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An MCell model of calcium dynamics and frequency-dependence of calmodulin activation in dendritic spines

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Abstract

Pairing action potentials in synaptically coupled cortical pyramidal cells induces LTP in a frequency-dependent manner (H. Markram et al., Science 275 (1997) 213). Using MCell, which simulated the 3D geometry of the spine and the diffusion and binding of Ca^{2+}, we show that pairing five EPSPs and back-propagating action potentials results in a Ca^{2+} influx into a model dendritic spine that is largely frequency independent but leads to a frequency-dependent activation of postsynaptic calmodulin. Furthermore, we show how altering the availability of calmodulin and the calcium-binding capacity can alter the efficacy and potency of the frequency-response curve. The model shows how the cell can regulate its plasticity by buffering Ca^{2+} signals.

Keywords: Calcium; Calmodulin; Buffering; Frequency-dependence; Long-term potentiation

1. Introduction

Hebbian synaptic plasticity in pyramidal neurons depends on the relative timing of presynaptic and postsynaptic activity [18]. Moreover, when action potentials are paired in synaptically coupled cortical neurons, long-term potentiation (LTP) is induced in a frequency-dependent manner [16]. Specifically, when 5 pairings of pre- and postsynaptic action potentials are presented at, or slower than, 5 Hz, no change in

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synaptic efficacy resulted, however 5 pairings at 10 Hz induces a persistent synaptic potentiation that increases with pairing frequency and saturated at 40 Hz. Calcium-calmodulin-dependent kinase II (CaMK II) in the postsynaptic density (PSD) is, at least initially, dependent on activated calmodulin (CaM) for its own activation, and implicated in the induction of LTP [12]. CaM activation requires the binding of four Ca^{2+} ions, and because CaM's affinity (17 μM) for Ca^{2+} is low relative to resting free intracellular Ca^{2+} concentration, typically around 50 nM, few of the sites are bound at rest [3]. When the cell is active, Ca^{2+} enters through either voltage- or ligand-gated channels [10]. CaM must compete with other intracellular Ca^{2+}-binding proteins (CBPs) for available Ca^{2+}, and only activates when all four sites are bound. Consequently, there is a highly non-linear relationship between Ca^{2+} influx and the activation of its downstream effectors.

If, for a given epoch of neural activity the total influx of Ca^{2+} is constant, then within a range, the total influx of Ca^{2+} depends linearly on the number of such epochs, but not on the frequency of their presentation; intracellular free Ca^{2+} concentration, however, is strongly dependent on pairing frequency [13]. First, the major source of Ca^{2+} in postsynaptic spines are NMDA channels [10,11], localized on the synaptic face [9], and Ca^{2+} diffuses through the cytoplasm from its site of entry. At high pairing frequencies, Ca^{2+} will accumulate near the NMDA channels before it has a chance to diffuse away. Second, pumps slowly return cytosolic Ca^{2+} concentration to resting levels by either pumping it out of the cell or sequestering it in intracellular stores [11]. The shorter the interval between successive pairings, less Ca^{2+} will have been pumped out of the cell since the previous pairing. Endogenous CBPs buffer free Ca^{2+}, but only indirectly affect the dependence of free Ca^{2+} concentration on pairing frequency. When Ca^{2+} first enters the spine it readily binds these proteins, however, their capacity saturates with sufficient Ca^{2+}, and the rate at which they recover is a function of both their K_{off} and the clearance rate via the pumps [13].

In this paper, we explore the relationship between Ca^{2+} dynamics (the activity-dependent influx of Ca^{2+} versus the cellular homeostatic mechanisms to maintain low levels of free intracellular Ca^{2+} and the activation of calmodulin, an intracellular Ca^{2+}-dependent effector protein. We first show how, for a given set of parameters, CaM activation is dependent on input-frequency. Then, by varying either the total amount of available CaM, or by changing the amount of CBPs, show how this frequency-dependence can be modulated. Previous models of Ca^{2+}-activation of calmodulin [6,7] are improved upon here by including individual channels rather than a non-specific Ca^{2+} flux into the cell and including the 3D-spatial organization of the spine. Furthermore, we include competition with other endogenous CBPs, where a previous model did not [6].

2. Methods

To examine the relationship between neural activity and CaM activation, we use MCell (www.mcell.cnl.salk.edu), a Monte Carlo simulator of microphysiology [2].

