Understanding Calcium waves and sparks in central neurons

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Preface

All cells use changes in intracellular calcium concentration $[Ca^{2+}]_i$ to regulate cell signaling events In neurons, with their elaborate dendritic and axonal arborizations, there are clear examples of both localized and widespread Ca^{2+} signals. $[Ca^{2+}]_i$ changes generated by Ca^{2+} entry through voltage gated and ligand gated channels are the best characterised. In addition, $[Ca^{2+}]_i$ can increase by release from intracellular stores. These signals have been less studied, in part because they usually are not associated with specific changes in membrane potential. However, recent experiments have revealed dramatic widespread Ca^{2+} waves and localized spark-like events, particularly in dendrites. Here we review emerging data on the nature of these signals and their functions.

Introduction

In most CNS neurons the best characterised intracellular calcium ($[Ca^{2+}]_i$) changes follow from opening voltage gated calcium channels (VGCCs) or ligand gated channels [1-3]. Action potentials (APs) generate widespread $[Ca^{2+}]_i$ increases in axons and presynaptic terminals and, when they backpropagate, over large regions of the dendrites. Synaptic potentials evoke localized $[Ca^{2+}]_i$ increases in the synaptic region. More localized Ca^{2+} signals result from Ca^{2+} entry through ligand gated channels. The classic example is entry through NMDA receptors on postsynaptic spines. These signals are brief and of moderate amplitude since the rise time of the Ca^{2+} transient is determined by the time course of the spike or synaptic potential and $[Ca^{2+}]_i$ is rapidly returned to resting levels through cytoplasmic buffers and efficient membrane and SERCA pumps. In some circumstances regenerative Ca^{2+} spikes or NMDA spikes, usually in the more distal parts of the dendrites, can generate much larger and longer lasting $[Ca^{2+}]_i$ increases [4,5].

Less is known about the $[Ca^{2+}]_i$ changes that result from Ca^{2+} release from internal stores. Although pharmacological and immunohistochemical evidence of their presence and potential significance has been clear for many years [2,6,7], direct observation and conclusions about these signals in intact neurons have been harder to realize, in part because they are not directly associated with membrane potential changes. With the advent of new technology and more sensitive Ca^{2+} indicators [Box 1] these $[Ca^{2+}]_i$ changes have begun to be characterized and have provoked renewed interest in the consequences of these changes. It is now clear that signaling mediated by Ca^{2+} release through the classic endoplasmic reticulum (ER) channels - ryanodine receptors (RyRs) and inositol trisphosphate receptors (IP_3Rs) - exists in most neurons. These signals can combine with or complement the voltage and ligand gated Ca^{2+} transients, (which together are often referred to as the "calcium toolkit") [8]. The great variety of neuronal cell types and their complex arborizations leads to diverse expression patterns of these signals and probably diverse functions, many of which are not yet rigorously established.

Calcium waves

Propagating Ca^{2+} waves are the most dramatic expression of Ca^{2+} release from internal stores. They reflect regenerative Ca^{2+} release, where elevated cytoplasmic Ca^{2+} induces further Ca^{2+} release (CICR) through a nonlinear cooperative process. There are

two types of waves. The most common are waves mediated through opening of IP_3Rs (Fig. 1). These waves were first described in non neuronal cells like *Xenopus* oocytes [9,10] and HeLa cells [11], where their main properties were characterized. In oocytes this $[Ca^{2+}]_i$ increase provides a developmental signal and in other cells, like exocrine gland cells, they can transfer information from one side of the cell to another [12]. The other type of Ca^{2+} wave, much rarer, is mediated by regenerative activation of RyRs. They have been observed in cardiac myocytes [13] but it is not clear if they occur under normal physiological conditions.

Ca^{2+} waves in neurons were discovered more recently. In these cells, with their spatially extended, intricate dendrites and axons, the properties of calcium waves and other $[Ca^{2+}]_i$ changes take on interesting forms. While detailed information about the spatial distribution of relevant channels and receptors involved in Ca^{2+} release in neurons is still lacking, it is clear that the molecular configurations are different in different regions of the cell and in different neuronal cell types [6,14] leading to different patterns of Ca^{2+} release.

Synaptically activated, IP_3 mediated Ca^{2+} waves (Fig. 2) have been observed in pyramidal neurons in the rodent hippocampus (CA1 and CA3 regions), cortex (L2/3 and L5), and principal neurons in the amygdala [15-20]. Interestingly, they have also been observed in pyramidal neurons in the turtle cortex [21]. Since turtles diverged from mammals over 300 million years ago and occupy a different ecological niche, this finding suggests that Ca^{2+} waves evolved early and are a robust and conserved property of pyramidal neurons and possibly other cell types.

The peak $[Ca^{2+}]_i$ amplitude of these waves can be over 5 µM if measured with non-buffering low-affinity Ca^{2+} indicators [16,22], much higher than the 0.15-0.3 µM signal that results from a VGCC mediated Ca^{2+} entry evoked by a backpropagating action potential (bAP) measured in the same dendritic region [23,24]. The synaptically evoked Ca^{2+} wave signal usually lasts much longer (0.5-1.5 s) than the brief Ca^{2+} transients (0.02-0.1 s) evoked by ligand-gated or spike evoked Ca^{2+} signals [25]. Typical propagation velocity of these waves is about 100 µm/s when measured with low affinity Ca^{2+} indicators [26] while bAP evoked Ca^{2+} signals propagate over this distance in 0.5 ms [27]. The large amplitude and long duration of the wave signals suggest that they should be effective activators of Ca^{2+} signaling pathways.

IP_3 mediated Ca^{2+} release occurs in other neuron types but may be expressed in different ways. Ca^{2+} release, but not in the form of propagating waves, is prominent in cerebellar Purkinje neurons, especially in spines [28,29] and has been observed in some interneurons [30]. The lack of propagating waves in these cells may be due to the high concentration of endogenous Ca^{2+} buffers [31,32], which could interfere with the regenerative character of CICR, since injection of exogenous buffers like EGTA can prevent wave propagation without preventing Ca^{2+} release [17,33].

From a molecular perspective Ca^{2+} wave generation in neurons conforms to the standard signaling cascade that has been described in many other cell types. An exogenous agonist (neurotransmitter) activates PLCβ, which generates IP_3 and DAG; IP_3 acts on the IP_3R to release Ca^{2+} from the ER; the released Ca^{2+} acts on nearby IP_3Rs to release more Ca^{2+} (CICR). Since activation of the IP_3R requires both Ca^{2+} and IP_3 [34-36] the initial Ca^{2+} release by mobilized IP_3 requires some Ca^{2+} in the cytoplasm. If the concentration of IP_3 is high enough then resting $[Ca^{2+}]_i$ can be sufficient [37]; at lower IP_3

levels additional Ca^{2+}, usually from Ca^{2+} entry through VGCCs is required. Once CICR is initiated regenerative propagation will continue as long as IP_3 is available; the level of IP_3 at rest is not sufficient. The requirement for coactivation by IP_3 and Ca^{2+} makes regenerative Ca^{2+} release a coincidence detector between mGluR or mAChR synaptic activation (which mobilizes IP_3) and postsynaptic Ca^{2+} signaling (from bAPs or dendritic Ca^{2+} spikes) in pyramidal neurons [17,22,38] and Purkinje neurons [39]. The timing window for this synergistic response varies from 100-500 ms [17,38,39]. The duration of the window is closely related to the lifetime of IP_3 in the dendritic region where the wave is generated, which is determined by a combination of IP_3 degradation, IP_3 diffusion, and IP_3 unbinding from the receptor [38,40]. If diffusion is the dominant factor, as appears to be the case in pyramidal neuron dendrites, then the timing window will be smaller if the spatial extent of Ca^{2+} release is more restricted since diffusional dissipation is more rapid in this case.

