SOCE in neurons: Signaling or just refilling?☆
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A B S T R A C T
In this review we describe the present knowledge about store operated Ca2+ entry (SOCE) in neurons and the proteins involved in this process: STIM, as well as Orai and TRP channels. We address the issue of whether SOCE is used only to refill Ca2+ in the ER or whether Ca2+ that enters the neuronal cell during SOCE also performs signaling functions. We collected the data indicating that SOCE and its components participate in the important processes in neurons. This has implications for identifying new drug targets for the treatment of brain diseases. Evidence indicates that in neurodegenerative diseases Ca2+ homeostasis and SOCE components become dysregulated. Thus, different targets and strategies might be identified for the potential treatment of these diseases. This article is part of a Special Issue entitled: 13th European symposium on calcium.

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1. Introduction
Calcium ions are a universal second messenger that regulates various processes, such as proliferation, transcription, contraction, exocytosis, apoptosis, the immune response, and neurotransmission [1-4]. The concentration of Ca2+ is tightly regulated spatially and temporally by multiple Ca2+ channel pumps, exchangers, and protein buffers. At rest, intracellular Ca2+ levels remain within the range of 100–300 nM and increase upon cell activation. An increase in cytoplasmic Ca2+ concentration can occur by the influx of Ca2+ ions through the plasma membrane (PM) and/or their release from intracellular stores, such as the endoplasmic reticulum (ER). This increase is usually in the form of microdomains with Ca2+ concentrations in the range of 50–100 μM [5–7].

Synaptic transmission and neuronal plasticity are based on several Ca2+-dependent processes, such as excitability, neurotransmitter release, and gene expression. The activation of presynaptic neurons leads to the release of neurotransmitters into the synaptic cleft via a Ca2+-dependent process. The released neurotransmitters, in turn, activate receptors in the PM of subsequent neurons, thus initiating signal transmission. In postsynaptic neurons, the activation of neurotransmitter receptors results in the generation of Ca2+ signals that trigger responses that are specific to the type of receptor (reviewed in [8]). The subsequent readout of Ca2+ signals employs Ca2+ binding protein sensors and downstream signaling proteins that transmit the Ca2+ signal to cellular effectors. This allows the generation of various Ca2+ signals with distinct spatial and temporal dimensions, amplitude, frequency (in the case of oscillations), and localization (reviewed in [9,10]). In addition to the mechanisms that activate neuronal pathways, Ca2+ homeostasis comprises mechanisms that turn off Ca2+ signals (reviewed in [10,11]).

Various Ca2+-permeable channels are present in the PM of neurons (reviewed in [12]). These can be roughly divided into voltage-operated Ca2+ channels (VOCCs), store-operated Ca2+ channels (SOCCs), receptor-activated Ca2+ channels (RACCs) such as glutamate receptors (e.g., N-methyl-D-aspartate [NMDA]) in hippocampal CA1 synapses; [13], and transient receptor potential canonical channels (TRPCs; [14–17]). The transient receptor potential (Trp) gene family encodes integral membrane proteins that function as ion channels. These channels are expressed from yeast to vertebrates and ubiquitously throughout the body of mammals. Only few TRP channels are highly Ca2+ selective, most are non-selective cation channels. TRP channels can be activated by diverse intra- and extracellular stimuli that are either of physical (temperature, osmotic pressure, mechanical stress) or chemical nature (pH, pO2, reactive oxygen species, neurotransmitters, growth factors and cytokines, environmental factors). The channels are involved in diverse physiological processes, and based on amino acid sequence homology, mode of activation and function they can be divided into seven subfamilies: TRPC, TRPV, TRPM, TRPP, TRPML, TRPA and TRPN (reviewed in [18]).

The ER also participates in the generation of Ca2+ signals by releasing Ca2+ via inositol-1,4,5-triphosphate (IP3) receptors (IP3Rs; reviewed in [19–21]) and ryanodine receptors (RyRs; [22]; reviewed in [23]) and through passive leakage [24–26]. Both types of ER receptors that are

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involved in Ca\(^{2+}\) release (i.e., IP\(_3\)Rs and RyRs) are functionally coupled to neurotransmitter-gated RACCs or VOCCs (reviewed in [27]).

The clearance of Ca\(^{2+}\) from the neuronal cytoplasm is attributable to the function of Ca\(^{2+}\) pumps, exchangers (e.g., the Na\(^+\)/Ca\(^{2+}\) exchanger; [28], the mitochondrial Ca\(^{2+}\) uniporter [29–32]), and Ca\(^{2+}\) binding proteins, such as calbindin D-28k, calretinin, and parvalbumin [8,33–35].

The storage capacity of the ER is limited, and Ca\(^{2+}\) release must be followed by Ca\(^{2+}\) replenishment. This is performed by Ca\(^{2+}\) reuptake pumps (i.e., SERCAs [sarco-endoplasmic reticulum Ca\(^{2+}\)-adenosine triphosphatases (ATPases)]; reviewed in [36–39]). The mechanism of subsequent Ca\(^{2+}\) entry from the environment is referred to as capacitative Ca\(^{2+}\) entry (CCE) or store-operated Ca\(^{2+}\) entry (SOCE). The refilling of Ca\(^{2+}\) in the ER occurs by pumping Ca\(^{2+}\) via SERCAs. Calcium then enters the cell through SOCCs located in the PM (reviewed in [40]). The identity of SOCCs and coupling between their opening and ER Ca\(^{2+}\) stores remained unidentified until the discovery of the Orai and stromal interaction molecule (STIM) proteins.

In this review, we evaluate data on STIM and Orai proteins in neurons to address the issue of whether SOCE in neurons is used only to refill Ca\(^{2+}\) in the ER or whether Ca\(^{2+}\) that enters the neuronal cell during SOCE also performs signaling functions. If the latter, then what type of function and through which pathways? The issue of the function of SOCE in neurons may sound simply academic, but resolving this issue has important implications for identifying new drug targets for the treatment of brain diseases. Evidence indicates that in such neurodegenerative diseases as Alzheimer's disease and Huntington's disease, Ca\(^{2+}\) homeostasis and SOCE components become dysregulated (reviewed in [48,81]). Thus, different targets and strategies might be identified for the potential treatment of these diseases (reviewed in [41]).

2. Store-operated Ca\(^{2+}\) entry before the discovery of STIM and Orai

Store-operated Ca\(^{2+}\) entry in non-excitable cells is a ubiquitous process (reviewed in [42,43]). It was earlier referred to as CCE [44] and described as an increase in Ca\(^{2+}\) influx across the PM when the ER Ca\(^{2+}\) pool was emptied by an agonist [44,45]. The proof that extracellular Ca\(^{2+}\) ions enter the cell to refill the pool was provided by the use of thapsigargin, which is a noncompetitive SERCA inhibitor derived from Thapsia garganica [46]. The use of thapsigargin provided evidence that the filling status of the intracellular store was responsible for Ca\(^{2+}\) influx via PM channels (reviewed in [47]).