Briefly, this program allows for the 3D-simulation of Ca^{2+} diffusion by Brownian dynamics random walk and kinetic state transitions of channels and reactive molecules as diffusion-driven bimolecular associations and probabilistic, unimolecular Markov processes. Here, we model Ca^{2+} influx into a postsynaptic spine, modeled as a cube, 0.5 μm along each side, and its binding to various intracellular proteins. Forty NMDA channels are placed on the synaptic face of the spine, and are gated by extracellularly released glutamate according to known rates [4]. High- (HVA) and low (LVA) voltage-activated Ca^{2+} channels are also placed at low densities on the spine membrane (1 μm^2) [14]. Voltage commands for the open probability of the voltage-gated channels and the Mg^{2+}-block of the NMDA receptors are taken from an earlier model [15] using the NEURON simulation environment [5]. Once in the postsynaptic structure, Ca^{2+} can diffuse out of the spine through a neck at the bottom, bind to non-specific CBPs, be extruded or sequestered by pumps or can bind to, and activate, CaM. Four different types of CBPs were distributed throughout the spine. These molecules all bind Ca^{2+} with the same affinity (K_D = 1 μM), but have different kinetics and concentrations: Fast-CBP, K_{on} 10^9 M^{-1} s^{-1}, 80 μM; Medium-CBP, K_{on} 10^8 M^{-1} s^{-1}, 80 μM; Slow-CBP, K_{on} 10^7 M^{-1} s^{-1}, 20 μM; Very-slow-CBP, K_{on} 10^6 M^{-1} s^{-1}, 20 μM [17]. Calmodulin (CaM) was also included in the postsynaptic density (PSD), the top 50 nm of the spine, each of which could bind four Ca^{2+} ions according to experimentally determined rates [3]. Unless otherwise indicated, CaM concentration in the PSD was 200 μM, corresponding to a total spine concentration of 20 μM.

If a response were not frequency-dependent, its expected magnitude would be the product of the number of events and the magnitude of a single event. To determine the frequency-dependence of Ca^{2+} influx into the spine following five pairings, we computed the charge accumulation through HVA, LVA and NMDA channels by integrating each of their currents. These values are then normalized by five times the charge through each of the channel types following a single pairing. Similarly, frequency-dependence of Ca^{2+} concentration and CaM activation are determined by integrating the $[Ca^{2+}]_i$ and [CaM-4] time series for each pairing frequency and normalizing by five times the respective values for a single pairing. Simulations were run on either PC workstations running FreeBSD 4.0 or on Bluehorizon, an IBM 1152-processor, massively parallel supercomputer, at the San Diego Supercomputer Center.

3. Results

To characterize the input frequency-dependence of calmodulin activation, we begin by simulating a single pairing of pre- and postsynaptic action potentials. The presynaptic action potential, represented as an excitatory postsynaptic potential (EPSP) in the postsynaptic cell, occurred 10 ms before the postsynaptic action potential (Fig. 1A). This generates an influx of Ca^{2+}, the details of which are described elsewhere (Franks et al., in preparation) Following a single pairing, most of the Ca^{2+}

