

Dendritic calcium dynamics

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Further characterization of the biochemical components that contribute to calcium handling, together with advances in optical imaging of ion concentration, are providing quantitative information on the dynamics of calcium in the dendrites of neurons in tissue culture, brain slices and *in vivo*. It has recently been demonstrated that strong spatial gradients and transient calcium elevations result from the geometry and membrane properties of dendrites. These studies are adding to our understanding of calcium's role in synaptic plasticity and in shaping the electrophysiological properties of neurons.

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Introduction

Calcium currents, the internal release of calcium, and the changes in intracellular free calcium ion concentration to which they contribute are known to be important for the control of neuronal excitability and synaptic plasticity. Characterizing and understanding calcium effects in nerve cell dendrites is complicated by the dendrites' extended size and complex geometry. Calcium influx through voltage-sensitive channels may be profoundly influenced by passive and active dendritic properties. Similarly, the spatial geometries of spines and dendrites contribute to the compartmentalization of calcium and affect the time course of intracellular free calcium ion changes. Sites of internal release may be non-uniform and affect compartmentalization. With so many factors contributing to calcium signaling in dendrites, direct measurements of the spatial distribution of calcium accumulations are essential.

Accommodation in hippocampal pyramidal cells is an example of an electrophysiological property that is shaped by calcium dynamics [1]. In response to a prolonged depolarizing current step, a hippocampal pyramidal cell initially fires action potentials at high frequency, but the frequency gradually declines. When calcium chelators are introduced to the cytoplasm, the cell continues to fire at high frequency throughout the current step; this behavior is called accommodation. This experiment suggests that calcium dynamics contribute to the time course of accommodation. Not only the presence, but also the spatial location, of calcium channels seems to play a vital role in shaping electrophysiological properties. For example, numerical simulation of the firing properties of CA3 pyramidal neurons suggests that the characteristic burst response observed in these cells is a consequence of voltage-dependent

calcium channels being located at sites electrotonically distant from the voltage-gated sodium channels that underlie fast spiking [2].

The spatial and temporal properties of calcium increases in dendrites also play an important role in synaptic plasticity. The central role of calcium in synaptic plasticity has been demonstrated in experiments where calcium chelators injected into postsynaptic cells block long-term potentiation (LTP) [3,4] and long-term depression (LTD) in the hippocampus [5], as well as a variety of other forms of plasticity [6–8]. Interestingly, as both LTP and LTD at hippocampal synapses seem dependent on postsynaptic calcium increases, it may be that a different activation pattern of calcium-dependent enzymes is produced by differences in the duration and amplitude of calcium transients that result from LTP and LTD stimulation paradigms [9].

These examples serve to illustrate a few of the reasons why quantitative measurements of dendritic calcium dynamics, and a better biophysical understanding of how they are produced, are important. In this review, we first briefly discuss the basic biophysical mechanisms contributing to dendritic calcium dynamics, and then review recent quantitative measurements of both the time course and spatial location of intracellular calcium ion changes in dendrites.

Factors contributing to dendritic calcium dynamics

Intracellular calcium dynamics in nerve cell dendrites is controlled by the same cellular components and biophysical mechanisms as in other cellular compart-

Abbreviations

AMPA— α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP5—2-amino-5-phosphonovaleate; IP₃—inositol 1,4,5-trisphosphate; LTD—long-term depression; LTP—long-term potentiation; NMDA—*N*-methyl-D-aspartate.

ments: calcium influx and internal release, buffering, uptake into intracellular organelles, and extrusion into the extracellular space. Calcium can enter dendrites through ligand-gated [10–12,13^{*}] and voltage-gated channels [14]; E McCleskey, this issue, pp 304–312). As shown in Fig. 1a, in the immediate vicinity of open calcium-permeable channels, intracellular calcium levels are thought to rise from resting levels, on the order of 100 nM, to close to millimolar levels [15,16^{**},17,18], creating a microdomain of high calcium. Cellular processes that require these high free calcium ion concentrations are only activated within this domain (reviewed in [19]). When calcium influx stops, radial gradients are expected to dissipate rapidly in dendrites. Recovery to resting levels occurs on a characteristic time scale of hundreds of milliseconds to seconds. The exact rate depends critically on the geometry of the structure, intracellular buffering and extrusion mechanisms.