The role of SOCE is well understood in various non-excitable cells, and its molecular mechanism has been recently described in several reviews [48,49]. The mechanism is based on the interaction between STIM proteins (STIM1 and STIM2) that sense Ca\(^{2+}\) levels in the ER and Ca\(^{2+}\) channels in the PM, such as Orai (Orai1, Orai2, Orai3) (reviewed in [50,51]). Originally STIM was discovered as a stromal cell-surface interacting protein [52], reviewed in [53]. Reports indicate that SOCE is also present in excitable cells, such as smooth muscle cells [54], cardiac muscle cells [55,56], skeletal muscle cells [57–59], and neuronal cells [60,61]. However, the physiological role of SOCE in excitable cells remains unclear [55,62] and has even been challenged [63]. On the other hand, P2Y receptor-induced signaling pathways, which play an important role in the proper development of neuromuscular junctions, depend on SOCE [64,65].

3. Store-operated Ca\(^{2+}\) entry in neurons after the discovery of STIM and Orai

Convincing results indicated the existence of SOCE in the nervous system (reviewed in [66,67]), but the mechanism of SOCE was unknown. To determine whether it is similar to SOCE in non-excitable cells, it was necessary to demonstrate that STIM and Orai proteins are expressed in neuronal cells and are operational. Studies by [68] and our group showed that STIM1 is present in neuronal cells in the brain [69,70], together with Orai2 [71]. More information was subsequently obtained about the neuronal expression of STIM1, STIM2, and three Orai proteins and their involvement in SOCE, indicating that this mechanism does in fact operate in these cells.

Growing evidence indicates substantial diversity among the proteins that are engaged in SOCE. Several sets of PM channels, such as Ca\(^{2+}\) release-activated channels (CRACs) and TRPC mediate this process and two types of Ca\(^{2+}\) sensors STIM1 and STIM2. CRACs are composed of Orai protein subunits, and TRPC channels are homo and heteromeric forms of TRPC proteins. Both types of channels, Orai and TRPC, are gated by the STIM1 protein, although it is managed by different mechanisms [72]; reviewed in [73,74]. TRPCs can be activated by PLC-derived signals, such as DAG, independently of Ca\(^{2+}\) store depletion and/or in a coordinated manner together with store-operated Orai channels (reviewed in [75]). STIM1 associates with TRPCs through electrostatic interactions. However, some of the channels of the TRPC family can also operate without STIM1 binding. The dual function of these channels appears to depend on stoichiometry (i.e., the STIM1/TRPC channel ratio; [76,77]). In many types of cells but not neurons, the activity of TRPCs after store depletion appears to also be Orai1-dependent [73]. We assume that STIMs, Orais, and some TRPC proteins are engaged in neurons in SOCE and contribute to neuronal processes (Fig. 1). If so, then they can also be involved in the etiology of some neurodegenerative diseases (reviewed in [78]).

The SOCE response was detected in neurons and neuroglial cells [69,79,80]. Although the major Ca\(^{2+}\) entry pathway in neuronal cells occurs through voltage-operated Ca\(^{2+}\) influx, recent reports suggest that SOCE constitutes a crucial source of Ca\(^{2+}\) when neurons are at rest [48,81]. Calcium microdomains were shown to be created through VOCCs in neurons (reviewed in [82,83]). Calcium microdomains may also be created through SOCE (reviewed in [84]).

The selectivity by which Ca\(^{2+}\) activates downstream pathways is encoded by the oscillatory amplitude and frequency and spatial profile of Ca\(^{2+}\) signaling (reviewed in [83,85]). Different agonists can induce versatile Ca\(^{2+}\) oscillation patterns (reviewed in [86,87]). In rat basophil leukemia (RBL) cells, the stimulation of cysteinyl leukotriene type 1 (cysLT1) or FcεRI receptors generates unique Ca\(^{2+}\) oscillation patterns and gene expression through the activation of different STIM proteins [88]. Agonist-induced Ca\(^{2+}\) entry with Orai1 or TRPC1 evokes different patterns of Ca\(^{2+}\) signaling that activate distinct Ca\(^{2+}\)-dependent gene expression pathways. Orai1-mediated Ca\(^{2+}\) oscillations appear to efficiently induce the nuclear translocation of nuclear factor of activated T-cells (NFATs), whereas Ca\(^{2+}\) entry via TRPC1 primarily regulates nuclear factor κB (NF-κB)-mediated gene expression [89].

4. Store-operated Ca\(^{2+}\) entry in polarized cells

Neurons are highly specialized cells with temporally and spatially separated Ca\(^{2+}\) responses. They are a classic example of polarized cells with long thin axons, a soma, and diverse forms of denticritic trees that are specialized to receive and process excitatory synaptic inputs. This neuronal polarity enables the directional flow of information that is required for communication between neurons or with effector cells. Secretory epithelial cells, such as salivary acinar gland cells, are also polarized. Sustained fluid secretion depends on the Ca\(^{2+}\) influx pathway that is mediated by SOCE (as reviewed in [90]). Neurotransmitter-generated Ca\(^{2+}\) signals in exocrine gland cells are crucial for the regulation of their secretory functions. The upregulation of fluid secretion in salivary glands is coordinated by autonomic sympathetic and parasympathetic stimuli. Major receptors associated with salivary gland fluid secretion are G-protein-coupled receptors (GPCRs) such as muscarinic M1 and M3, α-adrenergic receptors (e.g., α1A), and some purinergic receptors.GPCR stimulation leads to the G-protein-mediated activation of phospholipase C (PLC)-dependent pathways (reviewed in [91]).

Distinct patterns of plasmalemmal channel localization involved in SOCE were revealed by immunostaining. TRPC1 is localized in the lateral
and basal regions of salivary acinar cells, whereas Orai1 appears to be localized in the lateral membrane toward the luminal side. STIM1 has diffuse localization in these cells before stimulation; after agonist stimulation, however, it moves to the lateral and basal regions of the cells and strongly co-localizes in the lateral membrane region with both channels. In the basal region, it co-localizes only with TRPC1 [92]. Thus, distinct localization of Orai1 and TRPC1 implicates compartmentalization of Ca\(^{2+}\) influx (reviewed in [91]).

Similar patterns of STIM1, Orai1, and TRPC1 distribution were shown to exist in pancreatic acinar cells, which are also highly polarized cells. Orai1 is predominantly localized in the apical pole of the lateral membrane and tight junctions where it co-localizes with tight junction protein ZO1. In resting cells, STIM1 is more diffuse, with intense staining in the basal pole. Following stimulation, STIM1 is recruited to the lateral and apical side. TRPC1 is expressed in both the apical and basolateral regions of the PM. Stimulation by carbachol in the presence of mycotoxin cyclopiazonic acid (CPA), an inhibitor of Ca\(^{2+}\)-dependent ATPases, enhanced the co-immunoprecipitation of STIM1 with Orai1 or IP3Rs and the co-immunoprecipitation of STIM1 with TRPC1. This interaction was diminished by 2-aminoethoxydiphenyl borate (2-APB), a SOCE inhibitor [93].