Spread of Ca^{2+} waves

The region where Ca^{2+} waves extend determines which downstream signalling mechanisms are activated by the $[Ca^{2+}]_i$ changes they generate. In many small cells the extent of propagation of this regenerative mechanism is not a critical issue; the released Ca^{2+} effectively spreads over the entire cell. In bigger cells, especially neurons with complex morphology, the distribution of the components of this signaling apparatus within the cell are critically important for determining whether Ca^{2+} release transforms into a propagating Ca^{2+} wave.

In pyramidal neurons in the hippocampus and cortex Ca^{2+} waves are usually detected in the primary apical dendrite, and sometimes in the soma, with slight penetration (10-20 μm) into the oblique and basal dendrites; the limits of propagation into the distal dendrites are not as precisely determined, but waves are rarely detected beyond the point where the thick dendrites begin to branch [16,26]. Similar patterns are observed in projection neurons in the basolateral amygdala, although the waves in the fine dendrites are not as easy to follow [41]. This restricted range for wave propagation is interesting because the ER is thought to extend continuously throughout the dendrites of pyramidal neurons [42] and Purkinje neurons [43,44]. Furthermore, it is exactly orthogonal to the location of branches with high concentrations of spines [45] and, consequently, the location of Ca^{2+} entry through NMDA receptors and regenerative NMDA spikes [26,46] (see Supplementary Information S1: movie). Although there are some spines on the apical dendrite and some Ca^{2+} release in the finer processes, the sharpness of the boundary between the territories of NMDA spikes and Ca^{2+} waves is remarkable [47]. In contrast, the spatial extent of $[Ca^{2+}]_i$ changes from bAPs and Ca^{2+} spikes is more diffuse and extends over both apical dendrites and fine branches .

The location and spatial extent of individual Ca^{2+} waves depend on the stimulation protocol. Waves detected following threshold synaptic stimulation can be as small as 5 μm, although this is not a well determined limit. These threshold waves can be generated in different locations in the apical dendrites, usually close to the site of stimulation. Waves generated by ionophoresis or puffing of metabotropic agonists show a similar distribution, except they easily activate waves in the soma if the agonist is released over that location. Bath application of agonists (trans-1-aminocyclopentane-1,3-

dicarboxylic acid (t-ACPD) or carbachol (CCh)), in low concentration, which do not activate waves by themselves, will robustly support Ca^{2+} waves if they are triggered by backpropagating action potentials (bAPs). These agonist generated waves extend over the apical dendrites and soma, i.e. over the full potential range of wave generation. Lastly, waves can be generated by uncaging IP_3 that has been loaded into the cells. These waves usually extend over the area of uncaging if the flash is confined to the main apical dendrite and the soma; uncaging IP_3 in the oblique or basal dendrites evokes only a weak or no Ca^{2+} response.

These patterns suggest that the main factors determining the extent of wave generation are the distribution of IP_3Rs in the cell and the locus and magnitude of IP_3 generation. Waves can only spread as far as IP_3 is generated and diffuses. This region is constrained when IP_3 is generated by focal synaptic stimulation, focal ionophoresis of metabotropic agonists like trans-ACPD [48,49] or muscarine [50], or by localized uncaging of IP_3 [19,38]. However, when IP_3 is mobilized all over the cell following bath application of agonists [22,50] or by uncaging of IP_3 over the entire neuron [51], the waves will spread as far as IP_3Rs are available in sufficient density to support regenerative CICR. The spatiotemporal patterns of Ca^{2+} release are the same whether t-ACPD or CCh are used in these experiments [22], suggesting that it is not the distribution of different kinds of metabotropic receptors that is critical.

Brief trains of glutamatergic [52] or cholinergic [50] synaptic stimulation (or both [41]) in hippocampal slices generate localized Ca^{2+} waves that are usually confined to the dendrites. The extent of propagation increases with increased stimulus intensity, which recruits presynaptic fibers over a larger area. These waves rarely extend into the soma following low intensity stimulation since there are few synaptic contacts there (e.g. Fig. 2). However, following strong and sustained stimulation enough IP_3 can be generated that allows waves to spread into the soma. In that case, an intense Ca^{2+} release response often is generated in the cell body and nucleus [19,50,52], reflecting the high concentration of IP_3Rs in the soma and nuclear membrane. In some cases a local synaptically activated dendritic Ca^{2+} wave can be induced to propagate into the soma in the presence of bath-applied CCh, which supplies IP_3 to the somatic region, allowing extended CICR [52]. There is also evidence that a higher level of store filling, as regulated by a previous history of spike firing, can promote propagation to the soma [19].

These considerations affect the potential functions of Ca^{2+} waves. It has been suggested that Ca^{2+} waves could be one mechanism that carries information from a synapse in the dendrites to the nucleus, where the large $[Ca^{2+}]_i$ increase could activate certain genes or transcription factors involved in synaptic plasticity [17,19,42,50]. However, the restriction of mobilized IP_3 to sites close to the activated synapse makes it unlikely that these waves can spread to the soma and transport a message from the synapse except under unusual circumstances. It has also been proposed that dendritically activated Ca^{2+} waves could spread to the soma in brain stem preBötzinger complex neurons activating TRPM channels in that location to drive rhythmic respiratory patterns [53]. But there is little evidence that waves spread this far in physiological conditions. Indeed, in the case of the brain stem neurons recent experiments [54] questioned whether Ca^{2+} waves exist in this system and argued that they are not involved in somatic activation.

Sites of wave initiation

Where waves initiate reveals information about the distribution of critical molecules and sources of synaptic input that activate metabotropic receptors. Synaptically activated Ca^{2+} waves preferentially initiate at branch points in the dendrites in pyramidal neurons, even when the stimulating electrode is not directly opposite a branch point [16,26,48]. With strong stimulation, activating many presynaptic fibers synapsing on several branches, multiple sites of initiation are observed (Fig. 2). One possible explanation for this pattern is that IP_3 is generated at highest concentration on oblique dendrites, near the sites of most synaptic contacts, and then diffuses towards the main dendrite where it contacts a high density of IP_3Rs and CICR is initiated. Indeed there are examples of waves initiating in the first few microns of the oblique dendrites, and then becoming much larger when they reach the main dendrite [16]. However, there is also a preference (although not as strong) for waves to be initiated at branch points when metabotropic agonists are bath applied [26]. This pattern suggests that IP_3Rs are more concentrated near branch points since the IP_3 generated in this kind of experiment is likely to be relatively uniform throughout the cell following diffusion from sites of mobilization, although other metabolic processes could modulate this distribution [55]. Consistent with this model of IP_3R distribution, synaptically activated wave propagation is often weaker in the region between branch points (designated as "cold spots") [48]. A speculative model of the distribution of relevant molecules involved in wave initiation and propagation based on Ca^{2+} imaging is shown in Fig. 3. There is more information from immunocytochemistry on cultured neurons about the receptors, channels, and accessory proteins that might affect the initiation site [55]. However, much less is known about the distribution of these molecules in central neurons in intact preparations [48,56,57].

