Forum

Neuronal SOCE: Myth or Reality?

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Store-operated Ca^{2+} entry (SOCE) is the primary Ca^{2+} influx pathway in non-excitable cells. Long thought to be absent in nerve cells, neuronal SOCE is gaining popularity. We argue here that the evidence for SOCE in neurons remains contentious, mostly because SOCE imaging assays are inadequate in these cells.

Introduction

SOCE refers to a unique Ca^{2+} entry route that is specifically activated by depletion of Ca^{2+} from the endoplasmic reticulum (ER). The existence of SOCE was first formulated by Jim Putney in 1986 in a visionary paper that transformed the Ca^{2+} signaling field [1]. SOCE (originally called capacitative Ca^{2+} entry) was later shown to be the primary Ca^{2+} entry pathway in non-excitable cells. Its tight dependence on ER Ca^{2+} levels, $[Ca^{2+}_{ER}]$, makes it an ideal feedback system to replenish Ca^{2+} stores following receptor-mediated ER Ca^{2+} release. The sustained rise in cytoplasmic Ca^{2+} levels, $[Ca^{2+}_{cyto}]$, that follows Ca^{2+} entry does more, however, than merely refilling stores – SOCE shapes Ca^{2+} signals in space and time, and regulates a broad range of cellular responses.

Twenty-five years after the first description of SOCE, the advent of RNAi screens enabled the discovery of its molecular constituents. STIM1 (stromal interaction molecule 1) was identified in 2005 as the ER-localized Ca^{2+} sensor that detects decreases in $[Ca^{2+}_{ER}]$ and activates SOC channels at the plasma membrane (PM). The identity of the SOC channel, Orai1, was revealed a year later. The coordinated recruitment of STIM1 and Orai1 to ER–PM contact sites, which follows store depletion, is one of the most striking displays of cellular dynamics and intermembrane communication (Box 1) (reviewed in [2]).

The most popular method to measure SOCE is the Ca^{2+} readdition (or addback) assay, initially developed in non-excitable cells but rapidly adopted by the neuroscience community. This two-step assay (Figure 1A) first involves shifting cells (loaded with a Ca^{2+} fluorophore) to zero extracellular Ca^{2+}, $[Ca^{2+}_{extr}]$, in the presence of a SERCA pump inhibitor (typically thapsigargin). Inhibition of the SERCA pump leads to depletion of $[Ca^{2+}_{ER}]$ (through an ill-defined ER leak), resulting in a transient increase in $[Ca^{2+}_{cyto}]$. After $[Ca^{2+}_{cyto}]$ returns to baseline levels, cells are shifted back to high $[Ca^{2+}_{extr}]$, and Ca^{2+} influx through SOC channels (SOCCs) triggers a sustained $[Ca^{2+}_{cyto}]$ signal. The robust rise in $[Ca^{2+}_{cyto}]$ should be absent, or negligible, in cells with intact (replete) stores, a control experiment that probes the store-dependence of Ca^{2+} entry (Figure 1B).

Despite clear limitations [3], the Ca^{2+} readdition assay has proved to be an excellent tool in non-excitable cells, largely because SOCE responses in these cells are robust and, importantly, are dependent on the STIM and Orai proteins, a molecular signature shared by the inward Ca^{2+} current (I_{CRAC}) underlying SOCE in immune cells. We argue here that the Ca^{2+} readdition assay faces additional limitations in neurons that stem from the intrinsic properties of these cells and that have been largely overlooked by the neuroscience community.

The Problem of Store Content

The most important criterion for a SOCE assay is to link Ca^{2+} influx to store depletion. As mentioned earlier, this is usually done by comparing Ca^{2+} influx in cells with intact or empty stores. This works reasonably well in non-excitable cells, whose stores are full by the time Ca^{2+} is

Box 1. Molecular components of SOCE: STIM and Orai

In resting cells, when stores are full, STIM (STIM1 or STIM2 in mammals) is Ca^{2+}-bound and dispersedly localized throughout the ER. Following depletion of ER Ca^{2+} (Figure I), STIM (now Ca^{2+}-free) oligomerizes and migrates to regions of the ER closely apposed to the PM. Repositioning of STIM triggers lateral recruitment of Orai1 to these ER–PM contact sites and activation of Orai1 through direct protein–protein interactions across the narrow cytoplasmic gap (~15 nm) between the ER and the PM. Ca^{2+} influx into the cytoplasm replenishes the stores and eventually leads to dispersion of STIM and Orai1, and inactivation of SOCE.

