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Synaptically Activated Ca^{2+} Release From Internal Stores in CNS Neurons

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SUMMARY

1. Synaptically activated postsynaptic $[Ca^{2+}]_i$ increases occur through three main pathways: Ca^{2+} entry through voltage-gated Ca^{2+} channels, Ca^{2+} entry through ligand-gated channels, and Ca^{2+} release from internal stores. The first two pathways have been studied intensively; release from stores has been the subject of more recent investigations.

2. Ca^{2+} release from stores in CNS neurons primarily occurs as a result of IP_3 mobilized by activation of metabotropic glutamatergic and/or cholingergic receptors coupled to PLC. Ca^{2+} release is localized near spines in Purkinje cells and occurs as a wave in the primary apical dendrites of pyramidal cells in the hippocampus and cortex. The amplitude of the $[Ca^{2+}]_i$ increase can reach several micromolar, significantly larger than the increase due to backpropagating spikes.

3. The large amplitude, long duration, and unique location of the $[Ca^{2+}]_i$ increases due to Ca^{2+} release from stores suggests that these increases can affect specific downstream signaling mechanisms in neurons.

KEY WORDS: dendrite; calcium; pyramidal neuron; IP_3.

Most investigations of the properties of dendrites concentrate on their electrical properties. This is appropriate since the major forms of signaling in the nervous system take place on a very rapid time scale that can only be mediated by action potentials and synaptic potentials. However, many events in neurons take place on a slower time scale. These include gene expression, protein synthesis, and changes in synaptic strength (synaptic plasticity). On this slower time scale other forms of signaling play a prominent role, e.g., enzyme activation by second messengers. One of the most studied signaling mechanisms involves changes in intracellular calcium concentration ($[Ca^{2+}]_i$). Increases in $[Ca^{2+}]_i$ are known to affect all the processes mentioned above.

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Three main synaptic mechanisms affect $[Ca^{2+}]_i$ in neurons. These are Ca^{2+} entry through voltage-gated channels activated by postsynaptic electrical activity, Ca^{2+} entry through ligand-gated channels, and Ca^{2+} release from internal stores, in particular, from the endoplasmic reticulum (ER). Although Ca^{2+} release has only been studied recently, it has now been described in pyramidal neurons from both the CA1 and CA3 regions of the hippocampus, from pyramidal neurons in layer 2/3 and layer 5 regions of the neocortex, from midbrain dopaminergic neurons, and from Purkinje cells in the cerebellum, suggesting that it is a widespread characteristic of neurons in the CNS. In this paper we will first briefly review the first two sources, especially with regard to $[Ca^{2+}]_i$ changes in the dendrites of pyramidal neurons. We will then review more extensively mechanisms involving release from internal stores, concentrating on results from our laboratory.

Ca^{2+} Entry Through Voltage-Gated Channels

Electrical events increase $[Ca^{2+}]_i$ in the dendrites by transiently opening Ca^{2+} channels. Synaptic activation usually initiates Na^+ action potentials in the axon hillock region in both CA1 pyramidal neurons (Turner *et al.*, 1991) and neocortical pyramidal neurons (Stuart and Sakmann, 1994), and these spikes backpropagate over most of the dendritic arbor (Stuart and Sakmann, 1994; Spruston *et al.*, 1995). In the hippocampus the extent of backpropagation can be modulated by activity (Callaway and Ross, 1995; Spruston *et al.*, 1995), by neurotransmitters (Tsubokawa and Ross, 1997; Sandler and Ross, 1999; Poolos *et al.*, 2002), dendritically targeted synaptic excitation (Magee and Johnston, 1995), and synaptic inhibition (Tsubokawa and Ross, 1996). Underlying these complex electrical properties is a heterogeneous distribution of voltage-dependent channels (Magee, 1999) including Ca^{2+} channels, which are found at all dendritic locations including spines (Yuste and Denk, 1995; Sabatini *et al.*, 2002).

Many other neurons have action potentials that backpropagate over the dendrites. These include mitral cells in the olfactory bulb (Chen *et al.*, 1997) and neurons in the substantia nigra (Hausser *et al.*, 1995), as well as several kinds of interneurons. However, not all neurons support backpropagating spikes. Most prominent of these is the Purkinje cell in the cerebellum, where spike propagation in the dendrites is entirely passive (Stuart and Hausser, 1994).

