was not sufficient to open HVA VDCCs (Fig. 2H), and LVA VDCCs were inactivated at rest (Fig. 2J) so the only active \( \text{Ca}^{2+} \) source was NMDARs located on the synaptic face of the spine. Quantal release of glutamate (2,000 molecules) resulted in a large and highly variable fluorescent transients in the spine (Fig. 5A), but not in the dendrite (data not shown). The average \( \Delta \text{F/F}_{\text{max}} \) of 30 traces was 1.6 \( \pm \) 0.53, whereas the \( \Delta \text{F/F}_{\text{max}} \) of the ensemble average was 1.1. Individual traces showed sharp fluorescence spikes when \( \text{Ca}^{2+} \) entered the cell (Fig. 5A), whereas the 20%-80% rise-time of the smoothed ensemble averaged transient was 24 msec.

Flickering of some NMDA channels hundreds of milliseconds after peak (Fig. 5B) contaminated the measure of a single exponential decay. If no NMDA channel opened for at least 500 ms before the end of the trace and if the \( (\Delta \text{F/F})_{\text{max}} \) was greater than 0.2, (8 of 30 traces) a single exponential was fit to the falling phase of each transient with a decay constant of 46 \( \pm \) 3.2 msec, which describes the extrusion of \( \text{Ca}^{2+} \) from the cytoplasm, and is similar to the extrusion rate following an action potential. The ensemble averaged transient peaked 80 ms after release and then decayed with a time constant of 103 msec, which reflected both the decay of the NMDA current and the extrusion of \( \text{Ca}^{2+} \) from the spine.

There was no change from baseline \([\text{Ca}^{2+}]_i\) in the volume-averaged dendritic shaft (data not shown). \( \text{Ca}^{2+} \) levels in the spine were highly variable, rising sharply immediately after the NMDARs opened, but decreasing rapidly as channels closed again and the \( \text{Ca}^{2+} \) was buffered. The mean peak \([\text{Ca}^{2+}]_i\) was 4.7 \( \pm \) 3.3 \( \mu \text{M} \) (data not shown) but the peak of the averaged \( \text{Ca}^{2+} \) transient was only 1.0 \( \mu \text{M} \) (Fig. 5B). Again, this difference arose through temporal smoothing of
the $\text{Ca}^{2+}$ spikes. $[\text{Ca}^{2+}]_{\text{pred}}$ more closely matched the temporal properties of free $\text{Ca}^{2+}$ during an EPSP than an action potential, but still greatly underestimated $[\text{Ca}^{2+}]_i$ due to volume-averaging the fluorescent signal from the entire spine (Fig. 3B).

**Measurement of binding ratio**

Transient fluorescent responses to single action potentials were simulated using different concentrations of indicator. Note that the amplitude of the simulated fluorescent transients was smaller and the decay took longer in both the spine and the dendrite with increasing concentrations of indicator (Fig 6A). This relationship between indicator response and the amplitude and decay of the transient allows for quantitative determination of the endogenous binding ratio (Neher and Augustine, 1992). We measured this parameter in the spine and dendrite shaft to verify the robustness, and to ensure that intracellular $\text{Ca}^{2+}$ was correctly handled in our model. Plotting $1/[\text{Ca}^{2+}]_{\text{pred}}$ versus added buffer capacity ($\kappa_B$) in the shaft (Fig. 6B) and spine (Fig. 6C) yielded the expected linear relationship, extrapolation of which allows estimation of the peak amplitude in the presence of zero added buffer, at the y-axis intercept. The predicted peak amplitude of the $\text{Ca}^{2+}$ transient in the dendrite and spine were 0.6 µM and 0.9 µM, in close agreement with experimental measures (0.7 µM and 1.1 µM, Sabatini et al, 2002), but dramatically underestimating the true peak $[\text{Ca}^{2+}]_i$. Extrapolating to the x-axis intercept, $\kappa_S$ values of 55 and 33 were predicted in the shaft and spine, respectively, consistent with recent measurements for CA1 pyramidal cell dendrites (Maravall et al., 2000; Lee et al., 2000; Sabatini et al., 2002). The model therefore produces quantitatively similar results to experimental measures of the peak amplitudes and decay rates of $\text{Ca}^{2+}$ transients following action potentials and EPSP, and of endogenous buffering constants.
Input-Dependent Calcium Gradients Across the Spine

