

Interactions of Mitochondria/Metabolism and Calcium Regulation in Alzheimer's Disease: A Calcinist Point of View

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Abstract Decades of research suggest that alterations in calcium are central to the pathophysiology of Alzheimer's Disease (AD). Highly reproducible changes in calcium dynamics occur in cells from patients with both genetic and non-genetic forms of AD relative to controls. The most robust change is an exaggerated release of calcium from internal stores. Detailed analysis of these changes in animal and cell models of the AD-causing presenilin mutations reveal robust changes in ryanodine receptors, inositol tris-phosphate receptors, calcium leak channels and store activated calcium entry. Similar anomalies in calcium result when AD-like changes in mitochondrial enzymes or oxidative stress are induced experimentally. The calcium abnormalities can be directly linked to the altered tau phosphorylation, amyloid precursor protein processing and synaptic dysfunction that are defining features of AD. A better understanding of these changes is required before using calcium abnormalities as therapeutic targets.

Keywords Calcium · Alzheimer's disease · Mitochondria · Endoplasmic reticulum · Presenilin · Amyloid

Abbreviations

| | |
|-------------------|---|
| A β | Amyloid β peptide |
| AD | Alzheimer's disease |
| CCE | Capacitative calcium entry |
| CREB | cAMP Response element-binding |
| ER | Endoplasmic reticulum |
| FAD | Familial Alzheimer's disease |
| IP ₃ | Inositol 1,4,5-triphosphate |
| IP ₃ R | Inositol 1,4,5-triphosphate receptor |
| KGDHC | α -Ketoglutarate dehydrogenase complex |
| LTP | Long term potentiation |
| MAMS | Mitochondrial associated membranes |
| PS-1 | Presenilin-1 |
| PS-2 | Presenilin-2 |
| PDHC | Pyruvate dehydrogenase complex |
| ROS | Reactive oxygen species |
| RyR | Ryanodine receptor |
| SACE | Store activated calcium entry |
| VDAC | Voltage dependent anion channels |

Introduction

Cellular calcium regulation and metabolism/mitochondria are abnormal in Alzheimer's Disease (AD). The understanding of the mechanism of these changes, their interactions and relationship to AD are limited. Both variables are dynamic and best measured in living cells. Since living neurons in AD brains cannot be studied, measures have to be made in "imperfect" model systems. Calcium homeostasis and mitochondria/metabolism are intimately linked. Most regulators of calcium homeostasis are modulated by metabolism including ATP, phosphorylation and reactive oxygen species (ROS). On the other hand, calcium directly effects mitochondrial metabolism and the production of

This paper is dedicated to Dr. Ursula Sonnewald. Her pioneering, unique and creative application of MR spectroscopy to neuroscience made it possible for us and others to understand fundamental changes in metabolism that are critical to our understanding of neurodegenerative diseases. The metabolic changes she helped us delineate underlie many of the calcium changes described in this review.

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oxidative stress [i.e., reactive oxygen species (ROS)]. AD-like changes in metabolism and oxidative stress can also lead to AD like change in calcium. An understanding of AD related changes in calcium and mitochondria/oxidative stress and their interactions will provide possibilities for new therapeutic intervention.

The changes in calcium and mitochondria/metabolism cause changes in protein processing that can lead to β -amyloid (A β) containing plaques and the hyperphosphorylated tau protein (i.e., tangles), the defining pathologies of AD. Calcium increases the formation of A β [1]. Oxidative stress is prominent in AD brains [2]. Enhanced ROS occurs in peripheral cells and is an early event that precedes plaque and tangle formation in transgenic mice [3–6]. The enzymes catalyzing A β formation are sensitive to oxidants so that any interruption of metabolism or abnormal ROS production increases A β production [7]. Reduced glucose metabolism always accompanies AD and has been directly linked to tau phosphorylation and the formation of tangles [8].

Cellular Calcium Regulation

Calcium regulates many cellular processes and a basic understanding of cellular calcium regulation is essential to understand the calcium abnormalities in AD. Calcium permeability channels including NMDA channels, AMPA receptors, nicotinic receptors and store operated calcium channels control calcium entry from outside the cell. An immense literature exists for each of these and they vary with each cell type. Calcium is also maintained by intracellular calcium signaling and the most replicable AD related changes are in intracellular calcium regulation. This review will focus on the aspects of calcium regulation that are well documented to be altered in AD and their interaction with metabolism.

Endoplasmic Reticulum (ER) Calcium Store (Fig. 1)

Endoplasmic reticulum (ER) is the main intracellular calcium store (Fig. 1) [9, 10]. ER calcium signaling maintains synaptic plasticity by regulating synaptic transmission and membrane excitability [11]. While cytosolic calcium is 100 nM, ER calcium may be about a 1000 times higher. The ER lumen contains high concentrations of Ca²⁺ binding proteins so the total amount of calcium in the lumen may be 1 mM. The concentration of free Ca²⁺ in the ER has been estimated to be between 100 and 700 nM [12]. High ER calcium is an important co-factor for ER chaperones [13]. ER calcium is maintained by very specific channels and transporters. The calcium is transported into the ER by a Ca²⁺-ATPase transporter. Calcium leaves the ER by two release channels on the outer membrane of the ER.