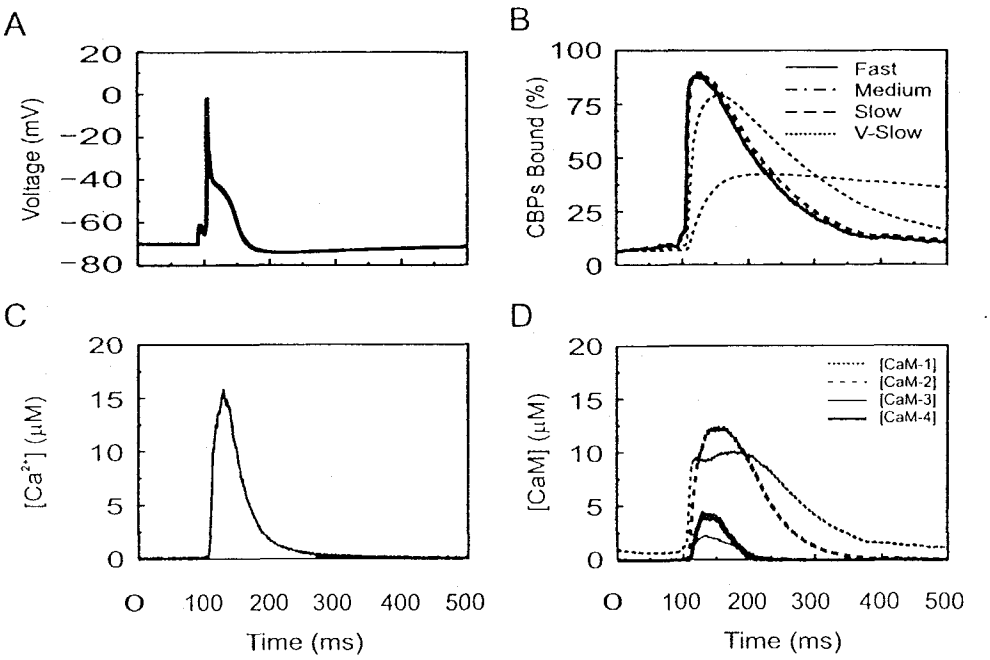

Fig. 1. A single EPSP and AP are paired, with the EPSP preceding the AP by 10 ms: (A) voltage trace, measured in the spine; (B) CBPs with different kinetics respond differentially to Ca^{2+} influx, (C) free Ca^{2+} concentration in the spine; (D) activation of CaM by Ca^{2+} binding. Calmodulin can be in a single, double, triple or, in its active, quadruple-bound form.

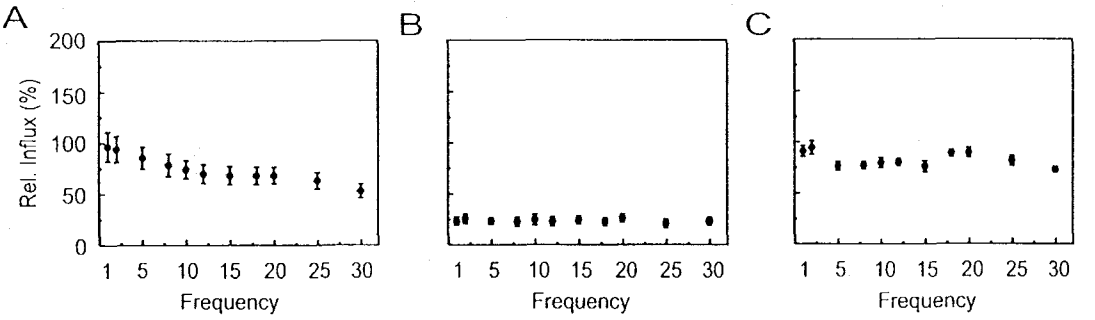

Fig. 2. Ca^{2+} influx is roughly frequency-independent: (A) Ca^{2+} influx from five pairings through HVA channels shows a modest frequency-dependence; (B) relative Ca^{2+} accumulation through LVA channels. These channels are largely activity-independent; (C) relative accumulation of Ca^{2+} through NMDA channels.

is buffered by CBPs, activating at different rates according to their kinetics (Fig. 1B). Some Ca^{2+} remains free, and a pairing results, on average, in peak free $[Ca^{2+}]_i$ of approximately 16 μM (Fig. 1C), corresponding to ~1200 free Ca^{2+} ions in the spine head. Calcium also binds to CaM, in the PSD. Fig. 1D shows the different stages of Ca^{2+}-CaM binding, with, on average, about 5 μM CaM activated by a single pairing.

Next, we presented five such pairings at frequencies ranging from 1 Hz to 30 Hz. Calcium influx through HVA channels is only slightly frequency-dependent (Fig. 2A).