We have shown that the sole source of $\text{Ca}^{2+}$ during an action potential are high-threshold, VDCCs (see Fig. 2 and Fig. 3), which are distributed across the entire surface of the spine. Thus, a backpropagating action potential induces $\text{Ca}^{2+}$ influx from multiple sites across the surface of the spine. To more clearly demonstrate this, we sampled $\text{Ca}^{2+}$ concentration in three distinct regions of the spine; at the PSD, in the middle and at the base of the spine (Fig. 7). No Calcium Green was present in these simulations. Figure 7A shows $\text{Ca}^{2+}$ concentration averaged across the entire spine resulting from a single action potential (i.e one trial). The instantaneous $\text{Ca}^{2+}$ current underlying the $\text{Ca}^{2+}$ influx is shown in Fig. 7B. The VDCCs generating this influx were distributed across the spine, so $\text{Ca}^{2+}$ influx during an action potential did not produce large gradients across the spine (Fig. 7C), but rather raised the $[\text{Ca}^{2+}]_i$ in the spine nearly uniformly.

In contrast, the only source of $\text{Ca}^{2+}$ influx during EPSPs was through NMDARs (Fig. 3) restricted to the surface of the synapse. During a single subthreshold EPSP, the volume averaged $[\text{Ca}^{2+}]_i$ (Fig. 7D) and instantaneous $\text{Ca}^{2+}$ current (Fig. 7E) in the entire spine were comparable in amplitude to that of the action potential, consistent with experimental data (Koester and Sakmann, 1998; Yuste et al., 1999; Sabatini et al., 2002). However, when the spine was again divided into three regions, a large $\text{Ca}^{2+}$ gradient was seen to extend across the spine during an EPSP. Specifically, $\text{Ca}^{2+}$ concentration in the PSD, where the NMDARs were located was extremely high, and decreased to near resting levels at the base of the spine (Fig. 7F).

That such $\text{Ca}^{2+}$ gradients in the spine occur following an EPSP is to be expected, given that NMDARs were located at one end of the structure. However, we have not yet shown whether or not these gradients are physiologically relevant. Is CaM was able to respond to the very rapid spikes in $\text{Ca}^{2+}$ concentration seen at the PSD following influx through flickering NMDARs? To address this, we examined the activation of CaM in the spine under two conditions; with CaM
either concentrated at the PSD (Fig. 8A) or uniformly distributed throughout the spine (Fig. 8B). Note, the total number of CaM molecules were the same in the two configurations. The distribution of CaM within the spine did not affect the Ca\(^{2+}\) dynamics during an action potential (Fig. 8Ci) or EPSP (Fig. 8Di). Synaptic clustering did not significantly affect the amount of active, fully calcified CaM (CaM-4) following an action potential (Fig. 8Cii), although there were slightly greater CaM-4 levels when CaM was uniformly distributed through the spine. An EPSP resulted in high levels of CaM-4 when synaptically clustered, however there was little CaM-4 when CaM was uniformly distributed throughout the spine (Fig. 8Dii). Therefore action potentials lead to nearly uniform increases in spine Ca\(^{2+}\), whereas EPSPs produce very large, very rapid increases in Ca\(^{2+}\) at the postsynaptic density, but almost no change in Ca\(^{2+}\) levels at the base of the spine. Furthermore CaM is sensitive to these large, local and rapid Ca\(^{2+}\) spikes near the source of entry. Thus even molecules with relatively slow mass-action kinetics, like CaM, can respond differentially to slow, global and rapid, highly local Ca\(^{2+}\) signals.
Discussion

Motivation for Monte Carlo Methods

Although Monte Carlo methods have been used to model postsynaptic receptor activation at central synapses (Faber et al., 1992; Wahl et al., 1996; Franks et al., 2002), this is the first use of these methods to model intracellular $\text{Ca}^{2+}$ dynamics in dendritic spines. Monte Carlo methods offer two important advantages over traditional, equation-based numerical methods: First, complex spatial configurations are preserved with little computational expense. These configurations can include both the complex diffusion spaces, for example, derived from serial-section reconstructed neurons or the important spatial relationships of signaling proteins within the neuron. Second, Monte Carlo methods preserve the discrete and stochastic nature of cellular signaling. This is particularly relevant when the number of participating molecules is small. For example, in the relatively large spine used in this model (volume 0.125 fL) a resting $[\text{Ca}^{2+}]_i$ of 50 nM entails between only 3 and 4 free $\text{Ca}^{2+}$ ions. This discreteness becomes increasingly important with detailed simulations using biologically realistic numbers of signaling molecules. Thus accurate modeling of postsynaptic $\text{Ca}^{2+}$ dynamics cannot be derived from using highly smoothed averages of $[\text{Ca}^{2+}]_i$ and $\text{Ca}^{2+}$-activated proteins across a large and non-homogenous volume.