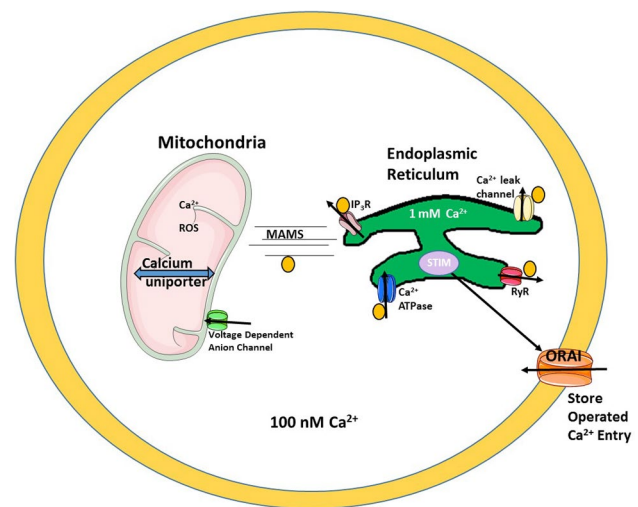


Fig. 1 Regulation of calcium in neurons. The details are provided in the text. The yellow circles indicate the process is regulated by ROS, ATP or phosphorylation and by presenilins. (Color figure online)

One is activated by IP₃ and one by ryanodine. In addition, a calcium leak channel has been proposed (Fig. 1).

Transport into the ER by the Sarco/Endoplasmic Reticulum Ca²⁺-ATPase (SERCA)

Resting cytosolic Ca²⁺ is maintained at low levels by sarco ER Ca²⁺-ATPase (SERCA) pumps [14]. The SERCA pumps have the highest affinity for Ca²⁺ removal from the cytosol. In combination with plasma membrane Ca²⁺-ATPases and transporters, SERCA pumps determine the resting cytosolic Ca²⁺ concentration. Three differentially expressed genes encode at least five isoforms of the SERCA pump. SERCA2b is ubiquitously expressed in smooth muscle tissues and non-muscle tissues including neurons [15]. Activators of SERCA have been used to treat diseases associated with ER stress [16]. SERCA is regulated by phosphorylation (ATP), ROS as well as calcium [17].

The Inositol 1,4,5-Trisphosphate Receptor (IP₃R) Activated Release Channel

The IP₃Rs, a family of Ca²⁺ release channels, are concentrated in the soma and localized predominately in the endoplasmic reticulum. The IP₃R channels are tetramers of IP₃R molecules of multiple isoforms [12]. Activation of these channels release Ca²⁺ into the cytoplasm in response to IP₃, which is produced by diverse stimuli such as bombesin or bradykinin. The channel gating is regulated not only by IP₃, but by other ligands as well, in particular cytoplasmic Ca²⁺. Low concentrations of cytoplasmic calcium stimulate IP₃R

whereas high concentrations inhibit. Other ligands such as ATP, regulate channel activity mainly by potentiating Ca^{2+} activation. Together, cytoplasmic free ATP and IP_3 act as allosteric regulators to tune the activation and inhibition. Phosphorylation by protein kinase A enhances single channel activity by approximately fivefold by increasing the apparent sensitivity to IP_3 [18].

Ryanodine Receptors (RyR)

RyR are primarily localized in the distal processes, spines, dendrites and presynaptic terminals, but they are also present in soma [19–23]. RyRs are homotetramers that are comprised of a huge N-terminal cytoplasmic domain that serves as a scaffold for channel regulators while the remaining domain is in the ER lumen. Ca^{2+} activates RyRs at low nanomolar concentrations. Caffeine and ryanodine are pharmacological modulators of RyRs. Caffeine-evoked $[\text{Ca}^{2+}]_i$ elevations are sensitive to pharmacological modulators interacting with RyRs or with SERCA pumps. These caffeine induced $[\text{Ca}^{2+}]_i$ responses are blocked by ryanodine, ruthenium red, and procaine and disappear after inhibition of SERCA-dependent ER Ca^{2+} uptake with thapsigargin or cyclopiazonic acid. Caffeine-induced Ca^{2+} increases occur in individual spines of cultured hippocampal neurons, which are rich in RyR. Ryanodine locks the RyR channel to an “open state” at low concentrations [23]. RyRs are also directly or indirectly modulated by other channels and kinases. CaMKII associates with, phosphorylates, and regulates the activity of RyR. Mutations in RyR or alterations in the post-translational modifications of RyRs (i.e. hyper-phosphorylation, oxidation and nitrosylation) can shift RyRs from a finely regulated state to an unregulated Ca^{2+} leak channel [23]. ROS can also modify calcium regulation. For example, H_2O_2 can modify the redox state of RyR by increasing its S-glutathionylation, which potentiate Ca^{2+} release [24].