Taking into account distinct distribution of STIM1, Orai1 and TRPC1 in epithelial secretory cells one can address several issues: (1) is this a unique feature of these cells or a more general rule for all polarized cells, including neurons, (2) whether STIMs and Orai are involved only in SOCE and (3) whether the SOCE response in the soma has the same function as the SOCE response in dendritic spines? Our group showed that the interaction between endogenous STIM2 and Orai1 under low extracellular Ca\(^{2+}\) conditions is increased in cortical neurons [94]. This is the location of the rough endoplasmic reticulum (RER). Because of the size of ribosomes, the RER does not permit direct contact between STIM and Orai proteins. However, the ribosome-free extension of the RER allows this organelle to form close junctions with the basolateral PM in pancreatic acinar cells. The overexpression of STIM1-YFP protein led to a two-fold increase in the density of ribosome-free terminals, demonstrating the role of STIM1 in the formation of ER–PM junctions [95]. This suggests that the RER, which is engaged in protein synthesis, folding, and transport might be employed in SOCE. Thus, an interesting line of investigation would be to explore whether Orai-mediated Ca\(^{2+}\) entry is involved in the regulation of protein synthesis in the RER.

5. SOCE in transcription factor signaling

Substantial evidence has been provided in many types of cells that proteins that are involved in SOCE, such as STIM and Orai, are engaged in various signaling pathways that play crucial roles in the regulation of cellular physiology and pathophysiology (reviewed in [96]). In addition to participating in calcium currents (I\(\text{crac}, I_{\text{arc}}\), I\(\text{rac}\)) these proteins have other functions. For instance, STIMs are involved in cell interaction [52], and Orais in the secretory pathway Ca\(^{2+}\)-ATPase-2 (SPCa2) [97]. In many animal models, including studies using knockouts of STIM or Orai genes, it was not unequivocally demonstrated that a particular result or phenotype is in fact due to the loss of SOCE.

One of the most studied Ca\(^{2+}\)-dependent signaling pathways where the SOCE response is well documented is the Ca\(^{2+}\)-calmodulin–calcineurin–NFAT pathway (reviewed in [98]). The stimulation of T cell receptors (TCRs) leads to the activation of PLC\(\gamma\)1. Among the products of PLC\(\gamma\)1 enzymatic activity are IP3 and diacylglycerol (DAG). IP3 binds to the IP3 receptor (IP3R) on the ER membrane, releasing Ca\(^{2+}\) from the ER into the cytoplasm. Depletion of the ER leads to the activation of CRAC channels on the PM, which are composed of Orai proteins. Upon the increase in Ca\(^{2+}\) influx via CRACs, calmodulin binds Ca\(^{2+}\) and forms a complex with the protein phosphatase calcineurin. This complex dephosphorylates cytoplasmic NFAT and triggers NFAT migration into the nucleus (reviewed in [99]). Nuclear NFAT activates gene transcription that is involved in many processes, including cell proliferation, cytokine production, growth arrest, and cell death (reviewed in [100]).

NFAT-dependent gene expression is known to be essential for the development and function of the nervous system. NFAT-luciferase transgenic mice were shown to exhibit much higher NFAT transcriptional activity in the brain than in most other tissues [101]. The NFAT signaling pathway is an important cascade in guided axonal growth during neuronal development and the integration of synaptic plasticity (reviewed in [102]). Sensory axon projections and commissural axon growth were impaired in NFATc2, NFATc3, and NFATc4 triple-mutant mice [103]. The response of axonal growth cones to neurophin stimulation is mediated by the calcineurin/NFAT cascade. Spinal cord explants from these triple-mutant mice exhibited dramatic impairment in axonal growth upon netrin-1 treatment compared with wildtype mice [103]. The NFAT signaling pathway is also known to be activated in neurons upon Ca\(^{2+}\) influx, which is mediated by L-type Ca\(^{2+}\) channels and NMDA receptors [104]. The calcineurin-dependent expression of IP3Rs
was increased in cultured cerebellar granule cells as a result of their depolarization [104].

A recent study showed that SOCE regulates calcineurin/NFAT-mediated gene expression and the proliferation of neural stem/progenitor cells (NPCs; [105]). Thapsigargin treatment of NPCs that were derived from embryonic, neonatal, and adult mice induced a prominent SOCE response via STIM1–Orai1 interactions. No difference was found in the amplitude of SOCE between embryonic and adult NPCs, suggesting that this Ca²⁺ entry mechanism is conserved throughout development. The pharmacological profile of SOCE in NPCs is characteristic of CRACs [105]. This was confirmed by the suppression or complete loss of SOCE in embryonic NPCs that were derived from mice that lacked either functional STIM1 or Orai1. However, in NPCs from adult Orai1 knockout mice, small residual Ca²⁺ influx was observed after thapsigargin treatment followed by extracellular Ca²⁺ re-addition. This response was sensitive to a low concentration of La³⁺, suggesting that this Ca²⁺ entry mechanism is conserved throughout development. The pharmacological profile of SOCE in NPCs is characteristic of CRACs [105]. This was confirmed by the suppression or complete loss of SOCE in embryonic NPCs that were derived from mice that lacked either functional STIM1 or Orai1. Moreover, this activation of SOCE led to high stimulation of NFAT-mediated gene expression, which was affected by the SOCE channel inhibitors 2-APB, SKF96365, and BTP-2 (Table 1). NFAT activation was also completely lost in NPCs that were obtained from Orai1R93W/R93W knock-in mice, which contain a loss-of-function R93W mutation.

The regulated activity of transcription factors by neuronal activity associated with membrane depolarization and Ca²⁺ signaling plays an important role in the development and function of the central nervous system. Sp4 transcriptional factor is highly expressed in neurons [106] and regulated by Ca²⁺, which likely occurs via Ca²⁺-store-operated Ca²⁺ entry (SOCE) [81]. Hypomorphic mice with reduced Sp4 expression exhibit impairments in spatial learning memory and synaptic plasticity. This might be associated with perturbations in dendrite patterning and contribute to the pathophysiology of several neuropsychiatric disorders [107–110]. Under hyperpolarizing (5 mM KCl) conditions, Sp4 was shown to be polyubiquitinated and degraded by the proteasome in cerebellar granule neurons (CGNs; [111]). In contrast, depolarizing conditions (65 mM KCl), which stimulate neuronal activity, significantly increased the abundance of Sp4. In both cases, the mRNA level of Sp4 was unchanged [81]. Furthermore, treatment with the SOCE inhibitors SKF96365 and 2-APB increased Sp4 abundance under depolarizing conditions and completely blocked the reduction of Sp4 in CGNs under resting conditions. These changes also occurred without changes in mRNA levels. SKF96365 treatment prevented the polyubiquitination and degradation of Sp4 in resting CGNs (5 mM KCl). Under these conditions, sustained Ca²⁺ influx occurred in CGNs and was prevented by SKF96365. The number of STIM1/Orai1/2 puncta was high in resting neurons with 5 mM KCl application, moderate with 25 mM KCl application, and very low in depolarized neurons with 65 mM KCl application. Altogether, these data indicate that SOCE in resting neurons may regulate Sp4-dependent transcription, which is consistent with the observation that a significant reduction of Sp4 degradation occurs upon the suppression of STIM1 levels [81].