Using biophysically realistic mechanisms for $\text{Ca}^{2+}$ influx, extrusion and buffering using these methods, we have reproduced experimental measures of intracellular $\text{Ca}^{2+}$ dynamics using fluorescent indicators. Although much has been learned from experiments, their spatial and temporal resolution are not sufficiently high to report on $\text{Ca}^{2+}$ dynamics in small structures, like the postsynaptic density at $\mu$s time-scales. Thus, if our model can quantitatively reproduce the macroscopic experimental results, we can then use the model to examine postsynaptic $\text{Ca}^{2+}$ dynamics at more microscopic levels.
Comparison with experiments

The peak amplitudes of simulated fluorescent transients elicited by an action potential was significantly greater in the spine than in the dendritic shaft, consistent with some studies (Yuste et al., 1999; Majewska et al., 2000a; Majewska et al., 2000b; Sabatini and Svoboda, 2000), although others report similar levels of fluorescence in both spines and their parent dendrite (Koester and Sakmann, 1998; Schiller et al., 1998). In all our simulations we have kept the density of HVA channels constant and equal in both the dendrite and the spine. Sabatini et al (2002) report a heterogeneous distribution of VDCC subtypes between the dendrite and spine (Sabatini and Svoboda, 2000), and we should therefore not necessarily expect that channel densities on the two structures are equal. They also report a greater signal variance in spines due to the small number and stochasticity of channels; this result is reproduced here. The rise-time of the fluorescent signal is rapid, at the level of resolution of, and consistent with, imaging studies (Koester and Sakmann, 1998; Majewska et al., 2000a; Sabatini and Svoboda, 2000). The decay of the transient in 100 μM Calcium-Green is fitted to a single exponential with a time-constant similar to that reported in the literature (Markram et al., 1995; Koester and Sakmann, 1998). Some studies report a slower, double-exponential decay of Calcium-Green transients in spines when higher concentrations of indicator are used (Majewska et al., 2000a; Majewska et al., 2000b).

It is understood that increasing exogenous Ca$^{2+}$ buffer decreases the amplitude and slows the decay of the [Ca$^{2+}$]$_i$ transient (Neher and Augustine, 1992), however it is nevertheless assumed that the fluorescent transients reflect the amplitude and time-course of the [Ca$^{2+}$]$_i$ transient for those conditions. Consistent with this, in our simulations, the amplitude of the transient decreased and decay time increased with increasing concentrations of Calcium Green. However, the amplitude of the transient estimated by extrapolating to zero added buffer still dramatically underestimated the true [Ca$^{2+}$]$_i$ in both the spine and dendrite. Furthermore, the decay of all
fluorescent transients could be fitted with single exponentials, whereas the \([\text{Ca}^{2+}]_i\) transient was fitted with a fast and slow double exponential decay not seen by indirect observation of \(\text{Ca}^{2+}\) by fluorescent indicators. The large differences observed in our simulations between the true and fluorescence-predicted peak \(\text{Ca}^{2+}\) concentration may be exaggerated by the assumption that the indicator was immobile. Since Calcium Green in cytosol is \(~3\) times slower than that \(\text{Ca}^{2+}\) in cytosol (Eberhard and Erne, 1991), and may be severely retarded in actin-rich spines (Konishi et al., 1989), the errors introduced by this limitation are expected to be small and qualitative. Nonetheless, it will be important to include the interaction between multiple diffusion species into future simulations of MCell to accurately account for these effects.

