New and Notable

The Calcium Code

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Calcium ions are perhaps the most versatile ions in the nervous system. In neurons (and other excitable cells) they participate directly in electrical events such as action potentials and slow oscillations, and at synapses they trigger the rapid release of neurotransmitter molecules. Furthermore, calcium ions act as important second messengers for regulating many biochemical processes including those associated with learning and memory and cell death (Ghosh and Greenberg, 1995; Teyler et al., 1994). It is no wonder then that biophysicists and neuroscientists are so interested in measuring changes in the intracellular concentration of calcium ions ($[Ca^{2+}]_i$). Through a number of important technical advances, rapid changes in $[Ca^{2+}]_i$ have recently been measured in synaptic nerve terminals (Regehr and Atluri, 1995; Wu and Saggau, 1994), in dendritic spines, and in the large dendritic trees of pyramidal neurons in the hippocampus and neocortex. In the paper by Helmchen et al. on page 1069 of the February, 1996 issue of the *Biophysical Journal*, the authors have further investigated dendritic $[Ca^{2+}]_i$ in pyramidal neurons. In so doing they have addressed a number of thorny issues in the field and have made a potentially important discovery of yet another role for calcium ions in neuronal information processing.

The ability to measure fast changes in $[Ca^{2+}]_i$ in neurons has come about principally through the development of very efficient indicator dye molecules and sensitive fluorescence detectors. Sakmann and his group, in collaboration with Hans-Ulrich Dodt, have also developed methods for making whole-cell patch recordings from dendrites of pyramidal neurons in normal brain slices. They and others have found that action potentials initiated near the soma back-propagate into the dendrites and elicit an influx of Ca^{2+} throughout most of the dendritic tree. With these techniques and background in hand, Helmchen et al. have addressed four important questions concerning dynamic changes in dendritic $[Ca^{2+}]_i$: How much Ca^{2+} enters the dendrites with each back-propagating action potential? What is the time course of the change in $[Ca^{2+}]_i$ following the action potential? How much of this entering Ca^{2+} remains free? and How much of the endogenous buffering of $[Ca^{2+}]_i$ is done by mobile buffers?

It should be obvious to most biophysicists that these are important quantitative questions. What may not be so obvious is why they have been so difficult to answer with fluorescence imaging techniques. To measure changes in $[Ca^{2+}]_i$, the indicator dyes must bind to the entering Ca^{2+}. The dye thus becomes a foreign Ca^{2+} buffer that alters normal Ca^{2+} homeostasis. For a given influx of Ca^{2+}, the amplitude and time course of the emitted fluorescence will therefore depend on the binding properties of the dye molecules as well as those of the endogenous buffers in the neuron. The higher the dye concentration, the more the fluorescence signals reflect the properties of the dye itself rather than those of the endogenous buffers. Ideally, the dye should be a passive observer of how the neuron handles an influx of Ca^{2+} rather than an active participant.

Helmchen et al. have attempted to overcome this problem by using increasingly low concentrations of their indicator dye (fura-2). They obtained relationships for amplitude and time course of their fluorescence signals as a function of dye concentration. By extrapolating to zero dye concentration, they were able to determine the maximum amplitude and shortest decay time constant that would have occurred with no dye in the neuron. Their estimates for the magnitude and decay time for the increases in $[Ca^{2+}]_i$ with each action potential are larger and shorter than those in previous publications where the dye concentrations were probably much higher.

With their (presumably better) measures of dynamic changes in $[Ca^{2+}]_i$, Helmchen et al. report a remarkably linear relationship between $[Ca^{2+}]_i$ and the frequency of dendritic action potentials. Furthermore, when they continuously varied the firing frequency of the neuron within the range of ~2 to 30 Hz, the changes in $[Ca^{2+}]_i$ were almost a direct replica of the instantaneous frequency (see Fig. 8). This is a highly significant result. Although the levels of intracellular $[Ca^{2+}]$ were previously assumed to follow in some way the number or frequency of action potentials, the relationship was not known. The findings of Helmchen et al. suggest that dendritic $[Ca^{2+}]_i$ actually encodes the frequency of firing. It now remains to determine what cellular processes use this information and how the Ca^{2+} code is deciphered by the neuron.

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This New and Notable paper addresses "Ca^{2+} Buffering and Action Potential-Evoked Ca^{2+} Signaling in Dendrites of Pyramidal Neurons," by Fritjof Helmchen, Keiji Imoto, and Bert Sakmann, which appears in *Biophysical Journal* February, 1996 (Vol. 70, pp. 1069–1081).

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REFERENCES

Ghosh A., and M. E. Greenberg. 1995. Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science*. 268: 239–247.

Teyler, T. J., I. Cavus, C. Coussens, P. DiScenna, L. Grover, Y. P. Lee, and Z. Little. 1994. Multideterminant role of calcium in hippocampal synaptic plasticity. *Hippocampus*. 4:623–634.

Regehr, W. G., and P. P. Atluri. 1995. Calcium transients in cerebellar granule cell presynaptic terminals. *Biophys. J.* 68:2156–2170.

Wu, L. G., and P. Saggau. 1994. Presynaptic calcium is increased during normal synaptic transmission and paired-pulse facilitation, but not in long-term potentiation in area CA1 in hippocampus. *J. Neurosci.* 14:645–654.