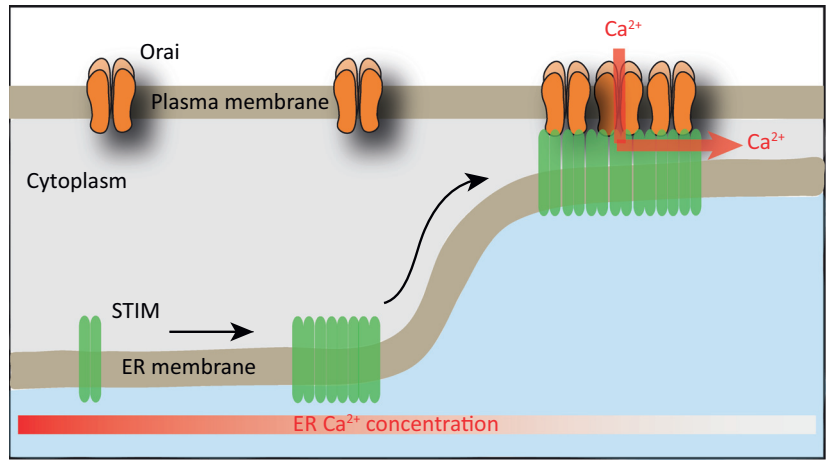

Figure I. Oligomerization and Redistribution of STIM in Response to ER Ca^{2+} Depletion.

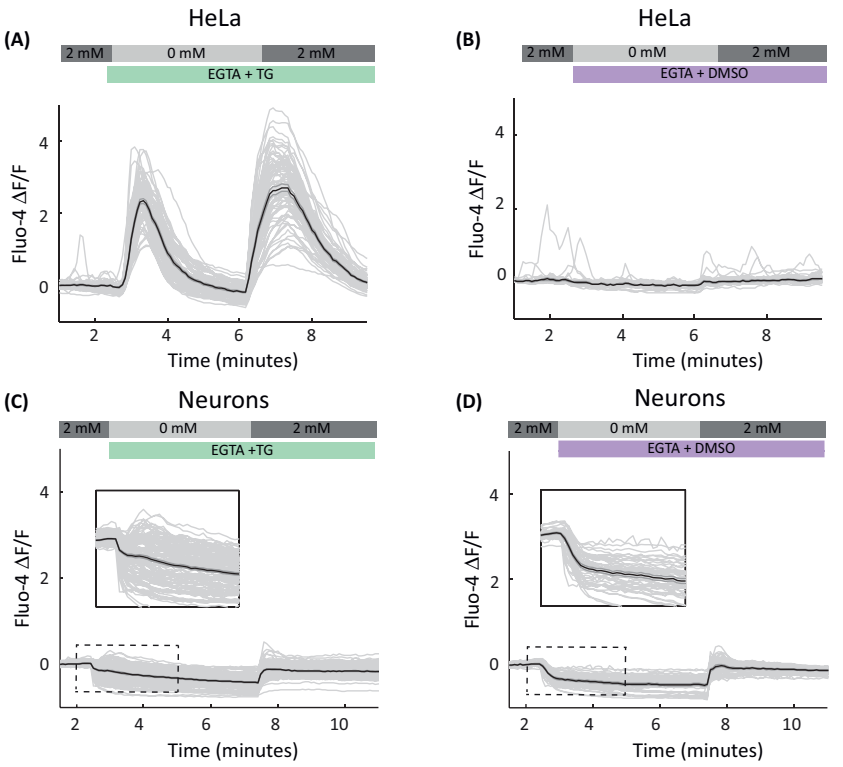

Figure 1. Store-Operated Ca^{2+} Entry (SOCE) in HeLa cells and Hippocampal Neurons. Ca^{2+} readdition assay in HeLa cells (A,B) and hippocampal neurons (C,D) loaded with the Ca^{2+} dye Fluo-4. [Ca^{2+}_{extr}] is indicated by grey bars above traces. Cells were switched to zero [Ca^{2+}_{extr}] (in the presence of EGTA to eliminate residual traces of external Ca^{2+}) and treated with thapsigargin (TG) (A,C) or dimethylsulfoxide (DMSO, vehicle, B,D). SOCE was then probed by adding back Ca^{2+} in the extracellular milieu. Note the sharp decrease in [Ca^{2+}_{cyto}] after removal of external Ca^{2+} in neurons (C,D), which partially occludes/masks TG-mediated ER Ca^{2+} release (C, inset). Ca^{2+} influx (upon Ca^{2+} readdition) in neurons is negligible compared to that observed in HeLa cells and is of similar amplitude in TG- or DMSO-treated cells (adapted, with permission, from [5]).

reintroduced unless previously exposed to a store-depleting drug.

This is not always the case in neurons. Whereas non-excitable cells are relatively impervious to manipulations of [Ca^{2+}_{extr}], switching neurons from high to zero [Ca^{2+}_{extr}] leads to a rapid decrease in both cytoplasmic and ER Ca^{2+} even in the absence of store-depleting agents (Figure 1D), as now documented by several groups in different types of neurons [4–8]. In fact, depletion of ER and cytoplasmic Ca^{2+} is so fast that it partially occludes or masks thapsigargin-mediated ER Ca^{2+} release (Figure 1C). This implies that SOCE, if present, should be at least partially activated by switching cells to zero [Ca^{2+}_{extr}], and the difference in SOCE magnitude between thapsigargin-treated and control cells is expected to be reduced. Aggravating this problem, store content in neurons appears to be activity-dependent [9,10], and stores may be partially depleted in resting cells [10]. Finally, further confusion arises from the lack of a systematic control experiment that probes Ca^{2+} entry in store-replete neurons. Taken together, these observations highlight that the observed Ca^{2+} influx in neurons may no longer be directly correlated to store content, arguably the best evidence for a causal relationship between store depletion and Ca^{2+} entry.