For these longer times, the simple single compartment model of Fig. 1b, which ignores gradients and diffusion, captures many of the main features of the calcium recovery in chromaffin cells [20^{**}] and nerve terminals [21^{*}], and should be applicable to recovery of calcium in the dendrite. Most of the calcium is bound to cytosolic buffers in cell somas and presynaptic terminals [20^{**},21^{*},22^{*},23^{**}]. As calcium is extruded from the cell, calcium returns to resting levels with an approximately exponential decay for low levels of calcium. The time constant of the decay depends linearly on the buffer capacity of the structure and is inversely proportional to the rate of calcium extrusion [20^{**},21^{*}]. Quantitative measurements of dendritic buffer capacity and extrusion have not been reported. It is known, however, that different types of neurons contain different calcium-binding proteins [24–28]. Ultimately calcium is removed from the dendrites and soma by extrusion into the extracellular space, most probably by the Na⁺/Ca²⁺ exchanger and the ATP-driven calcium pump.

One way in which the model of Fig. 1b is over simplified is that dendrites also contain internal calcium stores [29–34]. As shown in Fig. 1c, the stores consist of membrane-bound compartments that can be loaded with calcium by a calcium ATPase. They contain a high concentration of calcium-binding proteins. Calcium can be released from the stores into the cytoplasm through second messenger gated channels on the surface of the compartment. Known second messengers include calcium ions and inositol 1,4,5-trisphosphate (IP₃) [35].

The unique geometry of dendrites plays an important part in determining calcium dynamics. Dendrites have an aspect ratio of length to diameter that typically exceeds two orders of magnitude and are often covered by small spine-like structures (Fig. 1d). It is estimated that spatial gradients within spine heads and in the radial direction of dendrites will dissipate extremely rapidly, within tens of milliseconds, as calcium diffuses. In contrast, along the length of a dendrite, gradients

can persist for seconds to minutes. The geometry of dendrites will also influence the characteristic recovery time to resting levels. Other factors being equal, calcium dynamics are more rapid in small structures that have a greater surface-to-volume ratio, being most rapid in spines and fine dendrites, and progressively slower in thicker dendrites and the soma. When combined with non-uniform distributions of the elements involved in calcium regulation, the dendritic geometry greatly complicates calcium transients within cells.

Quantitative measurements of dendritic calcium dynamics

Measurements of calcium dynamics in dendrites have primarily been performed using optical methods having a spatial resolution on the micron scale and a temporal resolution in the tens of milliseconds to seconds time scale. Thus, they are insensitive to the microdomains and radial calcium gradients discussed above, but good information is provided along the axial dimension of the dendrite and the time course of calcium extrusion can be accurately determined. We will first discuss optical methods currently being used and then examine the results obtained for specific types of dendrites.

Microfluorimetry using calcium indicator molecules

Fluorescent indicator molecules for calcium ion concentration, such as fura-2, have revolutionized the study of calcium transients in dendrites [36,37]. In most applications, a calcium indicator is introduced into the intracellular compartment of the cell of interest by iontophoresis from a sharp intracellular microelectrode, or diffusional exchange from a patch electrode, although other methods may also be used [38]. Following a short incubation period (typically 10–30 minutes) the indicator diffuses into the dendrites and fills the dendritic arbor. Calcium transients are then detected by microfluorimetry, typically monitoring changes in indicator fluorescence with a sensitive camera, a photomultiplier tube, or photodiodes, often while simultaneously recording from the cell in voltage or current clamp. Confocal microscopy is sometimes used for high spatial resolution imaging of structures in a thin optical section of the preparation.