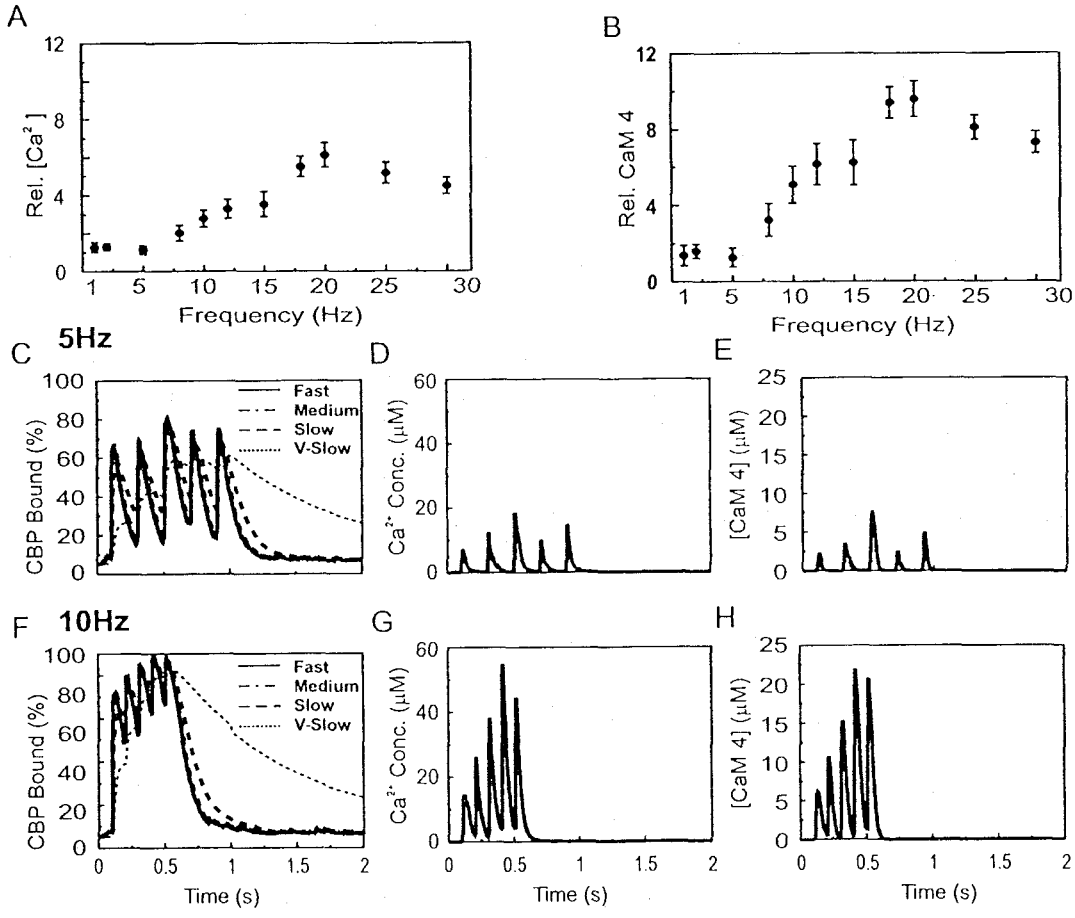

Fig. 3. Ca^{2+} concentration and activation of CaM-4 are strongly frequency-dependent: (A) frequency dependence of $[Ca^{2+}]_i$ in the spine through a range of pairing frequencies; (B) frequency dependence of CaM-4 in the PSD; (C,F) Percentage of different CBPs bound during 5 and 10 Hz trains, respectively; (D,G) Ca^{2+} concentration in the spine during 5 and 10 Hz trains, respectively; (E,H) CaM-4 in the PSD during 5 and 10 Hz trains, respectively.

The modest frequency-dependent decrement in influx is due to channel inactivation (data not shown). Integrated and normalized LVA-mediated Ca^{2+} influxes have values of 0.2 for all frequencies (Fig. 2b), suggesting that Ca^{2+} influx through these channels is activity-independent. Indeed, current through these channels is almost negligible at a resting potential of -65 mV, as most channels are inactivated at this potential (data not shown). Finally, charge through NMDA receptors is also only weakly frequency-dependent (Fig. 2C) due to both interference and receptor desensitization (data not shown).

Both $[Ca^{2+}]_i$ and the quarternary-bound, activated form of calmodulin (CaM-4), on the other hand, are strongly frequency-dependent (Fig. 3A and B). The mechanisms underlying this result are shown to depend upon clearance rates and buffering capacities by comparing CBP occupation at two different frequencies. Following a train of pairings at 5 Hz, the CBPs track the Ca^{2+} influx and unload before the next