Store Operated Calcium Entry (SOCE), Which is also Known as Capacitative Calcium Entry (CCE)

Depletion of ER calcium stores triggers influx of extracellular calcium into the cytosol that is rapidly used to refill the internal calcium stores. SOCE provides slow responses in calcium and are necessary to support the stability and maintenance of mushroom spines on neurons [25] and the proper function of calcium/calmodulin dependent processes including protein kinase 2 and calcium/calmodulin [25]. Stromal Interacting Molecules (STIM1 and STIM2) are required for SOCE (Fig. 1) [26, 27]. These proteins have a single transmembrane region with a putative Ca^{2+} binding domain in the lumen of the ER. Calcium store depletion causes a rapid translocation of STIM1 and STIM2 into puncta that accumulate near the plasma membrane. This leads to the subsequent activation of

the plasma membrane ORAI Ca^{2+} channel to replenish Ca^{2+} stores in the ER [28].

STIM2-nSOC-CaMKII pathway plays an essential role in maintenance of mushroom spines in healthy neurons [29]. Postsynaptic spines contain multiple potential sources of Ca^{2+} influx, such as NMDAR, AMPAR, and voltage gated calcium channels [30], which provide rapid and massive Ca^{2+} influx during stimulation and remain silent at rest. Thus, these channels are poorly suited to support long-term maintenance mechanism. Steady-state CaMKII activity in the spines depends on continuous Ca^{2+} influx via STIM-regulated SOCE pathways [29].

Mitochondrial Associated Proteins and the ER

Multiple proteins in the cytosol serve as signaling proteins to and from the mitochondria. These proteins also interact with the ER. For example, the BCL-2 family of proteins, which interact with mitochondria in cell death pathways, also alter ER calcium homeostasis [31]. Cells lacking Bax/Bak have a decreased ER calcium [32]. Bcl-2 inhibits IP_3 induced calcium release [33].

Mitochondria and ER calcium are linked functionally through energy dependent processes such as ATP and ROS, and morphologically through mitochondrial associated membranes (MAMS) (Fig. 1). Several proteins participate in stabilization of MAMS and alter Ca^{2+} transfer between ER and mitochondria. Glucose Regulated Protein 75 (GRP75) regulates the coupling of the voltage dependent anion channel of the mitochondria to the ER IP_3R [34]. MAMS are enriched in mitofusin2 and dynamin related GTPases. ER mitofusin2 interacts with mitochondrial mitofusin1/2. Mitochondrial Fis1 and ER-located Bap31 are proposed as ER–mitochondria tethering proteins [35]. MAMS are also enriched with presenilins. Presenilins have been shown to affect ER–mitochondria associations and related functions as measured by cholesteryl ester and phospholipid synthesis. The interactions are increased significantly in presenilin-mutant cells and in fibroblasts from patients with AD [35, 36]. Similarly, alterations in the ER–mitochondrial associations and functions are seen in amyloid precursor protein (APP) transgenic mouse models, and treatment of neurons with $\text{A}\beta$ alters ER–mitochondrial–ER contacts [37].

Mitochondrial and ER Interactions in the Regulation of Cellular Calcium (Fig. 1)

Mitochondria are also Important in the Regulation of Cellular Calcium

Voltage dependent anion channels (VDAC) regulate calcium across the outer mitochondrial membrane potential.

Several proteins such as the BCL-2 family of proteins can regulate permeability by binding to VDACs [34] and these same proteins also bind to ER protein. The inner mitochondrial membrane potential is regulated by the calcium uniporter which removes calcium from the mitochondria. Calcium transport into the mitochondria is regulated by the mitochondrial membrane potential [38].

Role of ER in Regulating Mitochondrial Calcium, ROS Production and Respiration

Calcium released from the ER has profound effects on mitochondria [10]. Calcium that is released from the ER does not mix with cytosolic calcium but is rapidly taken up by mitochondria in close proximity [39]. In many cell types, agonist induced calcium signals appear in the mitochondria [39, 40]. Isoform specific IP₃R are localized close to mitochondrial calcium uptake sites [39]. Suppression of the IP₃R leads to suppression of calcium transfer to the mitochondria. At high concentrations calcium regulates the permeability transition which leads to release of calcium and many apoptosis inducing factors. The ER-originating calcium signals and the subsequent increase in mitochondrial calcium will activate the α -ketoglutarate dehydrogenase complex (KGDHC) and the pyruvate dehydrogenase complex (PDHC). Both PDHC and KGDHC produce ROS and NADH [41]. Calcium activation can increase mitochondrial NADH to exceed that needed to sustain oxidative phosphorylation, and lead to an enough electron-saturated respiratory chain that results in increased mitochondrial ROS production. The Ca²⁺ sensitive sites include several dehydrogenases and substrate transporters together with a post-translational modification of F1-FO-ATPase and cytochrome oxidase [42, 43].

Role of Mitochondria in Regulating ER Calcium

Mitochondria regulate ER calcium by regulating cytosolic calcium and by regulating all of the proteins that regulate calcium through ATP and ROS. Since the Ca²⁺-ATPase pumps and the IP₃R of the ER are regulated by calcium, the local concentration of calcium around the mitochondria will determine the refilling of the ER and spatial temporal calcium signal. Both the SERCA pump, IP₃R and RyR are regulated by ATP, which depends on the mitochondria. IP₃ receptors are extensively phosphorylated and are controlled by phosphorylation [10]. Hyperphosphorylation of IP₃R decreases ER calcium stores. Local NADH levels can also alter IP₃ levels. Thus, mitochondria control the ER calcium through ATP, ROS and calcium.