Following the generation of a Ca^{2+} wave, which releases massive amounts of Ca^{2+} from the ER, a new wave cannot be generated for about 20-60 s [22,58], probably because ER stores are depleted at critical sites. In pyramidal neurons the main mechanism for refilling the stores ("priming") is by Ca^{2+} entry through VGCCs into the cytoplasm, which then gets pumped into the ER [15,19,51,59,60]. Action potentials are the most effective priming mechanism [59] but Ca^{2+} entry following subthreshold depolarization or even at resting potential is sometimes sufficient [58,60]. The importance of Ca^{2+} entry through store operated channels (SOCE) for refilling the ER in these neurons has not been demonstrated.

Function of Ca^{2+} waves

One obvious function of Ca^{2+} waves is that the large $[Ca^{2+}]_i$ increase generated by these waves activates Ca^{2+}-dependent membrane conductances. Indeed, many laboratories found that SK type K^+ channels, blocked by apamin, are specifically opened by IP_3 mediated waves in a variety of neurons (CA1 pyramidal neurons [61,62]; cortical pyramidal neurons [19,63,64]; midbrain dopamine neurons [65,66]; projection neurons in the BLA [20]). There is little evidence that Ca^{2+} waves activate BK type K^+ channels. An interesting aspect to these experiments is that the waves are most effective in causing an SK channel mediated after-hyperpolarization (AHP) in the neurons only if the wave is prominent in the soma. Dendritic Ca^{2+} waves that do not propagate into the soma, even if

they evoke a larger dendritic $[Ca^{2+}]_i$ increase than a somatic wave, cause a much weaker AHP response [19,20,61-63,67]. This result suggests that the density of SK channels is lower in the dendrites than in the soma, which is consistent with immunohistochemical observations [68]. However, the density in the dendrites cannot be too low since recent experiments showed that SK channels in spines, activated by Ca^{2+} entry through NMDA receptors, play an important role in regulating EPSP amplitude [69,70]. The (mostly) nonoverlapping locations of weak synaptically activated Ca^{2+} waves and SK channel distribution suggests that wave-evoked AHPs are not prominent in pyramidal neurons, and therefore would not have a strong modulatory effect on firing patterns in physiological conditions.

A second potential consequence of the rise in $[Ca^{2+}]_i$ from Ca^{2+} waves is that the released Ca^{2+} could directly inhibit Ca^{2+} entry through Ca^{2+} channels (CDI) [71,72]. In support of this hypothesis synaptically activated Ca^{2+} waves locally suppress bAP evoked $[Ca^{2+}]_i$ increases in the dendritic regions of pyramidal neurons where the waves are largest [47]. As expected for this form of inhibition other ways of causing large $[Ca^{2+}]_i$ increases also suppressed bAP evoked Ca^{2+} signals in the dendrites, showing that it is the Ca^{2+} and not some other component of the signaling cascade that affects the bAP signal.

Most forms of synaptic plasticity require a rise in postsynaptic $[Ca^{2+}]_i$. Over the years pharmacological evidence has implicated a role for Ca^{2+} release from stores in the induction of LTP and/or LTD [73-79]. Since Ca^{2+} waves generate a large and long lasting $[Ca^{2+}]_i$ increase in the dendrites it is reasonable to suggest that these waves could induce plasticity. In addition, the synergistic action of mGluR activation and postsynaptic Ca^{2+} entry through VGCCs in generating Ca^{2+} release, suggests that Ca^{2+} waves are a natural substrate for Hebbian plasticity mechanisms [17,39]. However, the current picture is confusing. There are few clear experiments where postsynaptic Ca^{2+} release from stores has been shown to be the main inducer for plasticity. Here we discuss a small number of examples, which highlight the lack of clarity in this issue. For other reviews of this subject see [80-83].

There are several cases where synaptically activated Ca^{2+} release has been shown to induce LTP. Many of these studies are controversial and there is no consensus on this issue. In one series of experiments mGluR evoked $[Ca^{2+}]_i$ changes, mediated through IP_3Rs, induced mossy fiber LTP in CA3 pyramidal neurons when other sources of postsynaptic $[Ca^{2+}]_i$ rise were blocked [84]. While these Ca^{2+} changes can be clearly seen and propagate as waves [18] the conclusion that these waves are relevant to LTP induction has been challenged [85]. The best evidence that Ca^{2+} waves can induce a form of LTP comes from recent experiments on pyramidal neurons in slices [86]. Those authors first induced LTP by the focal application of a muscarinic agonist to the dendrites, which also evoked a Ca^{2+} wave. Pharmacological dissection showed that the Ca^{2+} wave and not some other signaling component was the primary inducer. NMDA receptor activation was not required. This conclusion was supported by the demonstration that Ca^{2+} waves evoked by uncaging IP_3 in the dendrites caused a similar enhancement of EPSCs, which shows that muscarinic activation was not necessary. Interestingly, this form of LTP does not occlude homosynaptic LTP induced by repetitive stimulation of the Schaffer collaterals. The study did not examine whether the Ca^{2+} wave invaded or came close to

the potentiated synapses. Also left unclear from these experiments is why the classic protocol for generating NMDA receptor dependent LTP (tetanic stimulation of the Schaffer collaterals), which regularly evokes Ca^{2+} waves in the same parts of the dendrites [17,61], does not appear to evoke this form of LTP.

One paper [87], with imaging data from spines, supports a role for nonlinear Ca^{2+} release that might contribute to tetanus induced NMDA receptor dependent LTP. It claimed that most of the synaptically activated $[Ca^{2+}]_i$ rise in spines following single synaptic stimuli was Ca^{2+} release from stores that can be blocked by ryanodine. The signal was local to spines and synchronous with the stimulus, without a delayed component typical of IP_3 mediated Ca^{2+} release or a Ca^{2+} wave. Another study examining tetanus induced $[Ca^{2+}]_i$ changes [88] agrees that NMDA receptor activated, ryanodine sensitive Ca^{2+} release is prominent in spines. This signal is thought to be important in the induction of early LTP, but only following suprathreshold stimulation. A second NMDA receptor dependent Ca^{2+} component, prominent in dendrites, was considered to be due to IP_3 mediated Ca^{2+} release because of its sensitivity to xestospongin-C. Both of these Ca^{2+} release signals were locked in time with the stimulus, similar to the results of Emptage et al. [87]. The possibility of Ca^{2+} wave generation was not examined because the Ca^{2+} measurements were made using 2-photon microscope line scans that had no extended spatial resolution. Other groups do not find a $[Ca^{2+}]_i$ rise due to activation of RyRs in or near spines [89-91] and claim that the stimulus locked rise in $[Ca^{2+}]_i$ is due to Ca^{2+} entry through NMDA receptor channels or VGCCs. This long standing conflict is still not resolved. Some problems affecting the resolution may result from differences in preparations and/or recording techniques [80,82] or differences in determining which form of LTP is induced [88].

The evidence for IP_3 mediated Ca^{2+} signaling in some forms of LTD is clearer but also not without controversy. The best example is LTD of the parallel fiber to Purkinje cell synapse, which is primarily induced by combined parallel fiber and climbing fiber activation [92]. Parallel fiber activation evokes mGluR and IP_3 mediated Ca^{2+} release in spines that do not spread as waves [28,29], and which can be synergistically enhanced by coactivation of climbing fiber input [39]. The timing window for this synergism is consistent with the timing window for LTD generation. A specific role for IP_3Rs in Purkinje cell spines was demonstrated in mutant mice that lack spine ER and that could not express LTD [83,93]. Recent experiments [94] in which the $[Ca^{2+}]_i$ increase was generated with controlled activation of caged Ca^{2+}, suggests that it is the integrated $[Ca^{2+}]_i$ increase in spines that evokes LTD and that IP_3 mediated Ca^{2+} release is only one way of achieving the $[Ca^{2+}]_i$ level required for LTD.