Single backpropagating action potentials generate very fast $[Ca^{2+}]_i$ changes, usually rising within 2 ms (Markram *et al.*, 1995; Sabatini *et al.*, 2002) and decaying in 100 ms or less when measured with low concentrations of Ca^{2+} indicators (Helmchen *et al.*, 1996) or low affinity indicators (Maravall *et al.*, 2000). Since the spikes usually propagate over the entire dendritic tree, the associated $[Ca^{2+}]_i$ increases are detected everywhere (Jaffe *et al.*, 1992; Schiller *et al.*, 1995; Larkum *et al.*, 2003), but the largest increases are in the proximal apical and basal dendrites (Jaffe *et al.*, 1992; Regehr and Tank, 1992; Schiller *et al.*, 1995; Larkum *et al.*, 2003). Spike-evoked Ca^{2+} influx is sufficient to synergistically enhance the release of Ca^{2+} from stores (Nakamura *et al.*, 1999) and can initiate changes in gene expression in some circumstances (Dudek and Fields, 2002). Furthermore, the spikes themselves enhance the entry of Ca^{2+} through NMDA receptors (Yuste and Denk, 1995; Koester and Sakmann, 1998).

Synaptic potentials themselves usually evoke very little or undetectable increase in $[Ca^{2+}]_i$.

Figure 1 shows an example of a rapid $[Ca^{2+}]_i$ increase in the dendrites of a CA1 pyramidal neuron generated by an action potential evoked by repetitive synaptic stimulation. Two different methods are used to display the increases. In one, the increases in selected regions of interest (ROIs) are plotted above the electrical recording in the soma. It is clear that the $[Ca^{2+}]_i$ changes are essentially synchronous at the two locations and occur at the time as the action potential. The other method uses a variation of the "line scan" where the amplitude at a string of selected pixels

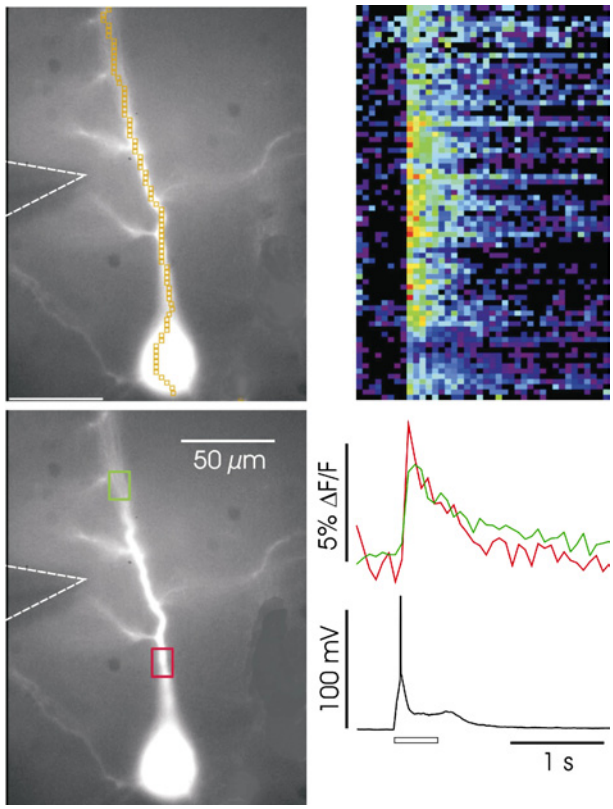

Fig. 1. Synaptically activated action potentials generate rapid $[Ca^{2+}]_i$ changes at all locations in the dendrites. Bottom: Repetitive synaptic stimulation (open rectangle)generated an action potential that evoked a rapid $[Ca^{2+}]_i$ increase in the dendrites. Essentially the same response was recorded at the two ROIs (red and green boxes). Top: The same data displayed in "line scan" mode. The pseudocolor image shows the $[Ca^{2+}]_i$ increases at all the locations along the dendrite shown with yellow boxes. Increases were detected at all dendritic locations. The somatic recording electrode contained 150 μM bis-fura-2. Watanabe *et al.* (unpublished data).

along the dendrite is displayed in pseudocolor as a function of time. From this display it is clear that the spike propagated over the entire dendritic region imaged by the camera and that there are Ca^{2+} channels at all locations.