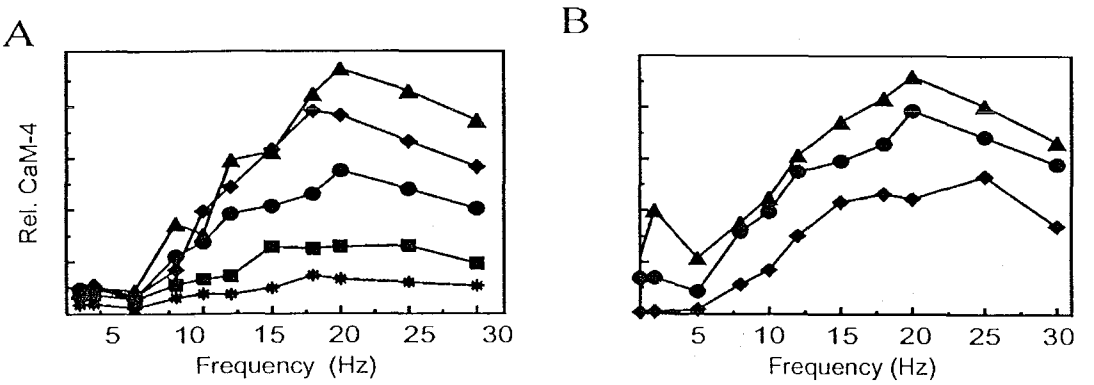

Fig. 4. Intracellular modulation of CaM activation: (A) shows the relation between $[CaM]_{total}$ and frequency-dependence of CaM-4 activation. CaM concentrations in the PSD are: *, 50 μM; ■, 100 μM; ●, 200 μM; ◆, 300 μM; ▲, 400 μM. Total CaM-4 is expressed as integrated CaM-4 molecules over 5 s; (B) Increasing the concentration of CBPs shifts the frequency-dependent CaM-4 activation curve down and right. Total [CBP] in spine: ◆, 100 μM; ●, 200 μM; ▲, 400 μM; represented as integrated CaM-4 over 5 s.

pulse by releasing Ca^{2+} and allowing its extrusion from the spine (Fig. 3C). The unloading of the CBPs between successive pairings results in an approximately constant Ca^{2+} buffering capacity at each pairing, and consequently, little cooperativity in either $[Ca^{2+}]_i$ or CaM-4 from pairing to pairing. Pairings at 10 Hz, however, rapidly saturate the buffering capacity of the CBPs as the pulses are presented before CBPs can unload from a previous pairing (Fig. 3F). Consequently, both $[Ca^{2+}]_i$ (Fig. 3G) and CaM-4 (Fig. 3H) show strong cooperativity, where peaks in both $[Ca^{2+}]_i$ and CaM-4 increase from pairing to pairing. Assuming identical initial conditions, the response to the first pairing should be identical for all pairing frequencies, and it is precisely the degree of cooperativity that endows the system its frequency dependence.

Finally, we further explore the frequency-response function for CaM-4; shown for one set of parameters in Fig. 3B. Total availability places a ceiling on the amount of CaM that can be activated. Changing the concentration of CaM in the PSD shifts the frequency-response curve up or down at high pairing frequencies (Fig. 4A). At saturating pairing frequencies the concentration of CaM-4 is proportional to the total concentration of CaM in the PSD. Changing the concentration of CBPs in the spine alters its Ca^{2+} buffering capacity. Halving or doubling CBP concentration shifts the frequency-response curve left and up, or right and down, respectively (Fig. 4B).

4. Discussion

The buffering actions of other CBPs and the necessity of quadruple Ca^{2+} binding entail a low probability of activating CaM after a single pairing. Similarly, if repeated pairings are presented sufficiently far apart, both Ca^{2+} concentration and CBP occupancy will have returned to resting levels before each next pairing, and the

pairings are read as independent events. As the interval between pairings gets smaller, residual Ca^{2+} from previous pairings decrease competition for CaM and the probability of its activation increases. If enough pairings are presented at a sufficiently high frequency, all CaM will be activated and increasing pairing frequency will have no additional effect on CaM activation. If the amount of CaMKII activation is a predictor of LTP induction, and its activation is CaM-dependent, then the spatio-temporal activation of CaM will govern whether LTP will be induced. CaMKII's requirement for multiple activated CaMs and its concentration in the PSD make it sensitive to a rapid influx of Ca^{2+} through NMDA channels. Here, we show that CaM activation is dependent on the pattern of neuronal activity as well as the Ca^{2+} buffering capacity of other endogenous binding proteins. Thus by regulating the amount of CBPs, a cell can modulate the frequency sensitivity of CaM activation, and consequently, its LTP induction threshold [1]. Such modulation may partly underlie periods of enhanced plasticity in the developing nervous system [8].

Acknowledgements

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