Experiments examining spike timing dependent LTD in the layer 4 to layer 2/3 synapse in the cortex suggests that IP_3 mediated Ca^{2+} release may play an important role in generating the endocannabinoids (eCBs) that are responsible for the depression [95]. This group found that LTD was blocked by intracellular heparin and thapsigargin, but not ryanodine, which is consistent with induction via the IP_3 pathway. However, similar experiments by another group [96] did not find LTD block by heparin. Furthermore, the same group [96] found that direct 2-photon imaging did not show a Ca^{2+} release component in the postsynaptic Ca^{2+} signal during the induction protocol. This issue has not been examined in more recent experiments and the conflict remains unresolved.

An interesting example of Ca^{2+} release-dependent LTD in the hippocampal CA3 to CA1 pyramidal neuron synapse was demonstrated in organotypic slices using 2-photon glutamate uncaging to activate individual spines [91]. Those authors found that the ER only invaded some dendritic spines. If glutamate was uncaged over those spines a delayed $[Ca^{2+}]_i$ rise, which resembled a Ca^{2+} wave (although the spatial extent of release was not determined), was observed in addition to a fast $[Ca^{2+}]_i$ change locked to the stimulus. A delayed signal was not observed in spines without penetrating ER. The delayed signal was blocked by intracellular heparin. In some cases the delayed signal could be observed following synaptic stimulation. This is the only published example of Ca^{2+} release in pyramidal neurons following the activation of a single spine. Generation of this signal was correlated with an NMDA receptor independent, synapse specific form of LTD. Both the delayed signal and LTD were blocked by mGluR antagonists.

Localized Ca^{2+} release events

Spontaneous localized Ca^{2+} release events, often called "sparks," were first discovered in cardiac myocytes [97] and soon after in frog skeletal muscle fibers [98] and other non neuronal cell types. They were immediately suggested to be the building blocks of the large regenerative Ca^{2+} release that controls contraction. These events are due to the opening of clusters of RyRs in the ER by local CICR. Although they occur at rest with external Ca^{2+} removed, their frequency in myocytes is sensitive to changes in membrane potential, primarily as a result of Ca^{2+} entry through VGCCs in the plasma membrane. Ca^{2+} waves can be observed in some conditions [13,99] but they rarely occur normally. In skeletal muscle the connection between depolarization and increased spark frequency is more direct, through coupling between dihydropyridine (DHP) receptors and RyRs.

Similar events, mediated through IP_3Rs, were first described in *Xenopus* oocytes [100] and in HeLa cells [11]. These events, ("puffs") have a number of similar features to sparks. They are localized, fast, and occur stochastically. One difference is that they require IP_3 in the cytoplasm, in addition to Ca^{2+}, to open the IP_3Rs. These events coalesce more easily into Ca^{2+} waves and will propagate throughout a cell as long as the levels of IP_3 and the density of IP_3Rs are high enough to activate regenerative CICR.

In general, Ca^{2+} sparks and puffs do not occur in the same cell types since the examined cells express either a great predominance of RyRs or IP_3Rs. However, there are a few cases, e.g. neonatal cardiomyocytes and oligodendrocyte progenitors [13,101], where interactions between these pathways have been observed. In some smooth muscle cells no interactions were noted, even though both events occurred in the same cells [102]. These and other properties of elementary events in non neuronal cells recently were recently reviewed [7].

Localized events in neurons that have spark-like and puff-like properties were first observed in differentiated PC12 cells and dissociated hippocampal neurons [103]. The organization of the receptor system underlying these events was not examined. Only a few spontaneous events were observed but their frequency was greatly enhanced by application of caffeine or bradykinin. These events were significantly larger (50-100 μm) and slower (100-160 ms rise time, 95-160 ms decay time) than classical sparks or puffs, although some of these numbers may reflect buffering by the acetoxymethylester (AM)-

loaded Ca^{2+} indicator and the speed of the confocal microscope used in the experiments. Interestingly, the events occurred more frequently at branch points, a property that was later observed in dendrites of intact pyramidal neurons in slices [104]. It is not clear if these events in cultured cells are a good model for events in intact preparations. There is evidence that Ca^{2+} release is much less robust in cultured cells [105].

Localized Calcium release events in presynaptic terminals and cell bodies

In several experiments Ca^{2+} release in presynaptic terminals was suggested to play a role in both spontaneous and evoked synaptic transmission and plasticity [106-109] although results in different preparations do not present a consistent picture. Localized events were first observed in a slice preparation in basket cell axons [110,111]. These transients, which may underlie some giant IPSCs in Purkinje neurons, had a spatial extent of 5-10 µm and duration of 0.2-2.0 s (probably lengthened by indicator buffering). The amplitudes of the largest events were comparable to the amplitude of spike evoked transients at the same locations. Interestingly, the frequency of these events was enhanced by low (10 µM) concentrations of ryanodine and suppressed at higher (100 µM) concentrations. These observations, together with strong immunostaining for RyRs in basket cell terminals, suggest that these were RyR mediated events. Similar spontaneous Ca^{2+} release events were observed in hippocampal presynaptic boutons in slice culture [112], although they were not studied in detail. Their role in synaptic function has been controversial [113].

Localized ryanodine sensitive events called "syntillas" were recorded in presynaptic terminals of magnocellular hypothalamic neurons [114]. Interestingly, their frequency could be enhanced by depolarization even in the absence of calcium influx, which differs from sparks in myocytes, where the function of depolarization is to promote Ca^{2+} entry through VGCCs. Searching for a function for these syntillas, the same group [115] detected vesicular transmitter release, but did not find a close correlation with the syntillas .

In contrast, Ouyang et al. [116] found RyR mediated sparks just under the plasma membrane of the somata of cultured DRG neurons that were correlated with exocytosis of neurotransmitter. However, the correlation was stochastic with about 1 vesicle released per 10 detected sparks. The frequency of these small (~2 µm) and fast (~40-50 ms) spark-like events was sensitive to Ca^{2+} entry through VGCCs, but not to depolarization alone, and was enhanced by caffeine. Similar localized events were observed in the cell bodies of hippocampal pyramidal neurons [117], but they were not characterized in detail.

Several groups found a ryanodine sensitive component to voltage-dependent Ca^{2+} entry in cell bodies or presynaptic terminals without describing a structure of localized events in this signal [118-120]. However, these measurements did not have the spatial or temporal resolution to resolve spark-like components in these signals.

Localized events in dendrites in intact preparations

Although Ca^{2+} signaling in dendrites has been studied intensely for more than two decades this is the last place local release events were observed. One laboratory [121]

observed local Ca^{2+} release in the dendrites of developing chick retinal ganglion cells (E13) that extended over a distance of about 10 µm, lasted about 10 s, and occurred at a low frequency of about 0.1 Hz. These events, whose frequency could be modulated by cholinergic synaptic transmission, stabilized the outgrowth of developing dendrites. Bonhoeffer's laboratory [122] then found that similar large, long lasting events (11 µm extent, 5 s duration) occurred in the dendrites of developing hippocampal pyramidal neurons in organotypic slice cultures prepared from P0-P2 newborn pups. These events were due to Ca^{2+} release from stores, and were closely correlated with the outgrowth of filopdia. Many were activated by GABAergic signaling, and occurred close to sites of putative synapse formation [123]. The events detected close to synapses had shorter durations (0.5-0.7 s).