Optical measurements of calcium levels are, however, not problem free. Quantifying calcium levels within cells using fluorescent indicators is always difficult [19,39^{*}], and in dendrites it is particularly challenging: it is extremely difficult to perform *in vitro* calibrations in cells within brain slices, apparently because calcium ionophores do not have access to structures deep within the slice. Also, background fluorescence of the slice can be quite intense and may not be stationary, and light scatter within a slice makes it hard to resolve fine structures in dendrites that are not at the

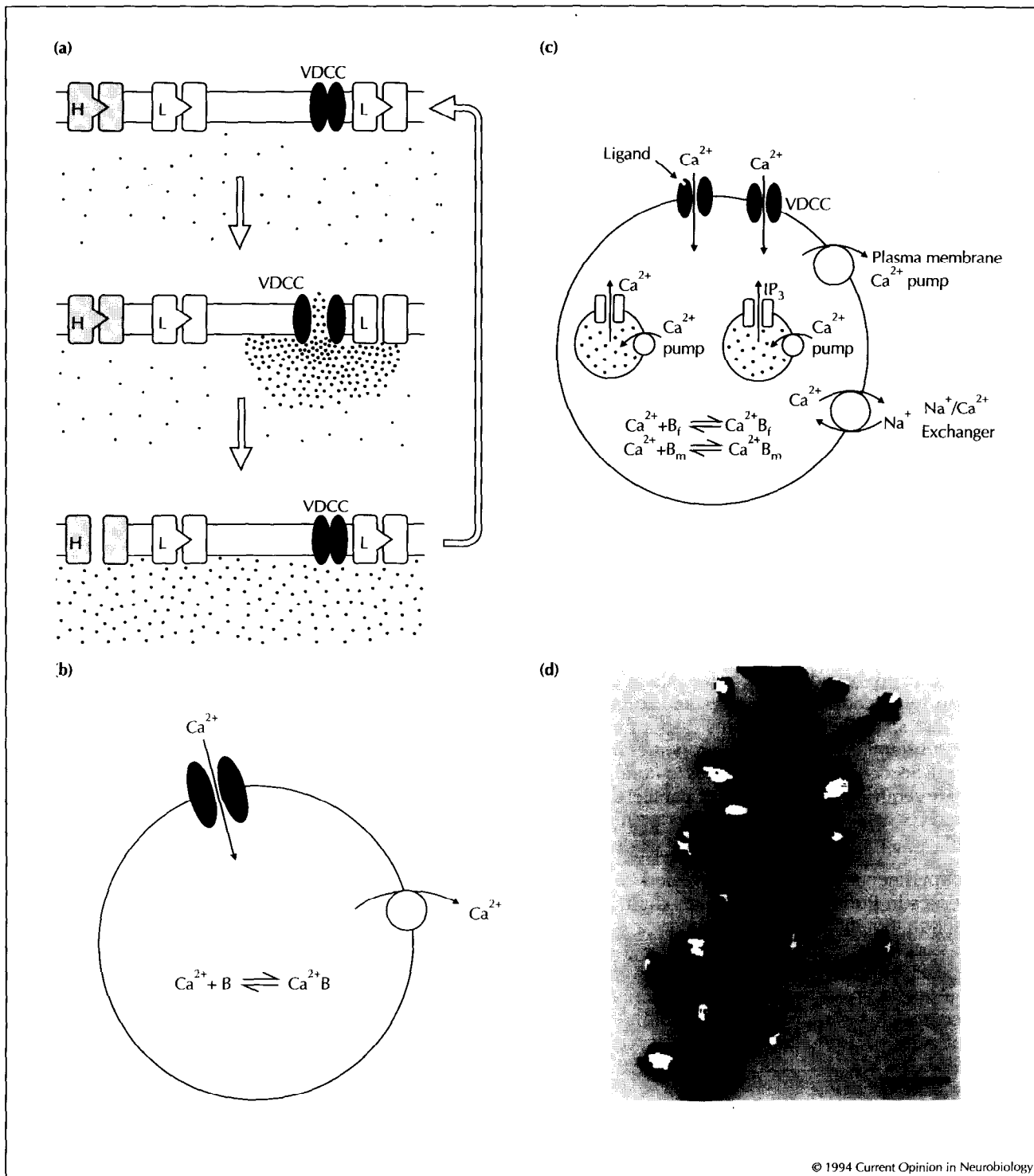


Fig. 1. Calcium regulation in cells. **(a)** A region of plasma membrane containing a voltage-dependent calcium channel (VDCC), and high-affinity (H) and low-affinity (L) calcium-activated channels. At rest, the calcium level within the cell is low. A brief opening of a calcium channel (VDCC) elevates calcium locally, opening a nearby, low-affinity calcium-activated channel. When the calcium channel closes, these local increases dissipate. With time, calcium diffuses throughout the cytoplasm, producing a widespread but modest calcium increase that activates high-affinity but not low-affinity calcium-activated channels. On a much slower time scale, calcium returns to resting levels. **(b)** Simplified model of calcium regulation in cells. Calcium enters the cell, binds to a buffer (B), and is then removed from the cell by an extrusion mechanism. **(c)** A somewhat more realistic representation of regulation inside a cell. Calcium enters the cell through ligand- and voltage-dependent calcium permeable channels, binds to both fixed (B_f) and mobile (B_m) calcium-binding proteins within the cell. It can then enter internal stores or is removed from the cell by calcium pumps on the surface membrane or with the Na^+/Ca^{2+} exchanger. In this diagram, internal stores are represented by two pools of calcium, one gated by IP_3 and the other by calcium. **(d)** A three-dimensional reconstruction of a CA1 hippocampal pyramidal cell dendrite. The dendritic shaft is indicated in dark gray, the spines in light gray and the postsynaptic density in white. This figure was obtained using serial electron microscopy and was provided by KM Harris [81]. The scale bar corresponds to 1 μm .

surface. In extreme cases, intense illumination of cells filled with high concentrations of indicator can produce phototoxicity, typically indicated by the inability to maintain resting calcium levels. This can be easily avoided, but limits signal-to-noise, particularly in small structures such as spines. A more subtle difficulty is that introducing calcium indicators into cells alters calcium dynamics. If the endogenous buffer dominates calcium handling, the