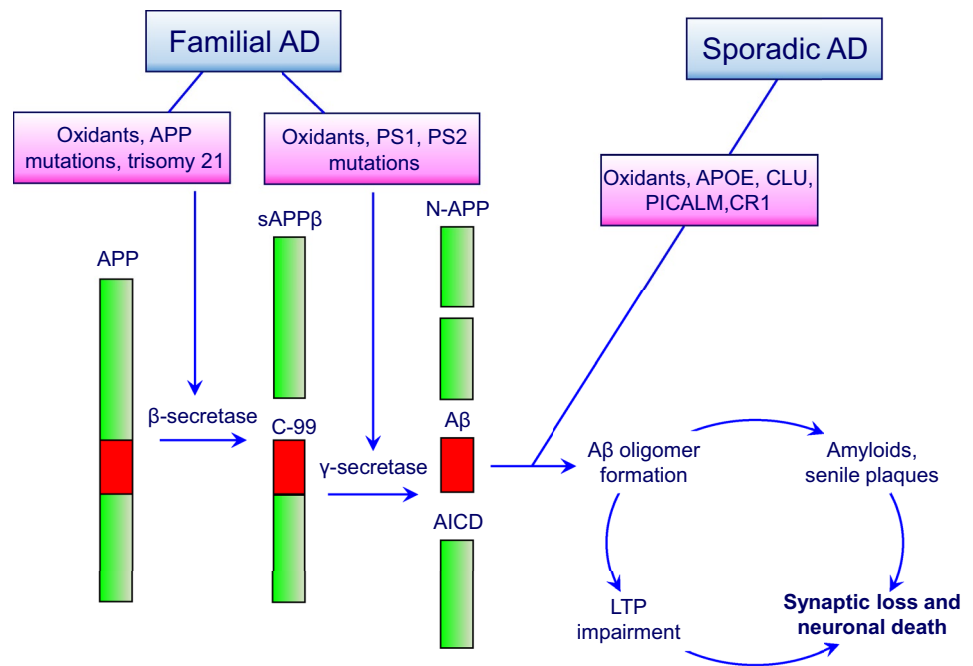
AD-Related Changes in Calcium Homeostasis and Their Interaction with Mitochondrial Function

Changes in calcium with aging and AD have been hypothesized to be important in AD for more than 30 years [44–46]. Initial studies showed that calcium uptake is diminished in fibroblasts from AD patients [46] and lymphocytes [47]. A large number of studies in fibroblasts, animal models of AD [48], autopsy brain studies and genetically manipulated cells of many kinds support the suggestion that abnormal calcium regulation accompanies AD. Calcium uptake is also reduced in brains from aged mice [49]. Mouse models harboring presenilin mutations accumulate calcium in the ER before pathology is present [50–52]. Such changes can be inferred in patients [53]. However, the findings have not lead to better treatments. Thus, a more detailed understanding of the changes as well as the details in calcium regulation in the right cellular systems are required. Each of the calcium regulatory systems outlined above have been implicated in AD.

Genetic engineering of cells and mice with presenilin mutations that cause AD is a powerful tool to study AD-related changes in ER and mitochondrial calcium. Mutations in presenilins 1 (PS1) or 2 (PS2) are the cause of most common genetic forms of AD. Presenilins are multifunctional transmembrane proteins that are synthesized and localized in the ER membrane and endosomes [54]. Presenilins have many functions including as a γ -secretase that cleaves several transmembrane proteins, including amyloid precursor protein (APP) [43, 55, 56] (Fig. 2). γ -Secretase plays a central role in regulating intramembrane proteolysis, cleaving the transmembrane domains of hundreds of proteins, including Notch and APP. β -secretase (BACE) processing of the latter exposes a 99-amino-acid C-terminal fragment to γ -secretase. This C-99 retains the transmembrane domain, which γ -secretase first cuts internally between amino acids 48 and 49, or 49 and 50 (so-called epsilon cleavage). This releases into the cytosol either of two APP intracellular domains, AICD49-99 or AICD50-99. Then, γ -secretase proceeds in carboxypeptidase fashion to chop off C-terminal amino acids from the remaining transmembrane domain until A β peptides are finally released into the lumen. Most mutations in PS1 or PS2 that lead to AD cause abnormalities in calcium, whereas mutations that do not cause AD do not change calcium. This has made these mutations powerful tools for looking at mechanism.

By contrast, few studies have been done to assess the effects of AD causing mutations in APP processing on neuronal calcium regulation. The one study in fibroblasts show the results to be quite different from those in cells from non-genetic forms or those bearing presenilin mutations [57].