6. Store-operated Ca²⁺ entry in guided axonal growth

Guided axonal growth and regeneration depend on the spatial and temporal regulation of Ca²⁺ signals (reviewed in [112]). This is a key component of the downstream signaling pathway that enables the proper navigation of axons through a complex environment to reach specific targets to form neuronal connections. The precise mechanism by which this regulation in neuronal growth cones is coordinated in response to guidance cues (e.g., netrins, semaphorins, and ephrins) is unknown. The Ca²⁺-dependent growth cone responses to netrin-1 and brain-derived neurotrophic factor (BDNF) are mediated by Ca²⁺-calmodulin dependent protein kinase II, and calcineurin and can be modulated by cAMP pathways [113], reviewed in [112]. Semaphorin-3A signaling depends on cyclic guanosine monophosphate (cGMP) and Ca²⁺ influx mediated by cyclic nucleotide-gated Ca²⁺ channels [114,115]. TRPC channels are involved in BDNF-mediated nerve growth cone guidance in cultured cerebellar granule cells. The pharmacological inhibition of TRPC3 channels or downregulation of its expression using siRNA disrupted BDNF-mediated elevations in Ca²⁺ and growth cone turning [116].

Accumulating evidence demonstrates the contribution of STIM, Orai, and TRPC proteins in neuronal growth cone and axon pathfinding but the mechanisms of their activity are unknown. Although their primary function is associated with SOCE activation, data suggest other possibilities, such as the involvement of STIM1 in cAMP signaling, ER modeling, and cytoskeleton reconstruction [117–119]; reviewed in [120,121].

Homer1 scaffolding protein in dorsal root ganglion (DRG) sensory neurons was shown to facilitate signaling at the dendritic postsynaptic density [122]. This occurred through the coupling of extracellular receptors, such as mGlurRs and TRPC family channels, to intracellular receptors, such as IP₃Rs. Isolated DRG neuronal growth cones turned toward micro-gradients of BDNF and netrin-1 and were repelled by semaphorin-3A. Homer1 knockdown had no effect on the growth cone response to semaphorin-3A treatment and dramatically reversed attraction to repulsion in response to BDNF and netrin-1. Moreover, the BDNF-induced elevation of Ca²⁺ influx was abolished in growth

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Table 1

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<tr>
<th>Agent</th>
<th>SOCE</th>
<th>Mechanism</th>
<th>References</th>
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<tbody>
<tr>
<td>2-ABP (2-aminoethoxydiphenyl borate)</td>
<td>Transient potentiation at low concentration (~5 μM)</td>
<td>Initially described as an inhibitor of IP₃R and CRAC channels</td>
<td>[228]</td>
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<td></td>
<td>Inhibition at high concentration (~50 μM)</td>
<td>Inhibitor of transient receptor potential canonical (TRPC) channels</td>
<td>[229]</td>
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<td></td>
<td></td>
<td>Complex effect on Orai3 channels</td>
<td>[230,231]</td>
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<td></td>
<td></td>
<td>Activator of transient receptor potential vanilloid (TRPV1, 2, 3)</td>
<td>[232]</td>
</tr>
<tr>
<td>SKF96365 (less specific to Ca²⁺ channels than 2-ABP)</td>
<td>Inhibition</td>
<td>Inhibitor of STIM1-mediated Ca²⁺ influx</td>
<td>[233]</td>
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<td></td>
<td></td>
<td>Inhibitor of TRPC channels</td>
<td>[234]</td>
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<tr>
<td>Thapsigargin</td>
<td>Potentiation</td>
<td>Inhibitor of voltage operated Ca²⁺ channels (VOCCs)</td>
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<td>Potentiation</td>
<td>Inhibitor of SERCA pump</td>
<td>[236]</td>
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<td>ML-9</td>
<td>Inhibition</td>
<td>Inhibitor of SERCA pump</td>
<td>[237]</td>
</tr>
<tr>
<td>Lanthanides</td>
<td>Inhibition</td>
<td>Inhibitor of STIM1-mediated Ca²⁺ influx</td>
<td>[238]</td>
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<td>BTP 2</td>
<td>Inhibition</td>
<td>Inhibitor of CRAC channels at low mM concentrations</td>
<td>[237]</td>
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<tr>
<td>3,5-bis(trifluoromethyl)pyrazole</td>
<td>Inhibition</td>
<td>Inhibitor of Crac channels (TRPC)</td>
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<tr>
<td></td>
<td></td>
<td>Inhibitor of Crac channels (TRPC)</td>
<td>[240]</td>
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</table>
cones with decreased expression of Homer1. This change was affected by SOCE inhibitors. Immunolocalization studies revealed a close association between Homer1 and TRPC1 and STIM1 in functionally relevant areas of growth cones, suggesting that the TRPC, Homer, and STIM1 complex might be crucial for proper axon guidance [122]. Store-operated Ca$^{2+}$ entry mediated by STIM1 and Orai1/Orai2 was shown to be necessary for growth cone turning responses to extracellular guidance cues, such as BDNF and semaphorin-3a [123]. Changes were observed in the morphology of growth cones, which exhibited different patterns of STIM1–Orai1/2 immunoreactivity. The majority of the growth cones displayed diffuse STIM1 and Orai1/2 immunoreactivity, which was very narrow and did not spread, whereas growth cones that displayed a punctate pattern of STIM1–Orai complexes presented spreading lamellipodia. STIM1 knockdown caused a reduction of the BDNF-induced elevation of Ca$^{2+}$ influx and changed the growth cones response from attraction to repulsion after BDNF treatment, with no response to semaphorin-3a exposure. Moreover, in the semaphorin-3a collapse assay, growth cones that had reduced STIM1 expression exhibited only minor suppression of spreading lamellipodial morphology compared with controls. These characteristics were sustained even in Ca$^{2+}$-free medium [123].

Growth cones were shown to require STIM1 and TRPC1 for attractive guidance responses [124]. TRPC1 knockdown by morpholino or the overexpression of dominant-negative STIM1 (STIM1-DN) abolished the detection of SOCE in spinal neurons. The overexpression of STIM1-DN also disrupted the sustained Ca$^{2+}$ elevation in neuronal growth cones in response to netrin-1. Localized and spontaneous Ca$^{2+}$ transients in filopodia were shown to be affected by the inhibition of STIM1 and TRPC1 function. This might indicate that STIM1- and TRPC1-dependent SOCE is essential for filopodial Ca$^{2+}$ entry and the maintenance of oscillatory filopodial Ca$^{2+}$ transient patterns. The incidence and frequency of filopodial Ca$^{2+}$ entry in response to netrin-1 were also impaired by alterations in STIM1 and TRPC1 expression. The authors determined that a large portion of Ca$^{2+}$ entry sites was located at the filopodial tip, suggesting that this is a primary site of SOCE-mediated Ca$^{2+}$ entry in nerve growth cones [124]. The observation that STIM1-DN overexpression completely abolished netrin-1–induced attraction, resulting in repulsion, is consistent with previous studies that reported that TRPC1 knockdown impaired netrin-1–induced attractive growth cone turning responses [125,126]. Altogether, these results suggest that proteins that are involved in SOCE may mediate axon pathfinding.

