

Imaging the changes in dendritic calcium ion concentration in response to single stimuli using calcium-sensitive dyes and whole-cell recording in rat hippocampal slices

Richard J. Adams* and Terrance J. Sejnowski
Computational Neurobiology Laboratory, Salk Institute, San Diego, USA and *University
Laboratory Of Physiology, Parks Road, Oxford OX1 3PT

Confocal fluorescence microscopy affords an opportunity to measure changes in calcium ion concentration in the dendrites of hippocampal pyramidal cells in live tissue slices. We have used whole-cell recording techniques to fill and record from single pyramidal cells in the region CA1 of transverse slices of rat hippocampus. Slices, 400 μ m thick, were cut from ether-anaesthetized, decapitated 14- to 28-day-old Sprague-Dawley rats. The pipette solution contained (in mM) potassium methanesulphonate, 120; KCl, 5; Hepes, 10; sorbitol, 12; MgCl₂, 2; Mg-ATP, 2; sodium GTP 0.4 and included 200 μ M EGTA and 200 μ M of either fluo-3 or Calcium Green (Molecular Probes) as a fluorescent calcium indicator. Once filled, confocal microscopy was used to image the cells during synaptic stimulation of the Shaffer collateral/commissural pathway or depolarization by direct current injection into the cell. Electrical activity was recorded under current-clamp conditions. Rapid changes in fluorescence in response to intracellular calcium ion concentration were imaged at 2 ms intervals using a line-scanning technique. Computer control of image collection and electrical stimulation and acquisition allows the simultaneous recording of cell activity and association of calcium ion changes with changes in membrane potential. Chief amongst our findings is that transient calcium elevations can be seen in the dendrites of pyramidal cells when the cell fires even a single action potential following orthodromic synaptic stimulation. This indicates to us that voltage-sensitive calcium channels are distributed, at least, several hundred micrometres from the soma. The rise in the calcium level takes place on a time scale that is more rapid than the time resolution of our method, and the fall time is approximately a single exponential with a time constant of 60-80 ms. Secondly, antidromic depolarization of the dendritic membrane by an action potential initiated close to the soma, is sufficient to activate these channels. This implies that a single action potential is capable of depolarizing the apical

dendrite of a pyramidal neuron well beyond that expected by the passive spread of current.

This work is supported by grants from the Human Frontier Science Program and National Institutes of Mental Health MH46482-01-A1.