PERSPECTIVES

Details of Ca^{2+} dynamics matter

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The analysis of transients in intracellular Ca^{2+} concentration ($[Ca^{2+}]$) has a long and – unfortunately – not so bright history. Although it was recognized early on that Ca^{2+}-indicator dyes act as Ca^{2+} buffers and distort the dynamical aspects of $[Ca^{2+}]$ transients (Timmerman & Ashley, 1986), this was largely neglected in the relevant literature. Researchers got away with ignoring such distortions, because of a peculiar property of Ca^{2+} buffers with respect to rapid $[Ca^{2+}]$ transients induced by action potentials: buffers reduce the amplitudes of such transients, but at the same time lengthen them by about the same factor. Therefore, the area under transients (or the integral over the deviation in $[Ca^{2+}]$ throughout the transient) is unchanged by buffers. Consequently, any Ca^{2+}-sensing effector process is unaffected by their presence, provided that it is linear in $[Ca^{2+}]$ (Neher, 1998). However, many processes in biology are non-linear, which means that quite often the details of the $[Ca^{2+}]$ signal are relevant. The paper by Aponte *et al.* (2008) in this issue of *The Journal of Physiology* is an excellent example of a growing list of studies, in which investigators have made serious and successful attempts to measure unperturbed Ca^{2+} signals. Not surprisingly, it turned out that the signals reveal detail and allow conclusions about roles of $[Ca^{2+}]$ in synaptic transmission that go far beyond those seen with normal (i.e. distorted) Ca^{2+} imaging.

Early work on several types of neurons and chromaffin cells was restricted to measurement of $[Ca^{2+}]$ by fura-2, a high-affinity Ca^{2+} buffer. When such a dye is infused into cells via patch pipettes the endogenous properties of cells are over-ridden by the buffering action of the dye, but they can be estimated by extrapolation of a concentration series to zero buffer concentration (Neher & Augustine, 1992; Helmchen *et al.* 1997; Lee *et al.* 2000). Using this approach, the buffering ratio (i.e. the incremental ratio of bound Ca^{2+} to free Ca^{2+}) of the cytoplasm of several cell types was found to be 40 or somewhat larger (see Neher, 1995 for review of early work). Fura-2 at 100 μM, which is often considered to be a low concentration of indicator dye, has a buffering ratio of approximately 330 at low $[Ca^{2+}]$. Therefore, the buffering by the indicator dye dominates the Ca^{2+} dynamics; in fact, a Ca^{2+} transient following an action potential is attenuated by the dye by almost a factor of 10. More recently, low-affinity indicator dyes have become available, allowing researchers to better match the affinity of the dye to the amplitude of the expected signal (The Handbook, Invitrogen; http://probes.invitrogen.com/handbook/). Thus, complications by saturation of the dye are avoided and buffering effects of the indicator dye can be minimized.

In their current study (this issue) Aponte *et al.* first used fura-2 to carefully estimate the endogenous buffering ratio (κ_s) in apical dendrites of fast spiking hippocampal basket cells. They found a relatively large value of 202, meaning that upon the entry of 203 Ca^{2+} ions, 202 will be bound to endogenous Ca^{2+}-binding sites and 1 will remain free. By extrapolation to zero fura-2 concentration they found the $[Ca^{2+}]$ increment during an action potential (AP) to be 43 nM and the unperturbed decay time constant of that signal to be 360 ms. The high value of κ_s made it relatively easy to also measure these properties directly, since the low-affinity dye fura-FF at 100 μM concentration has a Ca^{2+}-binding ratio of only 24. Analysing averages from 10 single AP responses, Aponte *et al.* arrived at a peak excursion value of 39 nM for a single AP, which appropriately is about 10% lower than the value obtained by extrapolation. The $[Ca^{2+}]$ increment increased linearly with the number of action potentials for short high-frequency trains, while during intense stimulation $[Ca^{2+}]$ signalling was shifted into a non-linear range. The relatively long duration of the transient was implied to set the time window for coincidence detection during LTP.

In contrast, a recent study on the calyx of Held (a giant, calyx-like nerve terminal in the brainstem) showed large transients and quite non-linear behaviour, even at the single-spike level (Müller *et al.* 2007). The calyx has a low intrinsic buffering ratio of 40, AP-induced Ca^{2+} transients of 400 nM, and a biphasic decay of the Ca^{2+} transient, which is governed both by Ca^{2+}-removal mechanisms and by slow binding of Ca^{2+} to the Ca^{2+}-binding protein parvalbumin. This decay correlates with a rapid decay of paired-pulse facilitation (see also Atluri & Regehr, 1996; Caillard *et al.* 2000). However, these properties can be observed only when extreme care is taken not to override the intrinsic buffers by the dye and not to 'washout' endogenous mobile buffers by diffusional loss through the patch pipette.

Both recent studies, discussed here, stress the importance of a subtler handling of Ca^{2+} signals. Only when care is taken not to overwhelm the cell by Ca^{2+} buffering of indicators can one reveal important dynamic aspects, which govern the kinetics of paired-pulse synaptic facilitation or the fine-tuning of LTP induction.

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