effect is small, but if the concentration of added indicator is high, calcium transients and, thus, calcium-dependent electrophysiological properties are significantly changed [20•, 21•, 40–42, 43•]. For example, the addition of an estimated 300 μM fura-2 greatly alters calcium transients within hippocampal pyramidal cell dendrites [43•]. On the other hand, this effect can sometimes be used to advantage: systematic variation of calcium dynamics by the controlled introduction of exogenous buffers can be used to determine if an electrophysiological response is produced by calcium [41, 42].

Calcium transients in Purkinje cell dendrites

The Purkinje cell, with its elaborate dendritic arbor integrating tens of thousands of parallel fiber inputs is largely contained in a single plane, making it particularly well suited for optical measurements. Calcium transients in these cells are interesting for two reasons. First, calcium-based action potentials have been observed in intradendritic recordings and it has been proposed that spatially non-uniform calcium currents contribute substantially to the firing properties of Purkinje cells [44]. Second, calcium increases in Purkinje cell dendrites are required for both LTD of parallel fiber inputs [6], and for activity-induced changes in inhibitory inputs [7, 8].

Imaging experiments have indeed revealed large calcium increases in Purkinje cell dendrites during spontaneous action potentials in response to a synaptic activation, and are seen in response to direct current injection ([6, 45–48, 49•]; see Note added in proof). Accumulations are not restricted to primary branches; significant accumulations appear to be occurring in even the finest dendrites, although imaging at the spine level has not yet been reported. Rapid-imaging techniques have demonstrated dendritic calcium transients associated with individual calcium spikes. Calcium changes in the soma measured with fura-2 are much smaller, and the removal of calcium is much slower than in the dendrites; very little calcium accumulation is observed in the axon.

Although transmembrane calcium currents through dendritic voltage-gated calcium channels [50] undoubtedly contribute to dendritic calcium accumulations, recent results suggest a role for internal release. Purkinje cell dendrites have extensive internal structures, which likely contain internal calcium stores gated by both IP_3 receptors and ryanodine receptors [30–33]. When metabotropic glutamate receptors are activated, calcium accumulations are observed in the soma and

restricted regions of the dendrites [51]. In calcium-free saline, the application of glutamate and quisqualate results in dendritic calcium accumulations [52]. Similarly, photorelease of caged IP_3 produces large dendritic calcium accumulations [53•].