More recently much faster and spatially more confined events, with different sensitivities to neurotransmitter antagonists, were observed in the dendrites of both hippocampal CA1 and CA3 rat pyramidal neurons and in L2/3 and L5 rat cortical pyramidal neurons in acute slices [104]. The events occur spontaneously at rest at moderate frequencies (1-2 Hz) at fixed locations in the dendrites. They occur at approximately the same frequency in cells from animals of all tested ages (P3-P80). However, their measured amplitudes are largest in younger animals (almost as large as the $[Ca^{2+}]_i$ change from a bAP at the same location) and are about 20% of this level in older animals, although the peak amplitude at the exact site of release is not known [124]. This pattern suggests that they are most important during development, but may continue to have a signaling role in mature animals, but there is no direct evidence for this connection. They have a spatial extent of 3-5 µm (Fig. 4, Supplementary Information S2 movie). They occur most often at branch points, which suggests that RyRs might be concentrated at those sites (Fig. 3), and may contribute to wave initiation at the same locations. They have fast rise times (less than 10 ms) and recovery times (~100 ms) largely determined by the time for Ca^{2+} to diffuse away from a localized source of 3-4 µm extent [124]. They are unaffected by TTX or any ionotropic transmitter inhibitors, and are only weakly affected by mGluR inhibitors.

Like sparks in myocytes the frequency of these events can be modulated by changes in membrane voltage around resting potential, largely by Ca^{2+} entry through L-type VGCCs. The increase in frequency continues at higher potentials but there is no evidence that this signal becomes regenerative. Sensitivity in the subthreshold potential range means that normal voltage variations in these CNS neurons, like changes from "up" to "down" states [125], could strongly affect event frequency. Interestingly, event frequency can also be modulated by low concentrations of IP_3, generated either by uncaging or by weak repetitive mGluR-mediated synaptic transmission. This dual modulation is unusual, but might resemble the crosstalk between RyR and IP_3R mediated events described in oligodendrocytes [101]. It is not yet clear if the IP_3-sensitive events are the same or different from the spontaneous voltage-sensitive events. Since sparks in myocytes are not modulated by mGluR signaling, these events may have some different molecular components than the localized events in myocytes.

The clearly different characteristics of these fast dendritic events (even those in very young animals) from the events described in developing retinal ganglion cells and pyramidal neurons, suggests they result from different signalling mechanisms. However, it is possible that differences in preparations (acute vs. cultured slices) and Ca^{2+} imaging

methods (AM-loaded vs. injected indicators) are responsible for some of the divergence. Future experiments, possibly using genetically encoded Ca^{2+} indicators, may determine if they have common origins.

The function of these localized events in the dendrites of mature neurons is unclear. One hypothesis is that they activate localized conductances or enzymes. In smooth muscle cells sparks frequently generate spontaneous transient outward currents (STOCs) [126-128]. These currents are due to activation of BK channels and lead to muscle relaxation. In a few neurons similar currents called SMOCs have been detected which activate either SK channels (rat medial preoptic neurons [129]) or BK channels (parasympathetic cardiac neurons [130]). These studies did not determine in which part of the neuron the currents were activated, or suggest a clear consequence of their generation. SMOCs have not been detected in pyramidal neurons.

Challenges in observing Ca^{2+} waves and localized Ca^{2+} events in physiological conditions

The dendritic Ca^{2+} waves and localized release events described in this review have not been observed in neurons *in vivo*, although no experiments have yet been specifically designed to look for them. It is reasonable to ask whether this absence is due to technical challenges or if the conditions for evoking these signals do not normally pertain in the intact preparations or in current experimental protocols.

Large amplitude Ca^{2+} waves have been observed in cortical L2/3 and L5 pyramidal neurons in slices [16,19]. Smaller Ca^{2+} signals from bAPs in the dendrites of these cells have been detected *in vivo* in many 2-photon imaging experiments [131,132]. So exceeding detection threshold *in vivo* is not an issue for these large waves. A second possibility is that they are not as locked in time to the stimulus as signals from bAPs and EPSPs, which have been recorded following sensory stimulation in other experiments. In slices the peak of Ca^{2+} waves often occur after a synaptic tetanus with variable latency; if the recording period *in vivo* is restricted the waves might be missed. A third, and more likely possibility, is that the synaptic pattern that is effective in evoking waves in slices is not the pattern that cortical pyramidal neurons receive during sensory stimulation. In slices Ca^{2+} waves are usually evoked synaptically with an extracellular stimulating electrode placed close to the dendrites of the examined pyramidal neuron. Cooperative activation of many fibers often is necessary to reach threshold for regenerative Ca^{2+} waves [17]. A reasonable hypothesis is that this cooperativity is achieved by combining the IP_3 mobilized at several synapses to reach threshold concentration. This summation can occur more easily with electrode stimulation since it activates bundles of presynaptic fibers that make contact on spines that probably are close together on a single dendritic branch. Consistent with this idea most experiments find that it is necessary to give repetitive synaptic stimulation to evoke waves [133], as if the IP_3 at the initiation site must be summated from several stimuli to reach threshold. In contrast, the first *in vivo* imaging studies of synaptic summation following sensory stimulation [134] suggest that activated synapses are widely distributed over the dendrites and are not activated in bundles on individual dendrites, a pattern less favorable for regenerative Ca^{2+} wave generation. Important exceptions to this perspective are the experiments of Holbro et al. [91]. In their

studies on CA1 pyramidal neurons in organotypic cultures they were able to evoke regenerative Ca^{2+} release signals in a subset of spines using 2-photon glutamate uncaging. The locations of the activated spines (oblique or main dendrite) were not indicated. Because only single spines were activated cooperative activation was not required. The spatial extent of Ca^{2+} release was not determined, but was probably small since IP_3 was generated at only one spine. In that case Ca^{2+} release, even if of large amplitude, would be difficult to detect without knowledge of the location of the activated synapse.

It is also possible that the chance of generating Ca^{2+} waves is affected by the modulatory state of the neuronal environment since several GPCR transmitters enhance wave generation in slices [22,50]. Therefore, it may be preferable to look for these waves in awake animals where the levels of these compounds are higher [135].

A different set of considerations affects the detection in dendrites of localized Ca^{2+} release events. These spark-like events occur spontaneously in pyramidal neurons in slices and do not require a specific stimulus. Since many aspects of cellular Ca^{2+} signaling in slices are reproduced faithfully *in vivo* [131] it is likely that these events also occur in neurons in the intact animal. The limitation in detection is probably the small size of the events (20-70% of the amplitude of a bAP signal in the dendrites), which might make them indistinguishable from noise in most *in vivo* 2-photon Ca^{2+} measurements. Also, the stochastic nature of their generation makes them hard to observe in experiments designed to look for signals linked in time to a stimulus. As systematic *in vivo* Ca^{2+} measurements are just beginning we may expect progress in detecting small events and waves in the near future.

Conclusions

Over the past decade increasingly detailed information about Ca^{2+} release in neurons and other cell types has accumulated. Some of this information has been summarized in several excellent reviews [2,6,7,8,14]. In this review I have concentrated on the properties and functions of Ca^{2+} waves and localized release events in intact preparations, primarily in neurons in brain slices. In these preparations some of the differences between neurons and other cell types and model cell preparations are clearly revealed. Among the interesting properties of Ca^{2+} waves are their large amplitude, their prominence in only subregions of the dendrites, and that they can be generated by coincident activation of mGluR inputs and postsynaptic Ca^{2+} entry. Among the major remaining questions two prominent issues are: what conditions evoke these waves during normal brain activity, and do they have a specific function? The spark-like events are just beginning to be examined. Although they were studied for many years in other cell types they managed to stay under the radar in neurons until recently. Since they occur spontaneously in the slice preparation they are very likely to occur *in vivo*. It is also interesting that their frequency can be modulated by normal synaptic activity and membrane potential changes. It is not yet clear if they have a specific function, like releasing neurotransmitter or triggering a developmental changes, or if they are just the building blocks for larger $[Ca^{2+}]_i$ changes.