There have been different proposals for the mechanism underlying the synaptic \(\text{Ca}^{2+}\) transient. In some cases influx through NMDA channels is assumed to be the primary source of \(\text{Ca}^{2+}\) entry (Koester and Sakmann, 1998; Yuste et al., 1999; Mainen et al., 1999; Kovalchuk et al., 2000). One report suggests that the dominant \(\text{Ca}^{2+}\) source is HVA VDCCs, activated by the depolarization caused by NMDA channels (Schiller et al., 1998). Another group, using imaging methods with lower spatial resolution, has argued that the subthreshold \(\text{Ca}^{2+}\) signal in dendrites is due to \(\text{Ca}^{2+}\) entry through LVA VDCCs (Magee and Johnston, 1995b). Some studies show a complete block of the \(\text{Ca}^{2+}\) transient using a NMDA receptor antagonist (Koester and Sakmann, 1998; Mainen et al., 1999) while others show a small residual influx pathway, possibly due to \(\text{Ca}^{2+}\)-permeable AMPA receptors (Yuste et al., 1999; Kovalchuk et al., 2000). Our model has not allowed for \(\text{Ca}^{2+}\) influx through AMPA receptors. The level of depolarization used in these simulations was not sufficient to activate HVA VDCCs, and LVA VDCCs were inactivated at resting membrane potential as no hyperpolarizing prepulse was delivered (cf Magee and Johnston, 1995). One group has argued that the \(\text{Ca}^{2+}\) influx through NMDA receptors required the AMPA-dependent EPSP to unblock NMDA channels (Yuste et al., 1999) whereas others have shown that most of the \(\text{Ca}^{2+}\) signal
persists after blocking AMPA receptors (Koester and Sakmann, 1998; Kovalchuk et al., 2000). Our simulations support the latter finding (data not shown).

This model does not yet allow for complex signaling pathways, such as Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular stores. Emptage et al (1999) reported that Ca\(^{2+}\) entering the spine through NMDARs mediated Ca\(^{2+}\) release from stores, which underlies most of the Ca\(^{2+}\) signal, however this result has not been reproduced (Mainen et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002). Moreover, our model, which quantitatively matched action potential mediated Ca\(^{2+}\) transients and buffering capacities was able to fully account for the magnitude of the EPSP-mediated Ca\(^{2+}\) transient using conservative estimates for NMDAR number and single channel conductance, therefore suggesting that Ca\(^{2+}\) influx through NMDARs alone is sufficient to account for the EPSP-mediated increase in postsynaptic Ca\(^{2+}\).

**Calcium microdomains**

Calcium is an important second messenger with diverse roles depending on selective activation of different intracellular signal transduction pathways. Specificity of these Ca\(^{2+}\)-dependent pathway activation can depend upon differences in spatio-temporal Ca\(^{2+}\) dynamics. However if the equilibration of Ca\(^{2+}\) within a single compartment is too rapid it would preclude any specificity imparted by spatial localization of different Ca\(^{2+}\) sensors. Indeed, some imaging studies report rapid, uniform increases in spine fluorescence (Yuste et al., 1999; Majewska et al., 2000a) suggesting that spines are well-mixed compartments with respect to Ca\(^{2+}\). However, these studies used high concentrations of high affinity and highly mobile indicators, which greatly increase the temporal aspect and spatial extent of the unperturbed Ca\(^{2+}\) signal (Sabatini et al., 2001). A single, well-mixed compartment also seems inconsistent with the remarkable structural organization of proteins comprising the postsynaptic density (PSD), which appear to form highly organized,
spatially-dependent signaling complexes (Kennedy, 2000; Sheng and Sala, 2001) with a laminar organization of signaling proteins associated with the NMDAR complex (Valtschanoff and Weinberg, 2001). Our simulations now demonstrate that Ca\(^{2+}\) gradients do occur in dendritic spines and, moreover, that these gradients are persistent and able to differentially activate downstream Ca\(^{2+}\) effector proteins. Thus, functional Ca\(^{2+}\) microdomains exist in spines and offer a simple mechanism for imparting selectivity to Ca\(^{2+}\)-dependent signal transduction cascades.

**Summary**

We have presented a Monte Carlo model of intracellular Ca\(^{2+}\) dynamics in spines typical of CA1 pyramidal cell dendrites. The model, which incorporated biophysically realistic mechanisms of Ca\(^{2+}\) influx, extrusion and buffering using experimentally constrained parameters for these processes, reproduced experimental measures of fluorescent Ca\(^{2+}\) transients following both action potentials and EPSPs. Our simulations revealed a large, rapid increase in [Ca\(^{2+}\)]\(_i\) following Ca\(^{2+}\) influx not predicted by the fluorescent transient. This difference between [Ca\(^{2+}\)]\(_i\) and [Ca\(^{2+}\)]\(_{pred}\) was greater following the rapid influx of Ca\(^{2+}\) during an action potential than during the prolonged influx following an EPSP. Closer analysis showed that action potentials resulted in relatively uniform increases in spine Ca\(^{2+}\) when the VDCCs were uniformly distributed at low densities across the cell membrane. By contrast, an EPSP resulted in a large gradient in spine Ca\(^{2+}\) because of the restriction of NMDARs to the synapse. Consequently, the distribution of CaM within the spine did not significantly affect its activation following an action potential. However, synaptic clustering of CaM dramatically increased its activation following an EPSP. Thus, we show that Ca\(^{2+}\) gradients in spines exist for sufficiently long to differentially activate its slow downstream targets. The model is currently being extended to include multiple steps in Ca\(^{2+}\)-activated signaling pathways in the postsynaptic density.
Acknowledgments