The spike-evoked transients are particularly fast in spines (Sabatini *et al.*, 2002), often decaying in less than 15 ms. The rapid kinetics of these Ca^{2+} transients have two important consequences. First, the $[Ca^{2+}]_i$ increase from a train of spikes does not build up to a high value because the transients from one spike would have almost recovered before a second one is initiated. Therefore, $[Ca^{2+}]_i$ changes from a train are rarely larger than 1 μM (Helmchen *et al.*, 1996). Second, the transients are too fast to effectively activate some enzymes, like calmodulin, that have slow kinetics (Klee, 1988; Sabatini *et al.*, 2002), making spikes a less effective signaling mechanism than events that generate slower Ca^{2+} transients. In addition, the $[Ca^{2+}]_i$ increases from spikes are highest just under the membrane and may not activate downstream mechanisms closer to the center of the dendrite or soma.

Ligand-Gated Ca^{2+} Entry

Synaptic activation or glutamate application can increase $[Ca^{2+}]_i$ in pyramidal cell dendrites by direct Ca^{2+} entry through glutamate receptors. Most entry is through NMDA receptors although recent experiments have demonstrated $[Ca^{2+}]_i$ increases from entry through Ca^{2+} permeable AMPA channels in aspiny dendrites (Goldberg *et al.*, 2003). The APV (an NMDA receptor blocker)-sensitive $[Ca^{2+}]_i$ increase is mostly in spines (Koester and Sakmann, 1998; Kovalchuck *et al.*, 2000; Sabatini *et al.*, 2002) with little subsequent diffusion into the dendritic shaft (Sabatini *et al.*, 2002; however, see Majewska *et al.*, 2000). Because of the small spine volume, the peak amplitudes reach more than 1 μM (Petrozzino *et al.*, 1995; Sabatini *et al.*, 2002). The transients begin without a significant delay following synaptic stimulation, but they last longer than the spike-evoked transients, mostly because of the long open times of NMDA receptor channels (Sabatini *et al.*, 2002). Since most spines are found on oblique dendrites in pyramidal neurons (Bannister and Larkmann, 1995) the $[Ca^{2+}]_i$ increases are largest in these oblique processes, and are of much smaller amplitude in the main apical shaft and soma (Nakamura *et al.*, 2002). This distribution of $[Ca^{2+}]_i$ changes means that downstream signaling molecules near spines will be activated by this pathway but molecules in other parts of the dendrites and soma may be unaffected. In particular, gene expression is unlikely to be directly activated by these localized $[Ca^{2+}]_i$ increases.

Figure 2 shows an example of a localized $[Ca^{2+}]_i$ increase in the dendrite of a pyramidal neuron recorded with high speed confocal microscopy. The highest increase was in the spine although there was some change in the dendrite close to the spine. The increase was blocked with the application of 50 μM APV showing that it was driven by Ca^{2+} entry through the NMDA receptor. This localized increase is in strong contrast with the widespread $[Ca^{2+}]_i$ increase due to backpropagating action potentials.