In other preparations, particularly cardiac myocytes and *Xenopus* oocytes, much more is known about the detailed microstructure of these Ca^{2+} signals and the molecules that underlie them. Experiments using currently available techniques, applying the lessons learned from these preparations, should supply much of the missing information

about waves and sparks in neurons. Improved Ca^{2+} indicators and imaging instrumentation will extend the reach of these experiments. In addition, the power of mouse genetics can be utilized since both Ca^{2+} waves and spark-like events have been detected with similar properties in murine pyramidal neurons (S. Manita, K. Miyazaki, W. Ross, unpublished observations). A big step forward will occur when they can be studied *in vivo*.

Box 1: Recording Calcium waves and sparks

Ca^{2+} waves and sparks are detected using variations of standard Ca^{2+} imaging methods. As with many forms of imaging, better results are obtained using techniques that maximize the sensitivity of the measurement with high spatial and temporal resolution, especially since signal averaging is not very useful in examining these events. Not all of these goals can be achieved simultaneously. Oregon Green BAPTA-1 is a good indicator for detecting the small $[Ca^{2+}]_i$ changes in sparks. However, the large $[Ca^{2+}]_i$ changes in Ca^{2+} waves saturate this high affinity indicator. Low affinity indicators like Oregon Green-BAPTA-5N or furaptra are better choices if quantitative assessment of wave parameters is the goal. Confocal microscopy has very good spatial and temporal resolution in the line scan mode and has been used in many experiments examining localized Ca^{2+} events. But this technique sometimes misses the extended spatial parameters of waves or the stochastic aspects of sparks. CCD cameras can have very good temporal and spatial resolution but usually not both. They are a good choice for capturing the spatial aspects of these events. They have poor spatial resolution in thick specimens. Sometimes choosing a thin specimen, like cultured neurons or dendrites in slices, can overcome these limitations. These issues have been discussed in several review articles [7,136] and imaging handbooks [137].

Glossary:

NMDA spike: A regenerative mechanism where the nonlinear component is the voltage dependence of the NMDA receptor. These spikes usually occur in dendrites, leading to large $[Ca^{2+}]_i$ increases but relatively small somatic membrane potential changes.

Coincidence detector: A term derived from electrical engineering where the output of a circuit depends on the simultaneous arrival of two (or more) inputs. The IP_3R is a coincidence detector for Ca^{2+} and IP_3.

Hebbian plasticity: A form of neuronal plasticity where a change in a property (often synaptic strength) results from the simultaneous activation (sometimes repetitively) of presynaptic and postsynaptic cells.

"Up" and "down" states: Persistent depolarizations and hyperpolarizations in neurons, which can differ by 10-20 mV. They are primarily observed in cortical neurons and are thought to be driven by network activity.

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Competing interest statement

The author declares no competing financial interests.

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Figure Legends

Figure 1. Model of regenerative Ca^{2+} release and wave propagation. (A) Opening the IP_3R requires both IP_3 and Ca^{2+}. The Ca^{2+} released through the receptor can act on the same IP_3Rs or other IP_3Rs to cause regenerative release (CICR). (B) By acting on other receptors Ca^{2+} release can propagate as far as IP_3 is available. The analogy to 'toppling dominos' (lower panel) is appropriate. Adapted from [138].

Figure 2. Synaptically activated Ca^{2+} wave in a neocortical pyramidal neuron. These waves can be easily evoked with repetitive focal synaptic stimulation in acute slices. In this experiment a pyramidal neuron was loaded with the low affinity indicator furaptra (300 μM) via a patch electrode on the soma and stimulated via a tungsten electrode (dotted arrow) at 100 Hz for 0.25 sec. Two views of the resulting changes in $[Ca^{2+}]_i$ are shown. Panel D shows the time course of the fluorescence changes at the regions of interest (ROIs) indicated by colored rectangles in panel C. The time courses and amplitudes of the delayed responses differ at the four ROIs. Panel B shows a pseudocolor "line scan" of the same data along a selected series of pixels in panel A. The timescale for panel B is the same as for the optical and electrical traces shown below (D). This figure illustrates that two waves initiated at different locations in the dendrites (close to branch points) can be propagated along the main dendrite in both directions. In this cell, the waves did not propagate into the soma. Figure courtesy of the author.

Figure 3. Model of the distribution of the ER and molecules related to the generation of Ca^{2+} waves and sparks in a hippocampal pyramidal neuron. The initiation of waves at branch points and the preference of localized events to occur at branch points suggest that IP_3Rs and RyRs are concentrated at those sites. Propagation of waves to the soma and nucleus indicates that the receptors are located in those compartments. mGluRs are located at the base of spines and at other extrasynaptic sites. The ER is continuous throughout the cell and connects to the nuclear membrane, but only invades some spines. Not all receptors are shown.

Figure 4. Spontaneous Ca^{2+} release events occur in localized regions of the dendrites of hippocampal pyramidal neurons. (A) The image shows a CA1 pyramidal neuron in an acute rat hippocampal slice filled with 100 μM Oregon-Green-BAPTA-1. Three regions of interest (ROIs) are marked. The string of pixels indicates the locations of the line scan images. In normal ACSF spontaneous increases in $[Ca^{2+}]_i$ were detected asynchronously at the three locations. There was no corresponding change in membrane potential. The pseudocolor 'line scan' image shows the increases at all locations along the dendrite. (B) Averaged records of event signals from nearby locations on a pyramidal cell dendrite. The data were recorded at 500 Hz and spontaneous event signals from 11 events at the same location were aligned at the time of the start of the rising phase of the fluorescence transient. The pseudocolor image shows the 'line scan' of the averaged signal. The traces show the signal at the center and neighboring (± 2 μm, ± 4 μm) locations. The data are consistent with Ca^{2+} release at the center and diffusion to nearby locations. Adapted from [104]. [Manita, S. & Ross, W.N. Synaptic activation and membrane potential changes modulate the frequency of spontaneous elementary Ca^{2+} release events in the dendrites of pyramidal neurons. *J Neurosci* **29**, 7833-7845 (2009), fig. 1A and 6B.

Supplementary Information S1 (movie). Synaptically activated Ca^{2+} wave in the dendrites of a CA1 pyramidal neuron. During tetanic stimulation at100 Hz $[Ca^{2+}]_i$ rises in the oblique dendrites. This increase is mostly due to Ca^{2+} entry through NMDA receptors as it can be blocked by APV (not shown). After a delay from the start of stimulation, a Ca^{2+} wave is generated on the main apical dendrite, which propagates towards but not into the soma. The extracellular stimulating electrode was over the oblique dendrites on the left side of the image. Vertical field of view ~120 μm; movie duration ~3.5 s.

Supplementary Information S2 (movie). Spontaneous localized Ca^{2+} release events along the main apical dendrites of a CA1 pyramidal neuron. Most of the Ca^{2+} release events are localized and do not propagate. There were no electrical transients at the times of these events as shown in the simultaneous somatic recording below the movie (vertical field of view, 110 μm; movie duration, 10 s; peak-to-peak voltage, 7 mV).