Fig. 2 Processing of amyloid precursor protein and its modification by presenilins



IP₃ Releasable Stores of Calcium

Exaggerated calcium signaling through IP₃R is a disease specific and robust proximal mechanism of AD [43]. ER calcium stores in fibroblasts from AD patients have an exaggerated calcium signal in response to agonists such as bradykinin or bombesin that produce IP₃. Early results suggest that the ER calcium pool is exaggerated [58]. Subsequent studies reveal that this exaggerated release is a very robust finding that is common and specific [19, 50, 59–61]. Studies on fibroblasts from hundreds of patients suggest the changes may be diagnostic for AD [43, 58, 62–64]. The abnormalities occur if the fibroblasts are taken before the patient developed AD, which suggests they are trait dependent markers, not state dependent markers [62]. The changes occur in fibroblasts from individuals that have presenilin mutations and those that do not. However, the changes do not occur in fibroblasts from patients bearing mutations in the processing of APP [57]. The increase in IP₃ releasable stores in fibroblasts bearing presenilin mutations can be reproduced in mice that have human mutant presenilin. As in humans, the changes occur in fibroblasts. Importantly, the same change occur in neurons from the same mice. Together, the results suggest that neurons in patients with AD bearing presenilin mutations likely have exaggerated internal stores of calcium [48].

These well documented changes in human fibroblasts stimulated experiments in many in vitro models with other cell types. Presenilin mutations increase IP₃ releasable calcium stores in multiple cell types including Sf9

cells, chicken DT40 B cells and human lymphoblasts from Familial AD (FAD) patients [20, 22, 43]. In *Xenopus* oocyte expression systems, photolysis of caged inositol IP₃ reveals that the PS1 mutations that cause AD potentiate IP₃-mediated release of Ca²⁺ from internal stores [59]. Single channel recordings of IP₃R show that presenilin mutations cause an apparent sensitization of the IP₃R channel to IP₃, which produces an enhanced IP₃R Ca²⁺ release channel gating [20]. Measures on the behavior of the IP₃R measured at the single channel levels also reveal exaggerated IP₃ calcium signals in lymphoblasts [43, 58] and other cell types [66, 67]. Further, genetic reduction of the type 1 IP₃R by 50% normalized exaggerated Ca²⁺ signaling. In PS1M146V knock-in mice, reduced IP₃R1 expression restores normal ryanodine receptor and cAMP response element-binding protein (CREB)-dependent gene expression and rescues aberrant hippocampal long-term potentiation (LTP) [67]. Presenilins interact with the gating of the IP₃R [22]. The IP₃R interacts with both wild type and mutant presenilin [20–22]. Electrophysiological recordings of IP₃ receptor in FAD patient B cells, cortical neurons of PS1 AD mice and other cells suggest a higher occupancy in a high open probability mode resulting in enhanced calcium signaling [43].

Presenilin mutations alter APP processing and lead to excess Aβ but the changes in calcium are not likely to be secondary to Aβ. Functionally, mutant PS1 has a gain of function consequences when binding to the channels and this was associated with exaggerated [Ca²⁺]_i signaling in intact cells [20, 22]. This was true even if the γ-secretase component of PS1 was inactive. Many FAD-linked PS

mutations have been shown to alter Ca^{2+} homeostasis by $\text{A}\beta$ independent mechanisms [43, 68–70]. The changes in calcium with mutated presenilins are also independent of the presenilin secretase activity [43]. Further evidence that $\text{A}\beta$ is not involved is that in fibroblasts from patients bearing the APP mutation, the increase in IP_3 mediated calcium release does not occur [57]. These observations provide molecular insights into the calcium dysregulation in AD pathogenesis and suggest novel targets for therapeutic intervention [20].

Ryanodine Receptors

The increased calcium release in AD models has also been attributed to enhanced release through ryanodine receptors [51, 60, 69]. Three RyR subtypes encoded by different genes were originally identified in skeletal muscle (type 1 RyR), heart (type 2 RyR), and brain (type 3 RyR) [71]. Ryanodine receptor activity is enhanced in dendrites and synaptic spines from pre-symptomatic mouse models of AD [72, 73]. Basal synaptic function and plasticity mechanisms appear similar between young non-transgenic and transgenic mice. However, when RyRs are manipulated, striking synaptic transmission and plasticity abnormalities are revealed in the transgenic mice. This is in large part due to calcium induced calcium release via the ryanodine receptor [51, 72, 73]. Electrophysiological recordings and two-photon calcium imaging in young (6–8 weeks old) 3xTg-AD and non-transgenic hippocampal slices reveal a marked increase in RyR-evoked calcium release within synapse-dense regions of CA1 pyramidal neurons in the AD mouse model. The RyR2 isoform is selectively increased more than fivefold in the hippocampus of 3xTg-AD mice. These novel findings demonstrate that 3xTg-AD CA1 neurons at pre-symptomatic ages operate under an aberrant calcium signaling and synaptic transmission system long before AD histopathology onset [51].

Several studies suggest precise mechanism for these changes. Caffeine evokes a peak rise of $[\text{Ca}^{2+}]_i$ in neurons from a 3xTg-AD that is significantly greater than those observed in non-transgenic neurons. This suggests that Ca^{2+} stores are greater in neurons from 3xTg-AD neurons or that the ryanodine receptor is altered. Protein levels of several Ca^{2+} binding proteins (SERCA2b, calbindin, calseminin and calreticulin) implicated in the pathogenesis of AD did not change, whereas ryanodine receptor expression in both PS1 Knock In and 3xTg-AD cortex increases. RyR expression and protein levels also increase in AD brains [74]. The results suggest that the enhanced Ca^{2+} response to caffeine observed in 3xTg-AD neurons may be attributable to an increase in the steady-state levels of the ryanodine receptor [19].