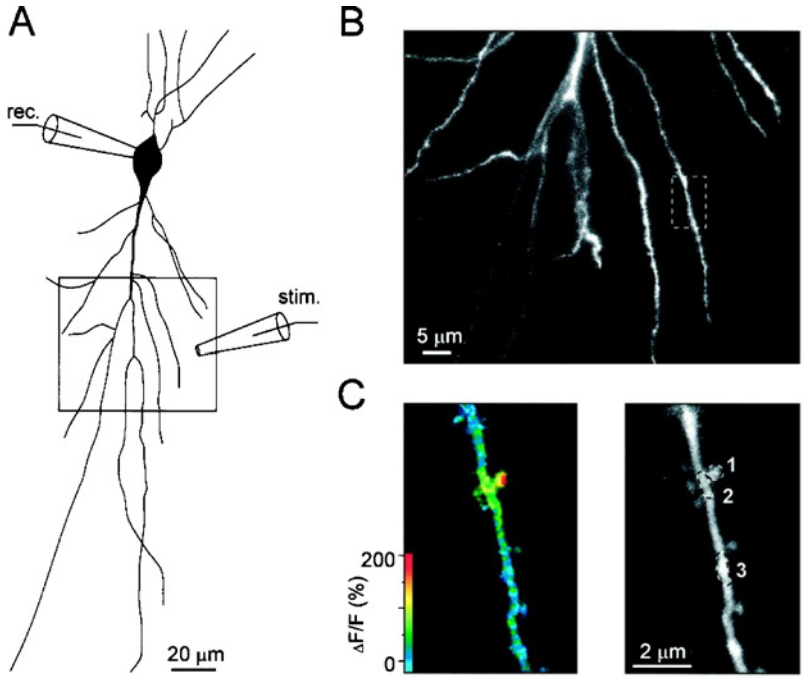

Fig. 2. Synaptically activated localized $[Ca^{2+}]_i$ increase in dendritic spines. (A) The drawing shows a hippocampal CA1 pyramidal neuron with a patch electrode on the soma and a stimulating electrode near an apical dendrite. (B) A confocal image of the dendritic region shown in the box in (A). (C, right): confocal image of the boxed region in B. (C, left): psuedocolor image of the $[Ca^{2+}]_i$ increase evoked by a single synaptic stimulation, measured at the time of peak response. The largest increase is localized around a spine. Adapted from Kovalchuk *et al.* (2000).

Ca^{2+} Release From Internal Stores

This source of postsynaptic Ca^{2+} has only come into focus in recent years. Early studies in culture (Shirasaki *et al.*, 1994; Seymour-Laurent and Barish, 1995; Irving and Collingridge, 1998) and in slices (Alford *et al.*, 1993; Jaffe and Brown, 1994; Pozzo-Miller *et al.*, 1996; Garashuk *et al.*, 1997) showed that Ca^{2+} could be released from stores in pyramidal neurons following synaptic stimulation or the application of metabotropic agonists like *t*-ACPD (acting on metabotropic glutamate receptors (mGluRs)) and CCh (acting on muscarinic receptors).

Recent experiments explored this mechanism in greater detail. Finch and Augustine (1998), Takechi *et al.* (1998), and Wang *et al.* (2000) showed that parallel fiber stimulation evoked Ca^{2+} release from stores in spines by activating mGluRs on cerebellar Purkinje cells. The activated mGluRs mobilized IP_3 to release Ca^{2+} from the ER. Ca^{2+} released in this way could contribute to the induction of LTD in these cells (Finch and Augustine, 1998). Later experiments (Wang *et al.*, 2000) showed that climbing fiber activation enhanced mGluR-mediated Ca^{2+} release in spines.

At about the same time it became clear that repetitive synaptic stimulation, at intensities that were often subthreshold for spike generation, could release Ca^{2+} from stores in hippocampal pyramidal neurons. Nakamura *et al.* (1999) found Ca^{2+} release in CA1 pyramidal neurons mediated by mGluR-mediated mobilization of IP_3. Yeckel *et al.* (1999) and Kapur *et al.* (2001) demonstrated similar mGluR-mediated release in CA3 neurons following typical mossy fiber stimulation. Power and Sah (2002) showed that synaptic activation of muscarinic receptors evoked Ca^{2+} waves in CA1 pyramidal neurons. Recently, Larkum *et al.* (2003) found that synaptic activation generated large amplitude Ca^{2+} release waves in both layer 2/3 and layer 5 pyramidal neurons in the neocortex and Morikawa *et al.* (2003) found evidence for similar events in midbrain dopamine neurons. These results suggest that Ca^{2+} release events or waves may be widespread in the CNS. All these examples involve Ca^{2+} release via the IP_3 receptor. At this time there is no clear evidence of calcium-induced calcium release (CICR) not mediated by IP_3 although ryanodine receptors are widely distributed in CNS neurons.

Figure 3 shows an example of synaptically activated Ca^{2+} release in the dendrite of a hippocampal pyramidal neuron. Note that, unlike the spike-evoked $[Ca^{2+}]_i$ signal, the increase does not occur synchronously at all locations. Rather, it spreads as a wave over a restricted region. The increases begin with a delay from the time of the start of tetanic synaptic stimulation and often do not begin until the stimulation is complete. The duration of these transients is typically more than 500 ms, substantially longer than the duration of transients due to spikes or ligand-gated Ca^{2+} entry.