Calcium transients in hippocampal pyramidal cell dendrites

Examination of calcium transients in the dendrites of hippocampal pyramidal cells has been driven in part by the desire to explore the involvement of dendritic calcium in LTP [54, 55]. According to the calcium induction hypothesis, activation of a synapse paired with depolarization of the postsynaptic cell relieves the magnesium block of the NMDA receptor. This allows calcium to enter the associated spine in the postsynaptic cell to trigger an enhancement of synaptic strength. Central to this hypothesis is the idea that spines act as distinct biochemical compartments thereby restricting calcium changes to individual spines. Numerical simulations have provided some important qualitative insights [54, 56, 57], but they have been limited by a lack of quantitative information on the location and density of the elements involved in calcium regulation. In high-resolution imaging experiments, calcium accumulations were primarily restricted to spine heads in synaptically activated hippocampal pyramidal cells [58], and spine head calcium appears to be isolated to some degree from that of the smooth dendrite [59]. Although these two studies support a segregation of calcium signaling, in cultured cortical cells it appears that calcium can escape from spines and spread to dendrites and nearby spines [60•]. This suggests that calcium compartmentalization is far from perfect. Maybe it doesn't have to be. The degree of compartmentalization required to maintain synapse specificity depends upon the sensitivity of the induction mechanism to calcium.

It appears that a large brief calcium signal is required to induce LTP. Imaging experiments clearly show that tetanic stimulation produces dendritic calcium transients, optically measured using high-affinity indicators, which last from hundreds of milliseconds to seconds, suggesting that calcium exerts its actions fairly rapidly within cells [43•, 61, 62, 63•, 64•, 65] (although see [66] for an alternative possibility). Consistent with this time scale is the observation that LTP is induced in cells where calcium accumulations are terminated after two seconds [67•]. Studies using a fluorophore that is sensitive only to very high calcium levels, fura-2 [43•], imply that fura-2 greatly underestimates the calcium levels reached in synaptically activated dendritic regions, suggesting that calcium can reach very high levels in spine heads. High-resolution imaging using such low-affinity dyes should give a better indication of the calcium levels reached in spine heads during synaptic activation.

It is not clear which calcium sources participate in the induction of LTP (i.e. NMDA receptors, voltage-dependen-