Presenilins interact with the gating of the RyR [22]. The PS1 N-terminal fragment 1–82 allosterically potentiates RyRs, but the channel still requires Ca^{2+} for activation [21]. Application of the PS2 N-terminal fragment 1–87 to the cytoplasmic side of the RyR significantly increases single channel activity by favoring higher sublevel openings. The Ca^{2+} activation and desensitization ranges for RyRs are unchanged. The results demonstrate that PS2 N-terminal fragment 1–87 facilitates RyR gating [75].

Store Activated Calcium Entry (SACE) or Capacitative Calcium Entry (CCE)

SACE is activated in response to lowering calcium content in the ER. The ER calcium overload in mice bearing the M146V presenilin mutation down regulates SACE [25, 43, 48, 61, 65, 70]. Deficits in SACE were evident after agonist stimulation, but not if intracellular calcium stores were completely depleted with thapsigargin. Treatment with ionomycin and thapsigargin revealed that calcium levels within the ER were significantly increased in mutant PS1 knock-in cells. Thus, the overfilling of calcium stores may represent the fundamental cellular defects underlying the alterations in calcium signaling conferred by presenilin mutations [48]. In the presence of IP_3 , mitochondria buffer the local Ca^{2+} released from ER to serve as a negative feedback to the SACE [76].

SACE has been examined in considerable morphological detail. Cultured hippocampal neurons expressing mutant PS1 have attenuated SACE that is associated with destabilized dendritic spines, and SACE is rescued by either γ -secretase inhibition or over-expression of STIM1. γ -Secretase activity may physiologically regulate CCE by targeting STIM1 [28]. PS1-associated γ -secretase activity may be important in this process because knockout of PS1 and PS2 or expression of catalytically inactive PS1 mutants (D257A or D385A) is associated with enhanced SACE [28]. Forms of PS1 with FAD-associated mutations enhanced γ -secretase cleavage of the STIM1 transmembrane domain at a sequence that was similar to the gamma-secretase cleavage sequence of APP [28]. Results indicate that γ -secretase activity may physiologically regulate SACE by targeting STIM1 and that restoring STIM1 may be a therapeutic approach in AD [28].

STIM2 has also been implicated in the mutant presenilin related calcium deficits [29]. Specific downregulation of STIM2 occurs in PS1-FAD patient lymphoblasts [77]. In PS-FAD and aging neurons, an increase in ER Ca^{2+} levels causes compensatory downregulation of STIM2 expression, impaired SACE, reduced steady-state CaMKII activity in the spines, and eventual loss of mushroom spines.

Loss of mushroom spines can be linked to memory impairment in aging and AD neurons [29].

STIM2 may also be involved in forms of AD not related to presenilin mutations. As emphasized elsewhere, elevated extracellular A β (perhaps due to oxidative stress) accompanies AD. Results are consistent with A β acting by down regulating STIM2. Extracellular A β may over activate mGluR5 receptor in vulnerable neurons, leading to elevated intracellular Ca²⁺ levels, compensatory downregulation of STIM2 expression, impaired synaptic SACE, and reduced CaMKII activity. Pharmacological inhibition of mGluR5 or overexpression of STIM2 rescues synaptic changes in internal calcium stores and prevents mushroom spine loss in hippocampal neurons [25].

Abnormalities in SERCA may also Underlie the Calcium Changes

Others have proposed that enhanced activity of the ER calcium pump [14] leads to enhanced loading of the ER lumen [19, 69]. SERCA pump activity is physiologically regulated by presenilin [14]. SERCA activity is diminished in fibroblasts lacking both PS1 and PS2 genes, despite elevated SERCA2b steady state levels. Presenilins and SERCA physically interact. Ca²⁺ homeostasis, overexpression of wild-type PS1 or PS2 in oocytes causes enhanced IP₃-mediated Ca²⁺ release, an effect that is exacerbated by mutations in both genes [59]. Enhancing presenilin levels in *Xenopus* oocytes accelerates clearance of cytosolic Ca²⁺, whereas higher levels of SERCA2b phenocopy PS1 overexpression, accelerating Ca²⁺ clearance and exaggerating IP₃-mediated Ca²⁺ liberation. The critical role that SERCA2b plays in the pathogenesis of AD is supported by findings that show modulating SERCA activity alters amyloid production. Considerable data suggests a physiological role for the presenilins in Ca²⁺ signaling via regulation of the SERCA pump.