Brief author biography

William Ross received his Ph.D. at Columbia University in high energy physics. After postdoctoral training with Larry Cohen at Yale Physiology and Ann Stuart at Harvard Neurobiology he joined the Physiology Department at New York Medical College, where he is now Professor. He spent two sabbatical years at the Hebrew University in Jerusalem and many summers at the Marine Biological Laboratory in Woods Hole. The research in his laboratory has emphasized the development and use of imaging techniques to understand calcium signaling and integration in dendrites.

Bulleted "summary at a glance"

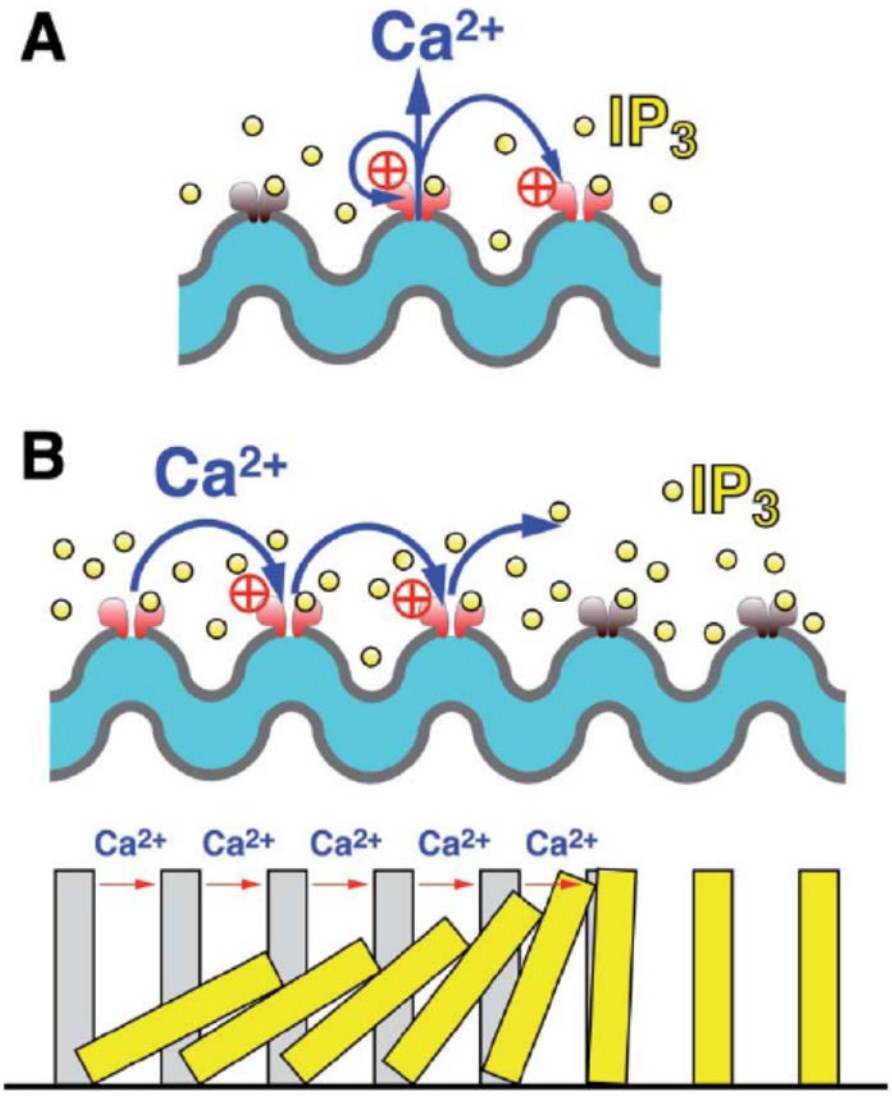
- Synaptic stimulation evokes postsynaptic $[Ca^{2+}]_i$ changes via Ca^{2+} entry through ligand gated channels, through voltage gated channels, and by Ca^{2+} release from internal stores. Ca^{2+} release can be widespread and substantial, but often has no correlated change in membrane potential.
- In many pyramidal neurons in the hippocampus, cortex, and amygdala Ca^{2+} release propagates as a wave in a restricted region of the dendrites. The waves are evoked by mGluR mobilization of IP_3 regeneratively releasing Ca^{2+} through IP_3 receptors.
- The range of wave propagation in the dendrites depends on the number and location of synaptic inputs and the influence of neuromodulators.
- Postsynaptic Ca^{2+} release and Ca^{2+} waves have been implicated in the modulation of membrane conductances and the induction of several forms of synaptic plasticity, including LTP and LTD. However, a number of these results are controversial.
- In addition to large amplitude, widespread Ca^{2+} waves, localized, smaller amplitude, spontaneous Ca^{2+} release events have been detected in the soma, dendrites, and presynaptic terminals of many CNS neurons. These events resemble "sparks" and "puffs," which have been observed in many non-neuronal cell types.
- The frequency of these events in dendrites can be modulated by changes in membrane potential in the subthreshold range, primarily by controlling Ca^{2+} entry through VGCCs. Their frequency also can be modulated by mGluR mediated mobilization of IP_3.
- These localized events appear to contribute to the generation of large amplitude IP_3 mediated Ca^{2+} waves. However, several of their properties implicate the involvement of RyRs, suggesting that they are more complex than IP_3 mediated puffs.
- Localized Ca^{2+} release events have been correlated with the generation of IPSCs at certain synapses and locally increase Ca^{2+}-activated K^+ conductances in some cells. But many of their functions remain to be determined.

TOC Blurb:

Understanding Calcium waves and sparks in central neurons

William N. Ross

There is increasing evidence of widespread Ca^{2+} waves and localized spark-like events in neurons, particularly in dendrites, however their origin and function is still poorly understood. This article reviews emerging data on the nature of these signals, their spatial distribution and potential roles.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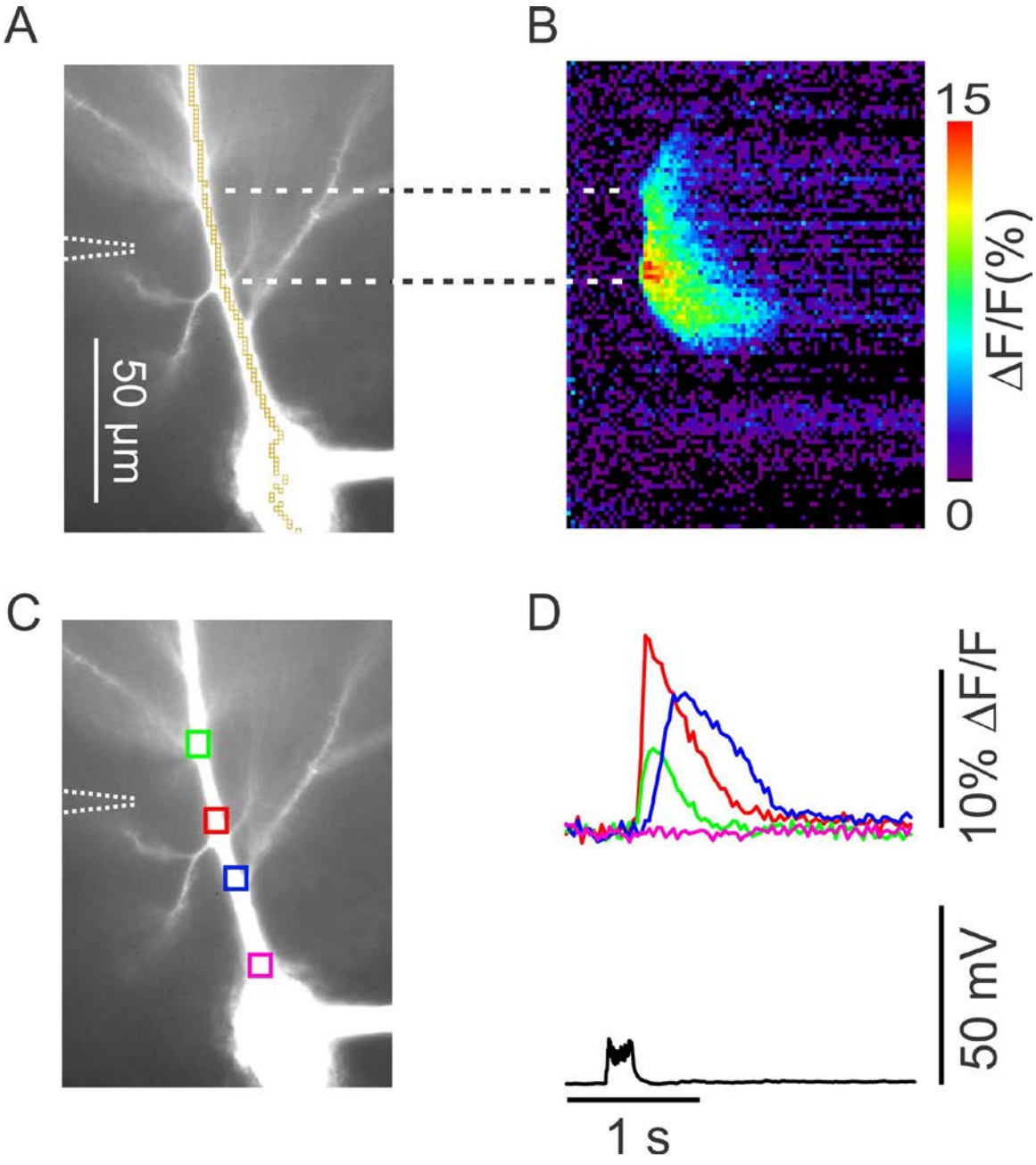
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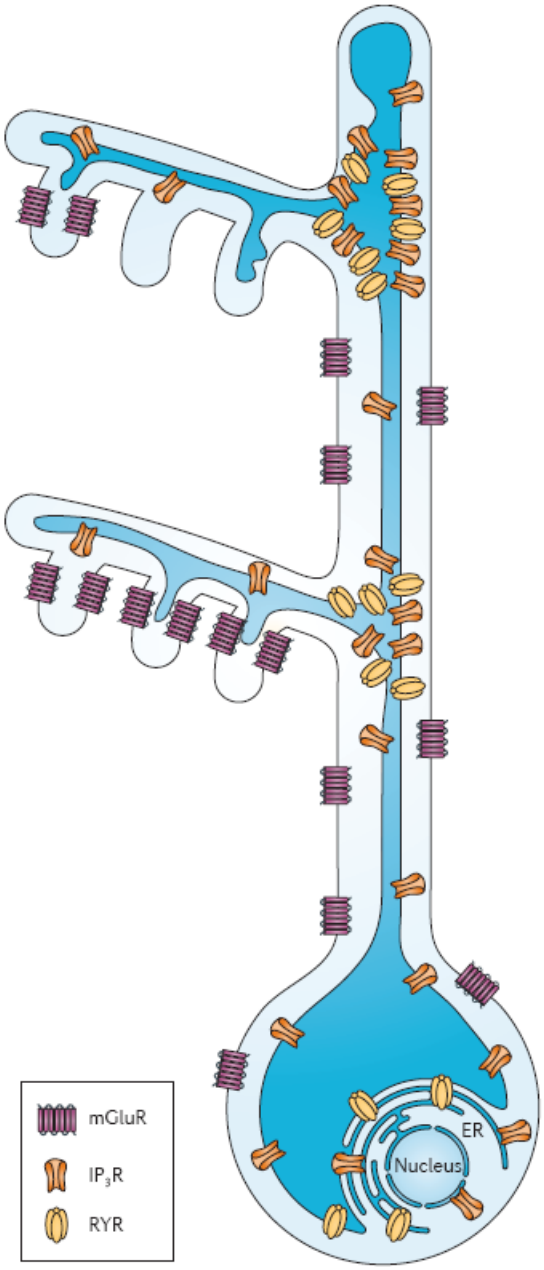
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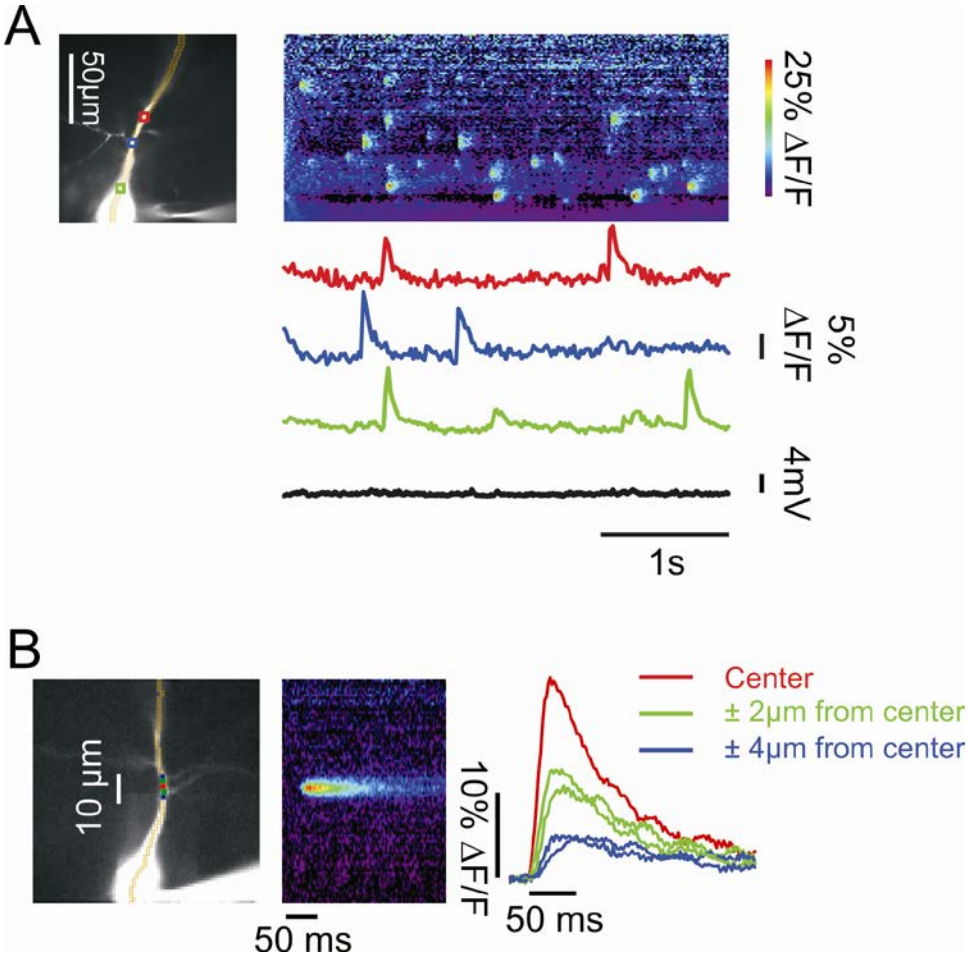
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