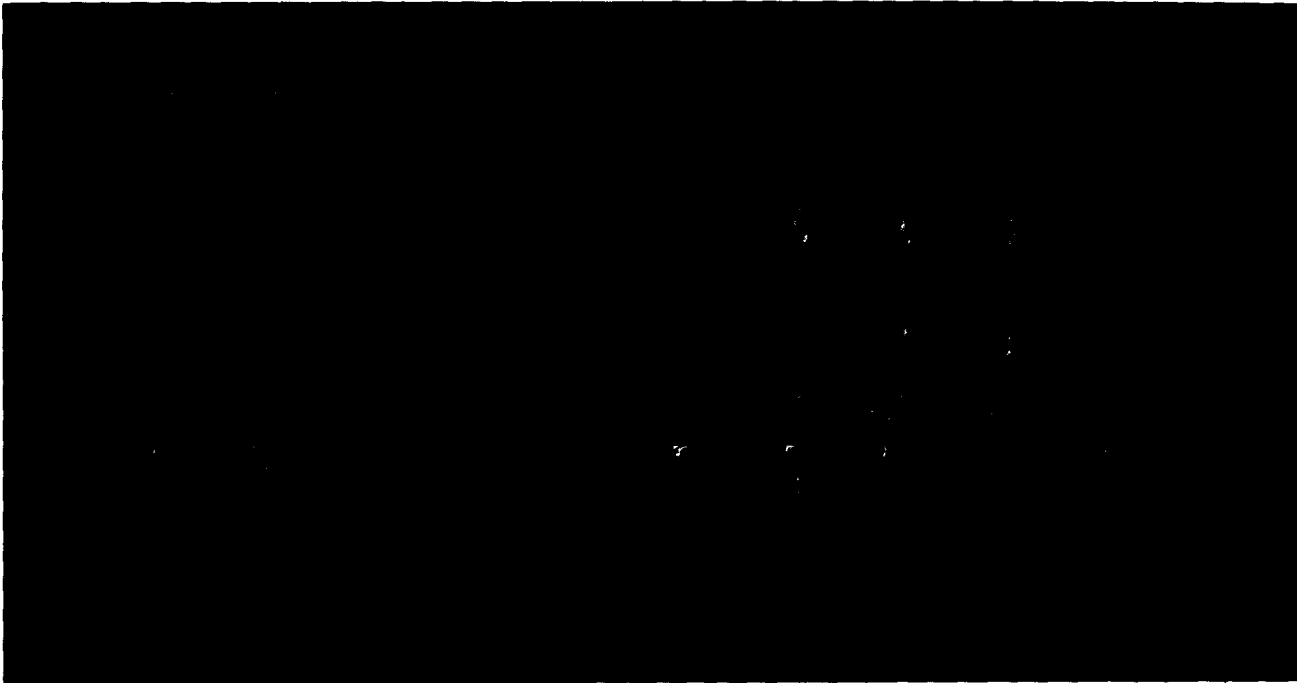


Fig. 2. $[Ca^{2+}]_i$ transients associated with miniature excitatory synaptic currents produced by spontaneous quantal neurotransmitter release in cultured cortical neurons. Images shown reflect $[Ca^{2+}]_i$ at the indicated time in a 10 s run. This dendrite exhibited three separate local increases in $[Ca^{2+}]_i$ within the sampling period. The first transient is initiated at a spine at $t = 4$ s. Over the next 1 s the rise in $[Ca^{2+}]_i$ spreads to involve $\sim 10 \mu m$ of the dendrite and 4–5 other spines. Additional transients are initiated at 7.3 s and 7.7 s. Over the next few seconds, the rise in $[Ca^{2+}]_i$ overlaps in the segment connecting them. These transients appear to be mediated by the activation of NMDA receptors, as they were completely blocked by the antagonist AP5. Calibration $5 \mu m$. Figure adapted with permission from [60**].

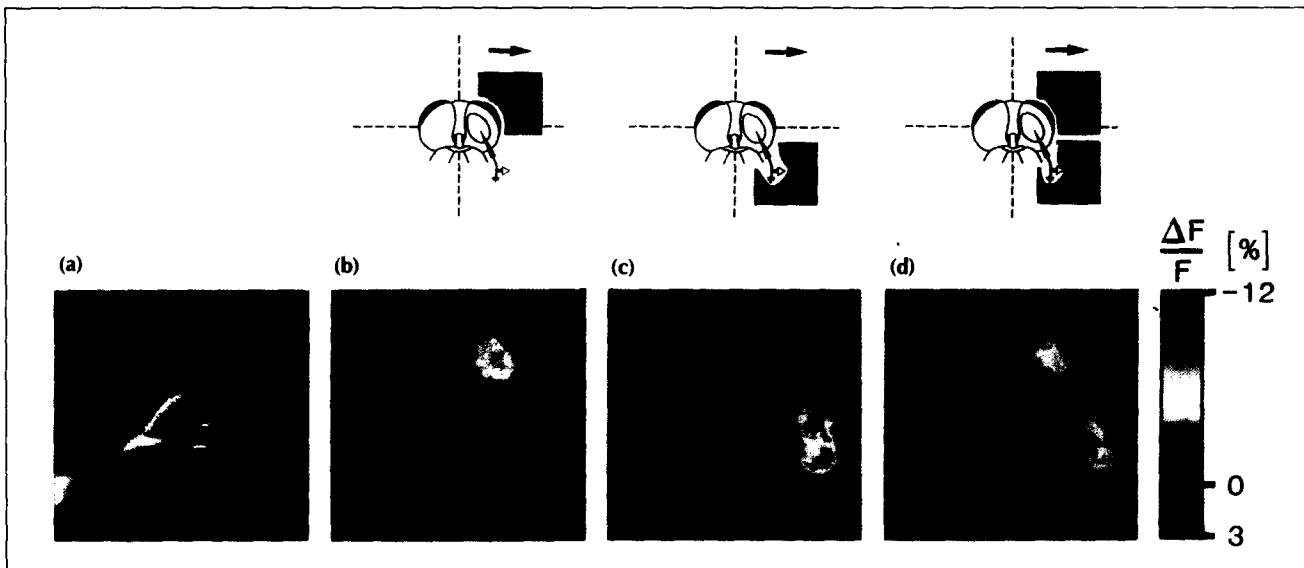


Fig. 3. Localized dendritic calcium increases produced by preferred direction of motion in subregions of the visual field of the fly. (a) Fluorescence image of a motion-sensitive cell filled with fura-2, taken *in vivo*. (b)–(d) Color coded change in fura-2 fluorescence produced by a moving stimulus in the (b) upper, (c) lower, and (d) combined visual fields, as indicated schematically above each figure. Negative $\Delta F/F$ values correspond to an increase in calcium. Figure adapted with permission from [78**].