Calcium Leak Channel

An alternative hypothesis is that presenilins form low conductance ER Ca²⁺ leak channels and that the mutations in presenilin that cause AD block the leak channels [78–81]. Although not without controversy [82], considerable data supports that conclusion. Some but not all genetic mutations in PS disrupt this function leading to overfilling of ER with calcium. In response, the cell upregulates the function of the ER calcium releasing channels such as IP₃ and ryanodine receptors. This leak channel function is independent of the gamma-secretase activity [78]. Crystal structures show that presenilin has a large hole that transverses the entire protein that is large enough to allow passage of small molecules [83]. The results are in agreement with other

studies utilizing mutagenesis [80]. The crystal structure of presenilin reveals a conspicuous pore in a bundle of nine α -helices, which was originally thought to adopt a novel protein fold. However, the presenilin fold is a variant of the ClC chloride channel/transporter fold. Several mutations that affect calcium leak activity line the presenilin “hole”. The fundamental architectural similarities between presenilin and ClCs may therefore have important implications for understanding the presenilin function, including its possible physiological role as a calcium channel [84].

The suggestion of a role for the calcium leak channel in the cells bearing presenilin mutations was verified by analysis of 250 small interfering RNAs on Ca²⁺ signals in single cells. The AD-linked protein presenilin-2 and the channel protein ORAI2 prevented overload of ER Ca²⁺ and that feedback from Ca²⁺ to membrane lipids [85].

What Causes the Change in Calcium in AD Fibroblasts Without Genetic Mutations?

The increase in releasable stores of calcium have been found in fibroblasts from the small percent (<4%) of patients bearing presenilin mutations as well as in fibroblasts from patients that do not have any known genetic cause who reflect the vast majority of patient population. The previous sections describe efforts made to understand the changes in greater functional and morphological detail in cells bearing presenilin mutations. The experiments described here were designed to determine what might cause the change in non-genetic forms of AD.

Oxidative stress is common in AD and oxidants can lead to changes in calcium that resemble those in AD. Multiple oxidants can induce the change in IP₃-releasable calcium stores (Fig. 3). The most effective oxidant in producing AD like changes in calcium is *t*-butryl-hydroperoxide [86]. Enhanced H₂O₂ production in cells expressing mutant PS1 requires the expression of the IP₃R. The agonist induced changes in ROS were also totally blocked by using BAPTA to buffer calcium [43]. This suggests the elevated calcium was stimulating ROS production by the mitochondria. Interestingly, SERCA2b is also the most sensitive of the SERCA pumps to oxidative stress [87, 88].

Thus, the findings support the hypothesis that the calcium changes are related to ROS in both cells bearing presenilin mutations and in cells with non-genetic forms of AD.

Lowering the activity of a key mitochondrial enzyme complex, the alpha-ketoglutarate dehydrogenase complex (KGDHC) also increases IP₃ releasable stores of calcium (Fig. 4). Neurons cultured from either embryonic or mature mice deficient in KGDHC have an exaggerated IP₃-inducible calcium change that resembles the

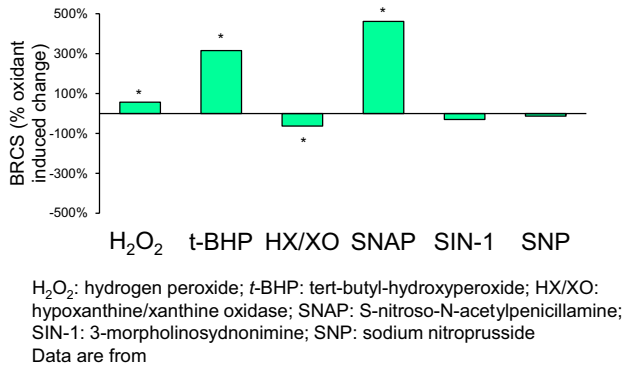


Fig. 3 Diverse oxidants induce select changes in releasable calcium stores. The indicated oxidants were added to cells. Internal calcium stores were measured exactly as in cell from AD patients. Data are from [86]

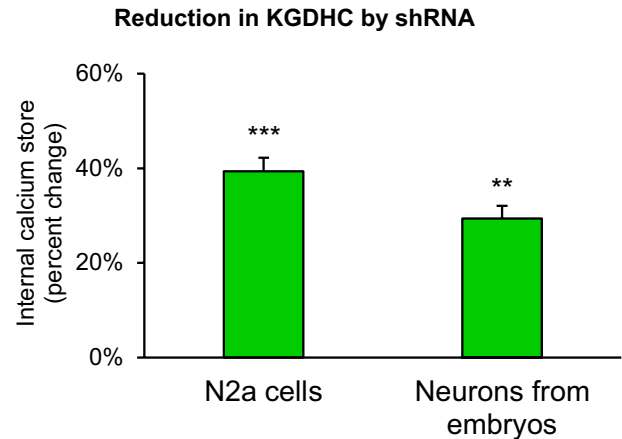
change in AD [89]. Reducing KGDHC in N2a cells by shRNA also increases IP₃ releasable calcium [89].

Relation of the IP₃ Releasable Calcium to Mitochondria and Mitochondrial Calcium Buffering

The many connections of the ER and mitochondria make it difficult to distinguish cause and effect relationships. Stable expression of PS1 or PS1 mutants does not alter H₂O₂ production in DT40 cells. However, upon activation both manipulations increase H₂O₂ production and the increase in cells bearing the PS1 mutation is greater. In the absence of IP₃R, the change is not apparent [43]. Qualitatively similar results occur in PC12 cells wherein the increase in ROS is blocked when calcium is buffered [43]. The excess release of calcium results in enhanced generation of ROS. However, in response to excess Ca²⁺ release, the Ca²⁺-induced increase in mitochondrial NADH can exceed that needed to sustain oxidative phosphorylation, and lead to an electron-saturated respiratory chain that results in increased mitochondrial ROS production [43]. The reduction in KGDHC leads to an exaggeration in the response to an oxidant challenge [90].