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References


Yuste, R., A. Majewska, S. S. Cash, and W. Denk. 1999. Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal

<table>
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Table 1: Geometric parameters used in MCell model.
## Table 2: Parameters used for Ca$^{2+}$ binding.

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<td>Medium CBP concentration</td>
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<td>(Cohen and Klee, 1988)</td>
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Figure Legends

Figure 1
Schematic representation of model. (A) A segment of dendrite contained a single spine (grey). A presynaptic bouton (green) was separated from the synaptic face of the spine by a 20 nm cleft. (B) VDCCs (yellow) were randomly distributed at low densities across the spine and dendrite membrane, whereas NMDARs (red) and AMPARs (not shown) were restricted to a disk centered on the synaptic face of the spine. The spine apparatus is visible through the translucent membrane of the spine head. (C) Extrusion pumps were distributed across cell membrane and surface of the spine apparatus (D) Intracellular CBP’s were uniformly distributed throughout the cytoplasm.

Figure 2
NEURON simulations for voltage-dependent channel gating. (A) Membrane potential measured in a dendritic spine located on the proximal part of the apical dendrite. Action potentials were evoked by short current injections into the soma. (B) EPSPs were simulated by placing 5 synapses at different locations in the dendritic arbor. (C) An action potential initiated in the soma (blue trace) propagated back into the dendrite (red trace). (D) Five EPSPs caused a small deflection in voltage at the soma but a large local increase in the spine. (E) P_{open} of HVA Ca^{2+} channels in the spine during the action potential. (F) Single EPSPs were too small to cause any significant increase in P_{open} of HVA VDCCs. At rest most of the LVA channels were inactivated. An action potential (G) or EPSP (H) only induced a nominal increase in P_{open}. (I) The Mg^{2+} block of the NMDA channel was transiently relieved during the action potential. The unblocked probability is expressed here as an unblocking rate. (J) A single EPSP caused only a small increase in the unblocking rate of NMDA receptors at the synapse.

Figure 3
Calcium currents through VDCCs. (A) Ensemble average (n=40) of open VDCCs across 1 µm² of membrane in response to a backpropagating action potential elicited by a current injection into the soma at t = 0 ms. Inset: Single trials show VDCC stochasticity. (B) Ensemble average (n=100) of open NMDARs across 1 µm² of membrane in response to the release of glutamate at t = 0 ms and a small depolarization. (C) Average rate of Ca^{2+} ion flux through a single VDCC as a function of voltage during an action potential. (D) Average
rate of Ca\(^{2+}\) ion flux through a single NMDAR as a function of voltage during an EPSP. Inset: Probability distribution of \(n\) for a 4.5 pS NMDAR at -65 mV. (E) Current density (solid trace) and cumulative flux of Ca\(^{2+}\) ions across 1 \(\mu\)m\(^2\) during an action potential (dashed trace). (F) Calcium current density from 20 NMDA receptors distributed across the PSD (solid trace) and cumulative flux of Ca\(^{2+}\) ions across the PSD during an EPSP (dashed trace, \(n = 100\)).

**Figure 4**

Calcium dynamics following an action potential. Volume-averaged Calcium-Green fluorescent transients measured in dendritic (A) shaft and (B) spine. Thin grey traces are single trials and thick black traces represent the ensemble average of 20 trials. Calcium concentration in the dendritic (C) shaft and (D) spine with or without 100 \(\mu\)M of Calcium Green. Also shown is the \([\text{Ca}^{2+}]_{\text{pred}}\) derived from the fluorescent transient. Action potentials were elicited by brief current injections to the soma at time indicated by arrows.

**Figure 5**

Calcium dynamics following an EPSP. (A) Volume-averaged Calcium-Green fluorescent transients measured in the spine. Thin grey traces are single trials and thick black traces represent the average 20 trials. (B) Calcium concentration in the spine with or without 100 \(\mu\)M of Calcium Green. Also shown is the \([\text{Ca}^{2+}]_{\text{pred}}\) derived from the fluorescent transient. EPSP were elicited by release of glutamate at time indicated by arrows.