7. Store-operated Ca$^{2+}$ entry in synaptic plasticity

Baba and coworkers showed that SOCE channel inhibitors, such as 2-APB and SKF96365, attenuated tetanus-induced dendritic Ca$^{2+}$ accumulation and long-term potentiation (LTP) at Schaffer collateral–CA1 synapses in the hippocampus [127]. This report, together with other results, demonstrated impairments in spontaneous Ca$^{2+}$ transients in synaptic boutons by blocking SOCE with La$^{3+}$ and [60] and suggested that SOCE may play a role in synaptic plasticity. Useful insights into the role of intracellular stores in neuronal function and plasticity were suggested by Narayanan and coworkers [128]. It was demonstrated that intracellular calcium store depletion in rat hippocampal neurons induces a perisomatic increase in the density of functional h channels (hyperpolarization-activated cyclic nucleotide-gated ion channels, HCN) mediated by Ca$^{2+}$ elevation through the activation of InsP$_3$,R and SOCE channels [128]. In the mammalian central nervous system, slow synaptic transmission involves the activation of mGlur1s [reviewed in (129)]. These are G-protein-coupled neurotransmitter receptors that are found in the presynaptic and postsynaptic densities of synapses (reviewed in [130]). They are implicated in many forms of neural plasticity, including hippocampal LTP [131–133], long-term depression (LTD; [134,135]), and associative learning [136,137]. TRPC1 was shown to be physically associated with mGlur1 and engaged in mGlur1-evoked slow excitatory postsynaptic conductance in Purkinje cells [138]. A subsequent report showed that TRPC3 is also important for mGluR-dependent synaptic signaling in mouse cerebellar Purkinje neurons [139]. A recent paper provided evidence that STIM1 is required for store refilling in resting Purkinje neurons and mGlur1-dependent TRPC3 activation [140]. The authors showed that the absence of STIM1 strongly attenuated IP$_3$-dependent Ca$^{2+}$ signals and mGlur1-dependent synaptic potentials. Notably, TRPC3 channels have been described as mediators of BDNF-mediated dendritic remodeling in hippocampal CA1 pyramidal neurons [141]. BDNF plays a significant role in modulating synaptic plasticity (reviewed in [142]) by enhancing synaptic transmission at excitatory synapses and altering dendritic architecture [143]. The role of interactions between STIM1 proteins and TRPC channels is under vigorous investigation (reviewed in [73]).

Interesting data show that synaptopodin (SP) may interact with SOCE machinery. Synaptopodin is an actin binding protein and marker of the enigmatic spine apparatus [144]. This cellular structure is composed of a stacked smooth ER [145]. Synaptopodin was shown to be essential for the formation of this organelle. For example, it is absent in SP-deficient mice [146]. These mice show deficits in synaptic plasticity and spatial learning [147]. Upon treatment with kainic acid or tetanic stimulation, the expression of SP was increased in hippocampal neurons [148,149]. Spines that contain SP generate a two-fold larger Ca$^{2+}$ response to flash photolysis of caged glutamate than SP-negative spines. This increase disappears with CPA treatment, indicating the involvement of the ER [150]. Thus, SP is involved in the glutamate response and appears to be associated with Ca$^{2+}$ stores [150]. Synaptopodin tends to accumulate in large-head spines [150,151]. A recent study demonstrated that STIM1 preferentially localized in spines that contained SP in cultured hippocampal neurons that were derived from SP knockout mice and transfected with a SP-green fluorescent protein (GFP) expression vector [119]. This association was especially prominent in mushroom spines. Consistent with this observation, a loss of STIM1 puncta was observed in mushroom spines without SP. Orai1 localization in this particular type of spine also appeared to be SP-dependent. These results indicate that SP may interact with SOCE in synaptic plasticity [119].

Non-receptor protein tyrosine kinases (PTKs), such as the Src family, are highly expressed in the brain. Their functions are postulated to be associated with the regulation of neuronal plasticity (reviewed in [152]), behavior [153], and cognitive deficits in schizophrenia [154]. Protein tyrosine kinase and protein tyrosine phosphatase (PTP) were shown to modulate Ca$^{2+}$ responses mediated by NMDA receptors (NMDARs; [154]; reviewed in [155,156]) and voltage-gated Ca$^{2+}$ channels [reviewed in (157)]. In pancreatic acinar AR42J cells and HEK293 cells, the SOCE response was reported to be modulated by protein tyrosine phosphatase 1B (PTP1B). Its overexpression triggered the inhibition of PTP1B plays a crucial role in the regulation of SOCE. Upon CPA treatment, Ca$^{2+}$ influx in cultured hippocampal neurons that were derived from PTP1B knockout mice was higher than in cells from wildtype animals. This indicates that this type of SOCE regulation is also present in neurons. Administration of the PTK inhibitor AgI26 suppressed SOCE in both wildtype and PTP1B knockout neurons. The global level of tyrosine phosphorylation was much higher in PTP1B knockout hippocampal neurons than in wildtype cells. This effect appeared to be at least partially mediated by the modulation of Src kinase activity [160]. Administration of sodium orthovanadate, an inhibitor of PTP, upregulated PTK activity in neonatal rat hippocampal neurons, which not only induced SOCE but also modulated NMDA-induced neuronal excitability. The authors suggested that the tyrosine-based phospho-regulation of SOCE might be involved in the phospho-tyrosine-dependent regulation of NMDAR-induced signaling pathways [161].
8. Effect of STIM1 on voltage-operated channels

The majority of Ca\(^{2+}\) influx in neurons occurs via VOCCs that are activated by depolarization and are essential for synaptic vesicle release, contraction, and electrical excitability (reviewed in [162]). One subtype of VOCCs, Ca\(_{\text{a}},1.2\) channels, is ubiquitously expressed in neuronal, cardiac, and smooth muscle cells (reviewed in [163]). STIM1 directly suppressed the depolarization-induced opening of voltage-gated Ca\(_{\text{a}},1.2\) channels [164]. The treatment of cultured rat cortical neurons with 1 μM thapsigargin led to an ~20% decrease in the initial slope of the Ca\(^{2+}\) rise, suggesting that store depletion inhibited the conductance by VOCCs. A deeper analysis of this phenomenon revealed that STIM1 altered the surface expression of Ca\(_{\text{a}},1.2\) by internalizing the channels. The authors provided a mechanism by which STIM1 was recruited to Ca\(_{\text{a}},1.2\) channels after store depletion and triggered Ca\(_{\text{a}},1.2\) internalization, leading to its complete loss of function. They also suggested that STIM1 acts as a switch that promotes Ca\(^{2+}\) entry through Orai channels and inhibits Ca\(^{2+}\) entry through Ca\(_{\text{a}},1.2\). This regulation of Ca\(_{\text{a}},1.2\) could be important after the activation of PLC-coupled receptors, such as dopamine D\(_2\) receptors [165]; reviewed in [166]. A similar observation of STIM1-dependent inactivation of VOCCs in vascular smooth muscle cells was made by another research group [167]. These authors found that STIM1 did not require Orai channels for the attenuation of Ca\(_{\text{a}},1.2\) channel activity. However, they found that Orai may enhance STIM1 recruitment to the proximity of Ca\(_{\text{a}},1.2\) channels by effectively trapping STIM1 in puncta. STIM1 has also been shown to interact with both types of channels through the short STIM–Orai activating region. The results of both research groups show that store depletion activates SOCCs and inhibits VOCCs, prompting Moreno and Vaca to introduce a novel term: store-inhibited channels (SICs) [168]. They postulated that the first member of this family is an L-type VOCC. STIM1 also inhibits T-type Ca\(^{2+}\) channels (Ca\(_{\text{a}},3.1\) in cardiomyocyte-derived HL-1 cells. Co-immunoprecipitation experiments revealed that STIM1 is associated with Ca\(_{\text{a}},3.1\) channels in HL-1 cells and reduces its activity to prevent excessive Ca\(^{2+}\) entry [169]. Harraz and Altier speculated that the STIM1-dependent modulation of Ca\(_{\text{a}},3.1\) could be involved in modulating the rhythmicity and excitability of cardiac pacemaker cells and may be involved in pathologic cardiac events (e.g., arrhythmias; [170]). One unresolved issue is the role of the long isoform of STIM1 (STIM1L), which is predominantly expressed in muscle cells and the brain [171–173] (Fig. 2). STIM1 appears to be a multifunctional protein in excitable cells (see Fig. 1). After store depletion, it activates CRACs and concurrently inhibits some VGCCs. The immunolocalization of STIM1 in the mouse brain has been described [70,174,175].