dent calcium channels, internal stores). Imaging experiments conducted in whole-cell patch clamp, under conditions that eliminate the contribution of voltage-dependent calcium channels, establish that synaptic activation results in large calcium accumulations that are blocked by the NMDA receptor antagonist AP5

(2-amino-5-phosphovalerate) [63*,68*]. These calcium transients are, however, drastically reduced by disrupting the intracellular calcium stores, suggesting that as much as two-thirds of this calcium signal might result from amplification via calcium-induced calcium release. In non-voltage clamped cells, acti-

vation of NMDA receptors may also result in calcium influx directly through the ligand-gated channels, and provide a sustained depolarization that enhances the influx through voltage-gated calcium channels [43*,61,62,64*,65,69]. In addition, recent experiments suggest that activation of the metabotropic glutamate receptor can contribute to calcium accumulation in hippocampal pyramidal cells [70]. Further experiments are clearly needed to clarify the relative contributions of voltage-dependent calcium channels, NMDA receptors and internal stores to the calcium signal that triggers LTP.

Another interesting issue with regard to hippocampal pyramidal cells is what controls the spatial distribution and amplitude of accumulations produced by influx through voltage-gated channels, which has a characteristic pattern that peaks in the proximal dendrites and decreases rapidly with distance from the soma [61]. Certainly the depolarization provided by sodium spikes is important in activating calcium channels and spike invasion will contribute to calcium channel accumulation [43*,64*,71*], however, a differential distribution of calcium channels may also be a factor. Immunohistochemical experiments suggest that in hippocampal pyramidal cells, L-type calcium channels are located on the proximal dendrites and soma [72,73], though N-type calcium channels are more widely distributed throughout the dendrites [74].

Measurements of dendritic calcium have been used to clarify the action of acetylcholine on the firing properties of hippocampal pyramidal cells. A calcium-activated hyperpolarizing current produced by somatic depolarization of the soma is greatly reduced in the presence of cholinergic agonists, thereby reducing accommodation in these cells. What is modified, calcium signaling or potassium channels? Imaging experiments demonstrated that accumulations in dendrites were enhanced by cholinergic agonists, presumably, at least partially, as a result of increased depolarization of voltage-gated calcium channels as the cell fired more action potentials. It was concluded that acetylcholine reduces the calcium-activated potassium conductance [75,76]. A similar approach was used to show that the absence of a slow after-hyperpolarization in non-pyramidal cells was a consequence of the absence of the underlying conductance, and not as a result of a lack of an appropriate calcium signal [77].

Using dendritic calcium transients to examine quantal synaptic transmission

A novel technique employing optical measurements of dendritic calcium transients has recently been developed to study synaptic transmission at individual synapses in cultured cortical neurons [60**]. For experimental conditions designed to maximize the calcium flux through NMDA receptors, calcium transients were detected in short sections of dendrites (Fig. 2) that probably result from the release of single quanta of neurotransmitter. An analysis of these transients sug-

gests that the probability of release varies greatly at different synapses and that individual synapses can be differentially enhanced.

In vivo measurements: functional correlates of calcium dynamics

Almost all of the experiments that have examined dendritic calcium dynamics have been performed in tissue culture or in *in vitro* systems, such as brain slice or isolated invertebrate ganglia. Recent progress has been made in measuring dendritic calcium dynamics *in vivo*. Experiments in two invertebrate systems have been reported: the fly visual system [78**,79] and the cricket auditory system [80*].