Two other aspects of these Ca^{2+} release signals are of interest. First, the amplitude of these $[Ca^{2+}]_i$ increases is significantly higher than the increases due to spike-evoked Ca^{2+} entry; they often have peak values of 5–10 μM in the proximal apical dendrites, while trains of spikes rarely generate increases larger than 1 μM (Fig. 4). Figure 4 also illustrates the second point that synaptically activated Ca^{2+} release can be synergistically enhanced by pairing tetanic stimulation with back-propagating action potentials. This enhancement is a Hebbian mechanism and is probably due to the fact that the IP_3 receptor requires both Ca^{2+} and IP_3 to open the receptor channel (Iino, 1990; Bezprozvanny *et al.*, 1991; Finch *et al.*, 1991; Yao and Parker, 1992), a form of coincidence detection. There is some evidence that Ca^{2+} entry through the NMDA receptor can play the same role as spike-evoked Ca^{2+} entry in enhancing Ca^{2+} release (Nakamura *et al.*, 2002).

One of the more intriguing properties of Ca^{2+} release in pyramidal neurons (Nakamura *et al.*, 2002) is that regenerative Ca^{2+} release waves are detected on the thick apical dendrites and soma, but rarely on the finer oblique branches where most of the synaptic contacts and NMDA receptor-mediated Ca^{2+} entry are found (Fig. 5). Thus, $[Ca^{2+}]_i$ increases due to these two different mechanisms are essentially segregated in different parts of the dendrites. Previously, there was little evidence that Ca^{2+} signaling might be different in different dendritic regions.

The importance of synaptically activated Ca^{2+} release in pyramidal neuron spines and Ca^{2+} release mediated through ryanodine receptors is controversial. Sharp *et al.* (1993) found ryanodine receptors but not IP_3 receptors in pyramidal cell spines. Emptage *et al.* (1999) detected Ca^{2+} release in spines mediated

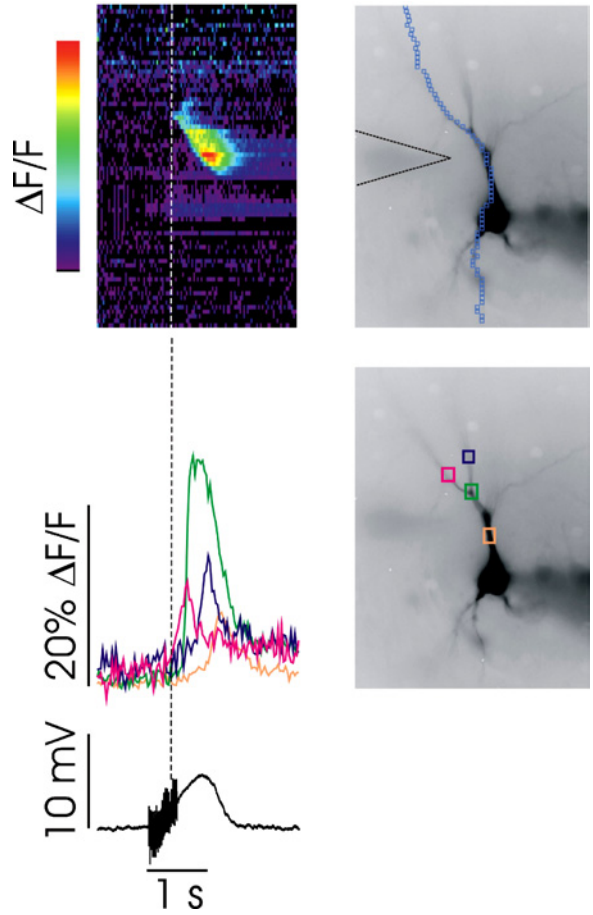

Fig. 3. Synaptically activated calcium wave in the dendrites of a CA1 pyramidal neuron. Image in upper right shows a CA1 pyramidal neuron with a patch electrode on the soma and the position of the stimulating electrode indicated with a vector (>). "Line scan" pixels shown in blue. Lower image shows the same cell with ROIs. Tetanic stimulation (100 Hz for 0.5 s) generates a large $[Ca^{2+}]_i$ increase that appears at different times in different locations. The line scan image shows that the $[Ca^{2+}]_i$ increase spread as a wave over a restricted region of the dendrites and initiated near a branch point. Modified from Nakamura *et al.* (1999).

through ryanodine receptors. However, both Kovalchuk *et al.* (2000) and Sabatini *et al.* (2002) challenged their results, specifically indicating that they could not find $[Ca^{2+}]_i$ increases in spines due to release from stores. Emptage *et al.* (1999) did their experiments in neurons in slice cultures and with sharp electrodes, which may explain the different results.