9. Store independent function of STIM and Orai proteins in non-neuronal cells

Arachidonate-regulated Ca\(^{2+}\) channels (ARCs) represent a member of highly selective Ca\(^{2+}\) entry channels in various cell types, including HeLa cells, COS cells, RBL cells, DT40 cells [176], and parotid and pancreatic acinar cells [177]. These channels, which are formed by a combination of Orai1 and Orai3 subunits [178], are activated by arachidonic acid independently of ER Ca\(^{2+}\) depletion. They are also insensitive to inhibition with 2-APB, unlike CRACs [176]. STIM1 plays a role in the regulation of ARCs. The minor pool of STIM1 that resides at the PM (PM-STIM1) is necessary for ARC activation in HEK293 cells [179]; reviewed in [180]. Leukotriene C\(_4\)-regulated Ca\(^{2+}\) channels (LRCs) share many ARC-like features, such as store independence and insensitivity to inhibition with 2-APB. In vascular smooth muscle cells (VSMCs), the activation of these channels by thrombin was shown to require STIM1, Orai1, and Orai3 [181]. Zhang and coworkers showed that unlike ARCs in HEK293 cells, the activation of LRCs in VSMCs by thrombin required ER-STIM1. However, PM-STIM1 could support thrombin-activated Ca\(^{2+}\) entry in these cells [182], suggesting a complex role for both pools of STIM1, in which endoplasmic and plasmalemmal pools are involved in the regulation of store-independent Orai1/Orai3 channels. One may speculate whether similar store-independent activities of STIM1, Orai1 and Orai3 exist in neurons.

10. Role of STIM2

STIM2 is a more sensitive Ca\(^{2+}\) sensor than its homolog STIM1. STIM2 is characterized by slower activation kinetics and activated by smaller depletion of the ER [183,184]. STIM2 is predominantly expressed in mouse brain, with abundant expression in hippocampal neurons [70,174,175]. STIM2-deficient mice develop normally but have a reduced life expectancy. Approximately 8 weeks after birth, sudden death is observed in homozygous mice (STIM2\(^{-/-}\)). In the Morris water maze (i.e., a standard test for hippocampus-dependent spatial memory), STIM2\(^{-/-}\) mice required a longer time to find the hidden platform, suggesting that STIM2 deficiency leads to cognitive impairment [174]. In cultured neurons from STIM2\(^{-/-}\) mice, the SOCE response was reduced following CPA treatment. This reduction was shown to be regulated by STIM2, with no contribution from STIM1 or Orai1. In cultures of cortical neurons derived from STIM1\(^{-/-}\) and Orai1\(^{-/-}\) mice, no changes were observed in the SOCE response induced by stimulation with CPA followed by the replacement of 1 mM ethylene glycol tetraacetic acid (EGTA) with 2 mM Ca\(^{2+}\) [174]. This observation may suggest that Orai2, the prominent expression of which was observed in the brain, may mainly contribute to SOCE in neurons, rather than Orai1 [71]; reviewed in [185]. In HEK293 and RBL cells, the ability of Orai2 to form CRACs depends on the cell background [71]. However, our research group used the Proximity Ligation Assay, co-immunoprecipitation, and single-cell Ca\(^{2+}\) imaging and found that endogenous STIM2 under low-Ca\(^{2+}\) extracellular conditions is associated with endogenous Orai1 in primary cortical neurons derived from rat embryos [94]. These results are consistent with our previous studies of ectopically expressed yellow fluorescent protein (YFP)-STIM2 and Orai1 in cortical neurons, in which the number of STIM2/Orai1 puncta...
increased under low Ca²⁺ conditions [175]. Surprisingly, a large difference was found between the number of YFP-STIM1/Orai1 and YFP-STIM2/Orai1 puncta following thapsigargin treatment. The reduced number of YFP-STIM2/Orai1 puncta was correlated with the suppression of Ca²⁺ entry after store depletion. The observation that neurons that were transfected with YFP-STIM2/Orai1 showed a greater increase in constitutive Ca²⁺ entry than YFP-STIM1/Orai1 suggests that STIM2 is involved in the regulation of resting Ca²⁺ levels in neurons [175]. One caveat should be considered regarding the activity of YFP-STIM2. The construct contained the signaling peptide of STIM1 [183]. Thus, the localization of STIM2 in transfected neurons might not fully resemble physiological conditions.

Under ischemic conditions, such as low glucose and low pH, cultured hippocampal neurons from STIM2−/− mice survived significantly longer compared with STIM1−/− and wildtype neurons. In vivo STIM2 deficiency was shown to protect mice from ischemic neuronal damage independent of functional alterations within the hematopoietic system. Histological analysis of the brains of mice subjected to transient focal cerebral ischemia revealed that infarcts in STIM2−/− mice were restricted to the basal ganglia, whereas they extended to the neocortex in wildtype mice. The authors suggested that STIM2 mediates neuronal SOCE, and this pathway might play a pivotal role in hypoxia-induced neuronal death [174].