The sharp decrease in [Ca^{2+}_{cyto}] following removal of extracellular Ca^{2+} creates yet another problem. Many studies take [Ca^{2+}_{cyto}] in the absence of extracellular Ca^{2+} as the baseline – often excluding (or not recording) [Ca^{2+}_{cyto}] before [Ca^{2+}_{extr}] depletion – and estimate SOCE based on the increase of [Ca^{2+}_{cyto}] (after Ca^{2+} readdition) relative to that baseline. Without knowledge of [Ca^{2+}_{cyto}] in the presence of external Ca^{2+} (the true baseline in our view), it is difficult to judge SOCE magnitude. Does Ca^{2+} entry simply readjust [Ca^{2+}_{cyto}] to levels before removal of external Ca^{2+} (Figure 1C,D), or does it elevate [Ca^{2+}_{cyto}] significantly above baseline – as typically observed in non-excitable cells (Figure 1A)?

The Difficulty of Assigning Ca^{2+} Influx to SOC Channels

Neurons express on their surface a variety of Ca^{2+} channels [voltage-gated Ca^{2+} channels (VGCCs), NMDARs, and Ca^{2+}-permeable AMPARs, to name a few] with conductance for Ca^{2+} several orders of magnitude higher than that of CRAC channels. In neuron cultures, the activity of these channels depends on the intrinsic excitability of the network, which, in turn, is strongly influenced by [Ca^{2+}_{extr}]. In the presence of physiological Ca^{2+} concentration (in the mM range), neurons plated at sufficient high density will frequently fire action potentials, with bursts of activity regularly spreading throughout the network. Such spontaneous firing is strongly inhibited by shifting cells to zero [Ca^{2+}_{extr}], but will quickly resume upon return to normal [Ca^{2+}_{extr}], resulting in high-amplitude Ca^{2+} signals. These large Ca^{2+} signals are likely to mask a potential SOCE response or, worse, may be mistaken for SOCE. The spontaneous activity of the network must therefore be silenced. This can be done pharmacologically by blocking voltage-gated Na^{2+} channels (to prevent the generation of action potentials) and various other relevant receptors/channels, such as VGCCs, NMDARs, and AMPARs. One important class of VGCCs rarely mentioned in the context

of the Ca^{2+} addback assay comprises T-type or low voltage-activated calcium channels. T-type channels are activated by small depolarizations of the plasma membrane, near the resting membrane potential. They are thus likely to be partially active during the Ca^{2+} addback protocol, even in 'silenced' neuron cultures. Even so, their involvement in Ca^{2+} entry is almost never investigated. The nSOCE field is now beginning to use blockers of neuronal activity, but there remains an abundant literature on nSOCE with no reported use of these inhibitors. We need to stress here that, although we feel the use of these inhibitors is warranted, they limit the analysis of SOCE to a silenced network, and may thus not reflect the activity of this Ca^{2+} entry route in all physiological states [10]. In addition, VGCC blockers can, at high doses, interfere with CRAC channels and should therefore be used with caution. Finally, SOCC blockers are often used to further probe the origin of Ca^{2+} influx. The lack of specificity of these drugs is, however, notorious and they cannot be used (in isolation) to infer the presence of SOCE in neurons.

These two pitfalls have a compounding effect – the difficulty of isolating SOCC-mediated Ca^{2+} entry is exacerbated by the lack of compelling evidence linking store depletion to Ca^{2+} influx. These limitations question the validity of the Ca^{2+} add-back assay in the nervous system.

The Variability and Magnitude of nSOCE

A survey of the nSOCE literature reveals a great deal of variability within a single neuronal cell type. SOCE responses in both cortical and hippocampal neurons (arguably the best studied cell types for SOCE) vary from negligible [5,11], to relatively weak [4,9,12], to levels approaching SOCE activity in non-neuronal cells [6,13,14]. The caveats associated with SOCE detection undoubtedly contribute to these discrepancies, but it is also possible that this variability arises from cell-to-cell differences in SOCE activity. Although rarely mentioned, the fraction of unresponsive neurons can be high (Figure 1C). SOCE may also depend on the activation state of a neuron [9,10], or perhaps even on its activity history, which may considerably vary from one cell to the next. Finally, nSOCE may be spatially heterogeneous [14]. There appears to be, therefore, an inherent cell-to-cell variability that further complicates the analysis of nSOCE, particularly if it is measured in a limited number of individual cells.

Time will tell if nSOCE holds up to scrutiny. Surprisingly, more than 10 years after the discovery of the SOCE components, no functional evidence has yet been reported for a role of Orai in nSOCE, while there is an ongoing controversy about the involvement of STIM in this pathway [5,13,14]. Ignoring the contentious nature of nSOCE may divert us from possible SOCE-independent functions of the STIM and Orai proteins in the brain. Putting an end to this debate will require new imaging assays that monitor nSOCE in a large number of individual neurons without manipulation of $[Ca^{2+}_{extr}]$. The recent development of optogenetic probes to activate SOCE may be an important step in this direction [15].

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