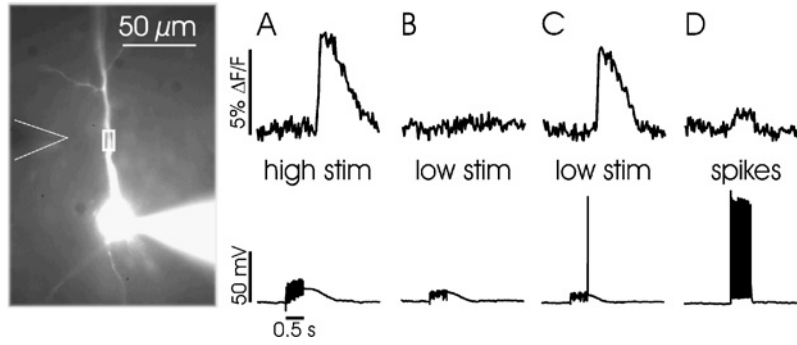

Fig. 4. Pairing action potentials with synaptic stimulation synergistically generates a large amplitude $[Ca^{2+}]_i$ increase due to Ca^{2+} release from stores. (A) Tetanic stimulation (100 Hz for 0.5 s) using 40 μA (*high stim*) evokes Ca^{2+} release in the dendrites. (B) Stimulation using 30 μA (*low stim*) is subthreshold for generating release. (C) Stimulation at the same intensity followed by a single intrasomatically generated action potential evokes release. (D) A train of 20 intrasomatically generated spikes evoked a smaller $[Ca^{2+}]_i$ increase. Pipette contained 500 μM of the low affinity indicator furaptra. With this indicator the peak release signal was measured to be about 5 μM. The bath contained 50 μM APV and 10 μM CNQX to block ionotropic glutamate responses. Modified from Nakamura *et al.* (1999).

Elementary Ca^{2+} Release Events

The spatial extent of the Ca^{2+} release events are variable even though they are regenerative. This result is consistent with the idea that the larger Ca^{2+} waves are built from fundamental or elementary release events, usually called "blips" or "puffs" (Berridge, 1998). The size and properties of these small events are interesting because they may correspond to the basic signaling units activated when mGluRs are stimulated during normal synaptic activity. Since the IP_3-mediated Ca^{2+} waves have many characteristics similar to Ca^{2+} waves found in other preparations (e.g. *Xenopus* oocytes, HeLa cells, astrocytes, RBL tumor cells, and adrenal chromaffin cells) information from these cells may be relevant to Ca^{2+} release events in pyramidal neurons. The elementary events in these cells are thought to represent the Ca^{2+} coming out of the ER through a group of IP_3 receptors that are clustered together (Bootman and Berridge, 1996).

The elementary events described in oocytes (e.g. Yao and Parker, 1992) and HeLa cells (e.g. Bootman *et al.*, 1997; Marchant and Parker, 2001) have dimensions of a few microns and duration of 100–300 ms. They often repetitively originate at the same discrete locations in the cytoplasm, suggesting that there is a morphological structure (e.g., a cluster of IP_3 receptors) determining their site of initiation. The spatial distribution of the elementary events may be different from the extent of regenerative Ca^{2+} waves (Callamaras *et al.*, 1998) since the regenerative waves require the clusters to be close together to allow Ca^{2+} released from one cluster to initiate release from the next cluster, while this requirement is irrelevant for release from single clusters. The similarity of elementary release events underlying Ca^{2+} waves in different cell types that vary by over 10^5 in volume suggests that they may