In immortalized lymphocytes from patients with familial Alzheimer’s disease, STIM2 levels decreased, whereas STIM1 levels remained unchanged [186]. Consistent with these observations, a recent study found that the level of STIM2 protein was dramatically reduced in the hippocampus in PS1-M146V KI mice, a model of familial Alzheimer’s disease, and in cortical samples from sporadic Alzheimer’s disease patients [187]. These changes correlated well with Mini Mental State Examination scores in Alzheimer’s disease patients that indicated the degree of dementia. The changes were also consistent with a reduction of the neuronal SOCE response in spines in PS1-M146V KI mice. In hippocampal neurons derived from PS1-M146V KI mice, a significant reduction of the fraction of mushroom spines was detected, together with an increase in the fraction of thin spines. The reduction of STIM2 also appeared to affect the morphology of dendritic spines in the hippocampus. STIM2 deletion in conditional knockout mice dramatically reduced the amplitude of synaptic SOCE and reduced CaMKII activity. Consistent with these observations, immunolocalization analyses showed that STIM2 was highly enriched in mushroom spines where it co-localized with pCaMKII. The Cdc42/Rac1 pathway, one of the way that is implicated in the maintenance of dendritic mushroom spines [187]. An interesting study that may have significant implications in our understanding of how STIM2 operates in neurons was conducted with HEK293 cells [188]. STIM2 was shown to exist in three distinct forms: ER resident STIM2, cytosolic preSTIM2, and cytosolic SPF. SPF is a ~91-amino acid short fragment of the STIM2 signaling peptide that is cleaved by signal peptide peptidases. All three forms are the result of STIM2’s possessing a very long (101 amino acid), highly conserved signaling peptide. PreSTIM2 is targeted to the inner leaflet of the PM, whereas SPF is distributed in the cytosol [Fig. 3]. PreSTIM2 can associate with Orai1 in the PM in a store-independent manner. The authors found that the regulation of basal Ca²⁺ influx by preSTIM2 regulated NFAT- and NF-kB-dependent transcription, whereas SPF slightly increased NF-kB-mediated transcription in a Ca²⁺-independent manner. Both preSTIM2 and SPF might bind calmodulin in the presence of Ca²⁺, indicating that they may regulate calmodulin-mediated processes. The authors proposed a new model whereby basal Ca²⁺ and SOCE are predominantly controlled by two distinct proteins, preSTIM2 and STIM2 [188].

11. SOCE in neurological disorders

Chronic increases in corticosteroid hormone levels in adult rats and humans make hippocampal neurons more sensitive to various neurological insults, which can lead to dendrite atrophy [189–191]. The chronic corticosterone treatment significantly increased SOCE in H19-7 cells, a cell-culture model of differentiating hippocampal neurons [192]. As previously demonstrated by Wu and coworkers, H19-7 cells undergo a dramatic increase in SOCE during differentiation, which is decreased by TRPC1/3 knockdown [193]. Surprisingly, TRPC1 knockdown did not block the enhancement of thapsigargin-stimulated Ca²⁺ entry caused by corticosterone treatment. The electrophysiological data indicated that in corticosterone-treated H19-7 differentiated neurons the store-operated Ca²⁺ currents exhibit the classic shape of Icrac. Consistent with this observation the authors also reported a dramatic increase in Orai1 immunostaining in corticosterone-treated cells compared with untreated cells. The results suggest that chronic exposure to high levels of stress hormones switched the SOCE response from the Icrac subtype to the Icrac subtype. The authors discussed the possible consequences of upregulating Icrac channels during periods of chronic stress and the implications for hippocampal neurons and memory retention [192].

The dysregulation of Ca²⁺ signaling has been shown to be a major factor in the development of pain behavior after peripheral nerve injury [194,195]. In dorsal root ganglion (DRG) neurons painful nerve injury disrupted Ca²⁺ levels in ER stores and decreased releasable Ca²⁺ from the ER [196]. Furthermore, peripheral nerve injury diminished the anatomical extent of the ER and reduced the expression of SERCA [197]. Recently, the same group showed that Ca²⁺ influx via SOCE after spinal nerve injury is needed for the preservation of intracellular levels of cytoplasmic and releasable pools of Ca²⁺ [80]. The expression of STIM1 and Orai1 in the DRG neurons was confirmed at both the mRNA and protein levels. An increase in SOCE was observed after thapsigargin treatment, which was affected by La²⁺ and 2-APB. In contrast, the inhibitor SKF96365 had no effect on Ca²⁺ influx stimulated by thapsigargin. SOCE was needed in the DRG neurons to replenish Ca²⁺ stores after a release event evoked by caffeine. The electrophysiological analysis revealed that store-operated Ca²⁺ currents in the DRG neurons exhibit the classic shape of the Icrac subtype. After peripheral nerve injury in rats by spinal nerve ligation (SNL; i.e., a standard model of neuropathic pain), amplification of the SOCE response was observed. An increase in Icrac currents was also detected after SNL. However, the mRNA and protein levels of STIM1 and Orai1 were unaffected by SNL. Moreover, in both cases, when store depletion was induced by thapsigargin, no differences in the SOCE response or Icrac currents were observed between control and affected DRG neurons. These results suggest that nerve injury activates SOCE, but the intrinsic capacity of SOCE remains unchanged. The level of cytoplasmic and stored Ca²⁺ in resting sensory neurons was diminished by injury. In fact, the termination of SOCE by removing Ca²⁺ from the medium led to much higher depression of Ca²⁺ levels in neurons after injury. This observation suggests that SOCE may play a compensatory role after injury. The upregulation of SOCE in injured neurons appears to be particularly involved in maintaining Ca²⁺ levels in the cytoplasm and stores [80].