In the fly work [78**,79], fura-2 was injected into a large visual interneuron located in the visual neuropil termed the lobula plate, as shown in Fig. 3. The cell imaged has a large arborization of neurites almost parallel to the surface of the brain and could be imaged in the living animal. Transient calcium changes were imaged during presentation of visual stimuli and spatially segregated regions of the arbor showed calcium accumulations for different classes of stimuli, for example, ipsilateral visual motion versus contralateral. These are the first measurements of dendritic calcium transients in cells driven by natural stimulation.

Calcium accumulations evoked by sound stimuli were imaged *in vivo* in the neurites of the omega neuron, a cricket auditory interneuron important in sound localization [80*]. The time course of an electrophysiological correlate of forward masking — a loud sound transiently suppressing the response to subsequent sounds — was demonstrated to be produced by a hyperpolarizing current activated by sound-stimulated dendritic calcium influx. The time course of the hyperpolarizing current, and thus the forward masking effect, were correlated with the time course of the return of residual calcium ion concentration to resting levels, as shown in Fig. 4. This work directly demonstrates a functional role of calcium dynamics in acoustic signal processing.

Conclusions

Optical imaging of calcium levels using fluorescent indicator molecules is providing quantitative measurements of dendritic calcium transients. The observed dynamics can be quite complex and exhibit spatial and temporal characteristics that reflect passive and active cable properties expected of dendrites. Compartmentalization of calcium has been directly demonstrated, both at the level of macroscopic gradients in long dendrites and in single spines. *In vivo* imaging of dendrites under neuroethologically relevant stimuli has recently been demonstrated in invertebrate preparations. These studies are adding to our understanding of the role of calcium dynamics in synaptic plasticity and in shaping the electrophysiological properties of neurons.

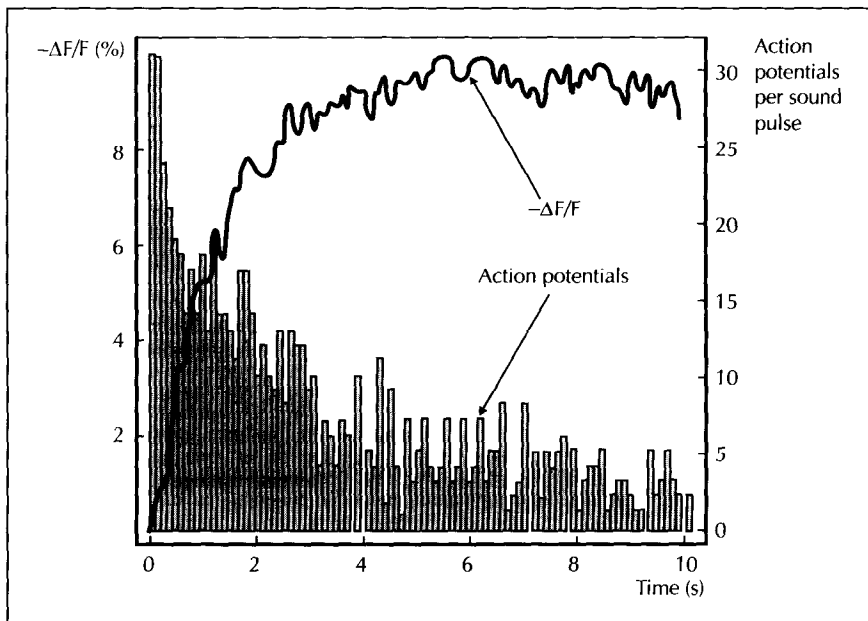


Fig. 4. Dendritic calcium accumulations are correlated with a decrease in firing frequency in the omega neuron, an interneuron in the cricket auditory system. Microfluorometric measurements of calcium accumulation (bold line, an increase in $-\Delta F/F$ of fura-2 indicates an increase in calcium) and the corresponding decrease in the number of action potentials per sound pulse (histogram) qualitatively follow the same time course. The cricket was presented with 4.8 kHz sound amplitude modulated into a pulse train which began at the origin (time=0). Experiments were performed *in vivo*. Figure adapted with permission from [80*].

Note added in proof

Recent studies have strengthened the physiological evidence for calcium-induced calcium release in Purkinje cells [82*].

Interesting results have also recently been obtained, similar to those of [6,45–48,49*], for dendrites of Purkinje cells in turtles [83].

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