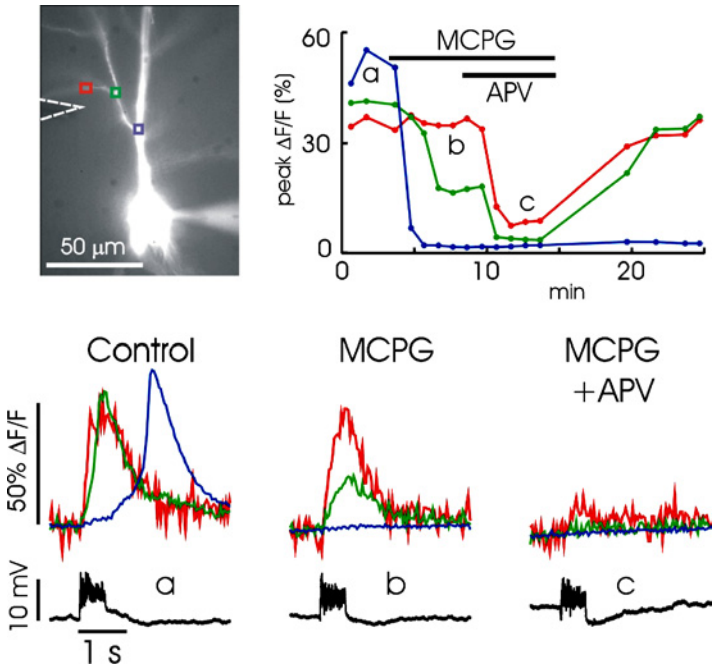

Fig. 5. Synaptically activated Ca^{2+} release occurs primarily on the main apical dendritic shaft while Ca^{2+} entry through NMDA receptors is predominantly on the oblique dendritic that have the highest concentration of spines. In *Control* conditions tetanic stimulation (100 Hz for 0.5 s) generates a rapid $[Ca^{2+}]_i$ increase on the oblique dendrites (red and green boxes) and a delayed $[Ca^{2+}]_i$ increase on the main shaft (blue box). Addition of 1 mM *MCPG* blocks the increase on the main shaft, has no effect on the side branch (red box) and reduces the increase at the intermediate location. Addition of 50 μM APV (*MCPG+APV*) blocks the remaining signal. The upper graph shows the time course of the experiment, indicating that the branch signals recovered after washout but the shaft signal did not. Modified from Nakamura *et al.* (2002).

also exist in pyramidal neurons. Indeed, there is some evidence for them in cultured pyramidal neurons (Koizumi *et al.*, 1999). Recent experiments (Watanabe and Ross, unpublished data) found spatially restricted synaptically evoked Ca^{2+} release events, usually located at branch points in the dendrites.

Functional Implications

The peak amplitude of the propagating Ca^{2+} waves can reach a value of several micromolar in the proximal and distal dendrites. In the somatic region and in the proximal dendritic region these $[Ca^{2+}]_i$ increases are much larger than the $[Ca^{2+}]_i$ increases generated by trains of backpropagating Na^+ action potentials. Ca^{2+} waves are more localized and longer lasting than the electrically generated Ca^{2+} transients. Unlike voltage-gated signals the $[Ca^{2+}]_i$ changes from release are not highly localized

just under the plasma membrane. For all these reasons the waves may activate downstream mechanisms that are untouched by the spike-induced $[Ca^{2+}]_i$ changes. For example, calmodulin has a K_D for binding Ca^{2+} in the micromolar range and slow rate constants for this reaction (e.g. Klee, 1988). Spike-induced $[Ca^{2+}]_i$ increases recover rapidly and do not reach these values (Helmchen *et al.*, 1996; Sabatini *et al.*, 2002). Synaptic activation of NMDA receptors also generates $[Ca^{2+}]_i$ increases in the micromolar range (Petrozzino *et al.*, 1995; Sabatini *et al.*, 2002). These increases are localized to spines and generally do not overlap spatially with the regions where waves are generated (Nakamura *et al.*, 2002). Therefore, they may activate different signaling mechanisms than the Ca^{2+} waves.

It is probable that the large and long-lasting $[Ca^{2+}]_i$ increases at the peak of Ca^{2+} waves activate many cellular functions. One simple consequence is that the Ca^{2+} modulates dendritic conductances (e.g. $g(K)_{Ca}$) and affects synaptic integration and electrogenesis in the dendrites in the immediate time frame following wave generation. Other targets for Ca^{2+} release from stores are mechanisms affecting synaptic plasticity (e.g. Reyes and Stanton, 1996; Nishiyama *et al.*, 2000; Daw *et al.*, 2002) and the strength of inhibition onto the neurons releasing Ca^{2+} (e.g. Brown *et al.*, 2003). These ideas remain to be definitively tested. In other neuron types a variety of more complex functions have been shown to depend on intracellular Ca^{2+} release. For example, in retinal ganglion cells intradendritic Ca^{2+} release controls the stabilization of new dendritic processes during development (Lohmann *et al.*, 2002). In spinal motoneurons Ca^{2+} waves are important in axon outgrowth (e.g. Lautermilch and Spitzer, 2000).

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