Recently Xia and coworkers revealed that a novel Ca²⁺ signal mediated by store operated Ca²⁺ entry is present in dorsal horn neurons and may play an important role in pain transmission [198]. Previously this group demonstrated that YM-58483 (BTP2), a potent SOC channel inhibitor, produces a strong central analgesic effect and prevents the development of chronic pain in mice [199]. This group confirmed the functionality expression of protein involved in SOCE — STIM1, STIM2 and all three isoforms of Orai in dorsal horn neurons. Reduction of STIM1 or STIM2 or Orai1 by knockdown using siRNA markedly decreased SOCE as well as the basal cytosolic Ca²⁺ level in these cells. It suggests that SOCE contributes in resting Ca²⁺ homeostasis. Treatment with substance P (SP), neurotransmitter of pain processing acting through the neurokinin 1 (NK1) receptors led to SOCE activation.
and in consequence to the excitatory action in dorsal horn neurons. Altogether these results indicate that SOCE may modulate the Ca\textsuperscript{2+}-dependent pathological events related to chronic pain\cite{198}. Parkinson's disease is one of the most common neurodegenerative disorders. It is characterized by the selective loss of dopaminergic neurons in the substantia nigra. The major symptoms involve motor dysfunction (reviewed in\cite{200}). One of the mechanisms that affect the survival of dopaminergic neurons in Parkinson's disease is associated with dysregulation of Ca\textsuperscript{2+} levels, especially in the ER and mitochondria\cite{201}. One of the consequences of perturbing Ca\textsuperscript{2+} homeostasis is the accumulation of unfolded/misfolded proteins in the ER lumen. To alleviate ER stress and improve cell survival, the unfolded protein response (UPR) is activated (reviewed in\cite{202}). However, prolonged activation of the UPR results in programmed cell death (reviewed in\cite{203}). The oxidative form of neurotoxin 1 (MPP\textsuperscript{+}) induced the selective loss of dopaminergic neurons in the substantia nigra, leading to the development of a mouse model that mimics Parkinson's disease. In SH-SY5Y neuroblastoma cells, C57/BL mice, and PC12 pheochromocytoma cells, MPP\textsuperscript{+} treatment decreased the level of TRPC1 and consequently increased neuronal death\cite{204,205}. The loss of SOCE, which was observed in SH-SY5Y cells upon MPP\textsuperscript{+} treatment, might be involved in the induction of ER stress and initiation of the UPR\cite{206}. Electrophysiological studies in SH-SY5Y cells revealed that store-operated currents are mediated by I\textsubscript{SOCE} channels and decrease with SKF96365 and MPP\textsuperscript{+} treatment. The interaction between STIM1 and TRPC1 is also reduced with neurotoxin 1 treatment. In contrast, the association between STIM1 and Orai1 was unaffected by MPP\textsuperscript{+} application, indicating that TRPC1 is essential for SOCE in SH-SY5Y cells. A decrease in TRPC1 expression was also observed in brain tissue derived from Parkinson's disease patients and mouse primary dopaminergic neurons treated with neurotoxin 1. In SH-SY5Y cells, the silencing of TRPC1 decreased SOCE and Ca\textsuperscript{2+} levels in the ER and induced the UPR. Several UPR markers, such as GRP78 and CHOP, were upregulated after the specific suppression of TRPC1 but not TRPC3. Consistent with these data, upregulation of the UPR after TRPC1 or STIM1 silencing was associated with the inhibition of protein translation mediated by increasing eIF2\textalpha phosphorylation. A similar observation was made in \textit{Trpc1}\textsuperscript{−/−} mice, which exhibited greater activation of UPR machinery compared with wildtype mice. Altogether, these results indicate that the inhibition of SOCE due to silencing of TRPC1 leads to the dysregulation of Ca\textsuperscript{2+} levels in the ER and induces ER stress. The overexpression of TRPC1 but not Orai1 in SH-SY5Y cells restored SOCE and attenuated MPP\textsuperscript{+}-induced ER stress. Moreover, MPP\textsuperscript{+} administration significantly decreased AKT1 phosphorylation without affecting total AKT1 levels in SH-SY5Y cells, where-in the relationship between AKT and neuroprotection is well established\cite{207}. Surprisingly, thapsigargin treatment in the absence of external Ca\textsuperscript{2+} abolished AKT phosphorylation in SH-SY5Y cells, indicating that SOCE plays a crucial role in this pro-survival pathway. This effect was shown to be mediated by TRPC1. In contrast, BDNF expression was not enhanced by TRPC1 overexpression, although MPP\textsuperscript{+} decreased BDNF expression. Moreover, a significant decrease in neuronal survival was observed with MPP\textsuperscript{+} application in SH-SY5Y cells, which was inhibited by TRPC1 overexpression. Furthermore, the suppression of AKT1 abolished TRPC1-mediated neuroprotection against MPP\textsuperscript{+} in SH-SY5Y cells. The results obtained in the Parkinson's disease mice model in vivo were consistent with studies on SH-SY5Y neuroblastoma.

Fig. 3. Molecular domains of STIM2 protein and its subcellular localization. A) Three forms of STIM2 protein: preSTIM2 localized in the cytosol and associated with PM, mature STIM2 localized in the ER, signal peptide fragment (SPF) localized in the cytosol. This is a result of productive or aborted translocation of nascent STIM2 polypeptides through the ER translocon; a signal peptide (SP), a canonical EF-hand motif (cEF), a hidden EF-hand (hEF), a sterile alpha motif (SAM), a transmembrane domain (TM), coiled-coil domains (CC1), a STIM1–Orai activating region (SOAR), and a proline-histidine-rich domain (PH). Arrows indicate cleavage of SP by signal peptide peptidases. B) Both STIM1 and STIM2 are localized in the ER membrane and sense Ca\textsuperscript{2+} level within the store. After ER depletion STIM proteins present in the ER oligomerize and translocate toward PM. STIM2 protein is activated by smaller changes in Ca\textsuperscript{2+} level within the ER. Both homologs could also be localized in PM, whereas STIM2 is localized in the cytosol.
cells [206]. Additionally, neurotoxin 1 impaired the functions of AKT/ mammalian target of rapamycin (mTOR) in dopaminergic neurons, which might induce neurodegeneration. TRPC1 overexpression in mouse substantia nigra upon neurotoxin 1 administration significantly restored the activation of mTOR and its downstream targets. These results indicate that TRPC1-mediated SOCE in dopaminergic neurons is necessary for the activation of AKT/mTOR-dependent neuroprotection. Thus, TRPC1-dependent SOCE appears to be necessary for the maintenance of Ca\(^{2+}\) levels in the ER. The suppression of its function activates the UPR pathway, resulting in the depletion of AKT and consequently leading to the neurodegeneration of dopaminergic neurons and Parkinson’s disease [206]. It is of interest that SOCE is dysregulated also in several models of Huntington’s disease [208–210].

12. SOCE components as therapeutic targets: conclusions and future directions

Ca\(^{2+}\) dysregulation has been observed in a broad range of diseases (reviewed in [211], including brain disorders [41,212–214]). The complexity of Ca\(^{2+}\) homeostasis and signaling makes it difficult to understand the relationships between all of the factors involved. Nevertheless, studying these processes is greatly important because of the potential of using the acquired knowledge to identify new drug targets and develop novel therapeutic strategies. In this article, we focused on SOCE, a mechanism with well-established significance in non-excitable cells, such as lymphocytes. Store-operated Ca\(^{2+}\) entry has also been recognized as important for excitable cells, such as neurons, indicating that SOCE has dual roles. One role is to refill Ca\(^{2+}\) that is released from the ER during cell activation, which initiates specific signaling pathways. The other role is to in parallel trigger various other signaling pathways.

Store-operated Ca\(^{2+}\) entry or its components are disturbed in many brain diseases and models of these diseases (reviewed in [8]), including Alzheimer’s disease [41,215,216], Parkinson’s disease [217], epilepsy [218,219], and Huntington’s disease [220]. In one hand, SOCE appears to be neuroprotective (e.g. in Parkinson’s disease) [206], while in other situations such as for instance under ischemic conditions, neuroprotection is achieved by blocking SOCE [174]. Dual form of SOCE action should be taken under consideration while debating SOCE elements as potential targets for new drugs. Nevertheless three classes of SOCE or SOCE-responsive proteins are potential targets for new drugs. One class is a group of Ca\(^{2+}\) sensors (STIM1, STIM2, and their isoforms) that are located in different cellular compartments. The second class consists of Ca\(^{2+}\) channels, which are either activated (Orai and TRPs) or inhibited (voltage-operated channels) by STIMs. The third class consists of proteins that sense Ca\(^{2+}\) entering the cell during SOCE and then can activate specific pathways. If new drugs can successfully target these proteins specifically, then they might improve or correct dysregulated Ca\(^{2+}\) homeostasis and signaling without major side effects. The discovery, mechanisms, and biological activity of some chemical compounds have been described [170,221–227]. The search for these drugs may contribute to the development of treatments for currently incurable diseases, such as Alzheimer’s disease.

### Transparency Document

The Transparency Document associated with this article can be found, in the online version.

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