**Figure 6**

Determination of Ca\(^{2+}\) binding ratios in the model. (A) Action potential evoked fluorescent transients in the spine (black line) and dendrite shaft (grey line) simulated with 50 \(\mu\)M, 200 \(\mu\)M and 300 \(\mu\)M Calcium Green. Arrows indicate time of somatic current injection. Note the decrease in amplitude and increased decay with increasing indicator concentrations. Each trace is the average of 20 trials. Inverse amplitude of \([\text{Ca}^{2+}]_{\text{pred}}\) as a function of \(\kappa_B\) in the (B) dendrite shaft and (C). Each point shows the mean ± SD for 20 trials with different concentrations of indicator (50 \(\mu\)M, 100 \(\mu\)M, 150 \(\mu\)M, 200 \(\mu\)M, 250 \(\mu\)M and 300 \(\mu\)M). A regression line (solid line) was fit to the data. Extrapolating this line, the y-intercept (\(\ast\)) gives the estimated amplitude of the unperturbed transient, the x-intercept (arrow) gives the estimated \(\kappa_S\).

**Figure 7**
Input-dependent calcium gradients across the spine. Action potentials did not result in \( \text{Ca}^{2+} \) gradients across the spine. (A) Volume averaged \( [\text{Ca}^{2+}]_{i} \) in the entire spine during an action potential. Time of somatic current injection is indicated by the arrow. (B) Instantaneous \( \text{Ca}^{2+} \) current through VDCCs in the spine during an action potential. (C) Schematic (top right) shows the spine subdivided into three distinct sampling regions; the PSD (red), the middle (green) and the base (blue) of the spine. \( \text{Ca}^{2+} \) concentration in each of the three subregions of the spine during an action potential. Color of traces correspond to the three sampling regions shown in schematic. EPSPs, however, resulted in large \( \text{Ca}^{2+} \) gradients across the spine. (D) Volume averaged \( [\text{Ca}^{2+}]_{i} \) in the entire spine during an EPSP. Arrow indicates time of glutamate release. (B) Instantaneous \( \text{Ca}^{2+} \) current through NMDARs during EPSP. (C) \( \text{Ca}^{2+} \) concentration in each of the three subregions of the spine during an EPSP. Note the large \( \text{Ca}^{2+} \) gradient across the spine.

**Figure 8**

Activation of CaM is sensitive to its distribution and the mode of \( \text{Ca}^{2+} \) entry. Schematic display of the two configurations tested, with CaM either (A) concentrated at the PSD or (B) uniformly distributed through the spine. (C) Action potential-evoked changes in (i) \( [\text{Ca}^{2+}]_{i} \) and (ii) CaM-4 with CaM either clustered at the PSD (red, \( n = 20 \)) or uniformly distributed throughout the spine (black, \( n = 19 \)). (D) EPSP evoked (i) \( [\text{Ca}^{2+}]_{i} \) and (ii) CaM-4 transients with CaM clustered (red, \( n = 19 \)) or uniformly distributed (black, \( n = 30 \)).
Franks et al, 2002.

4.1 VDCCs and NMDARs

A) Bouton and Spine

B) NMDARs and VDCCs

C) Spine apparatus and Pumps

D) CBPs
Franks et al., 2002.
ΔF/F_0.5
200 ms
no indicator
100 µM indicator
pred. [Ca^{2+}]
0.2 µM
200 ms
A
B

A

ΔF/F
0.5
200 ms

B

no indicator
100 µM indicator
pred. [Ca^{2+}]
0.2 µM
200 ms

A

B

no indicator
100 µM indicator
pred. [Ca^{2+}]
Franks et al, 2002.

A

CaM Clustered

B

CaM Diffuse

Ci

[Ca\(^{2+}\)] (µM)

0
5
10

0
100
200

Time (ms)

Clustered

Diffuse

AP

Cii

[CaM-4] (nM)

0
50
100

0
100
200

Time (ms)

Clustered

Diffuse

AP

Di

[Ca\(^{2+}\)] (µM)

0
0.5
1.0
1.5

0
400
800

Time (ms)

Clustered

Diffuse

EPSP

Dii

[CaM-4] (nM)

0
20
40

0
400
800

Time (ms)

Clustered

Diffuse

EPSP