The Changes in Calcium can be Directly Linked to AD like Pathophysiological Changes

The increase in ER calcium release results in altered presynaptic and post-synaptic transmission mechanisms [51, 91, 92]. Studies of neurons in hippocampal slices from AD transgenic mice and appropriate controls reveal that upon theta burst stimulation or high-frequency stimulation, input-specific LTP in AD mice have a larger initial



Neuronal cultures from mice with reduced KGDHC

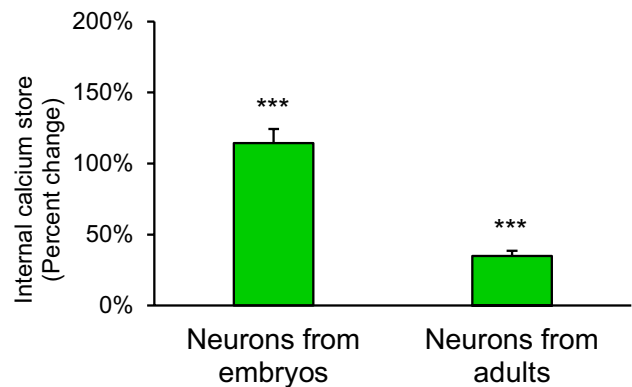


Fig. 4 Bradykinin or caffeine releasable calcium stores are exaggerated in response to reduced KGDHC. In the *top panel*, KGDHC was reduced with shRNA in either N2a cells or cultured neurons. In the *lower panel*, neurons were cultured from mice with diminished KGDHC [89]

amplitude and are more persistent than that in controls. These data suggest that the AD mutations lead to higher degree of LTP induction [91]. Additional studies of either presynaptic (CA3) or postsynaptic (CA1) neurons of the hippocampal Schaeffer-collateral pathway reveal that long-term potentiation induced by theta-burst stimulation decreased after presynaptic, but not postsynaptic deletion of presenilins. Moreover, presynaptic but not postsynaptic inactivation of presenilins alters short-term plasticity and synaptic facilitation. Depletion of endoplasmic reticulum Ca²⁺ stores by thapsigargin, or blockade of Ca²⁺ release from these stores by ryanodine receptor inhibitors, mimics and occludes the effects of presynaptic presenilin inactivation. Collectively, these results indicate a selective role for presenilins in the activity-dependent regulation of neurotransmitter release and long-term potentiation induction by modulation of intracellular Ca²⁺ release in presynaptic terminals, and further suggest that presynaptic dysfunction

might be an early pathogenic event leading to dementia and neurodegeneration in AD [92].

Two-photon calcium imaging, flash photolysis of caged glutamate, and patch-clamp electrophysiology in cortical brain slices reveal increased ryanodine receptor-evoked calcium signals within dendritic spine heads, dendritic processes, and the soma of pyramidal neurons from AD transgenic mice. In addition, synaptically evoked postsynaptic calcium responses and calcium signals generated from NMDAR activation are larger in the AD strains. Concurrent activation of RyRs with either synaptic or NMDAR stimulation generate a supra-additive calcium response in the AD strains, suggesting an aberrant calcium-induced calcium release effect within spines and dendrites. The results suggest that presenilin-linked disruptions in RyR signaling and subsequent calcium-induced calcium release via NMDAR-mediated calcium influx alters synaptic function and serves as an early pathogenic factor in AD [73].

The exaggerated calcium store is likely coupled to the pathology. Elevating calcium increases A β production [1]. In 3xTg mice, reduced IP₃R1 expression profoundly attenuated A β accumulation and tau hyperphosphorylation and rescued hippocampal LTP and memory deficits. The critical role that SERCA2b plays in the pathogenesis of AD is underscored by findings that modulating SERCA activity alters A β production [14]. Thus, reduction of the calcium change ameliorates the pathology in animal models [67].

Another possible way that the calcium changes contribute to AD is by altering ROS production [43]. Oxidative damage due to ROS is very common in brains of patients that died with AD. Enhanced generation of ROS is believed to be an important component in AD pathogenesis [93–96]. Exaggerated Ca²⁺ signaling results in enhanced generation of ROS. The increased ROS production in PS1 mutants depends entirely on expression of the IP₃R [43]. The reduction in KGDHC that accompanies AD leads to an exaggeration in the response to an oxidant challenge [90]. The calcium changes are present from birth in mice, and perhaps in humans, but the brain compensates. The compensatory process then exaggerates the pathophysiology. The initial response is increased post-synaptic small conductance potassium activated calcium currents and increased presynaptic vesicle release which lead to synaptic depression and increased long term depression [11].

Possible Therapeutic Approaches to Study the Calcium Change

Since the AD related changes in calcium are accompanied by an increase in IP₃R and RyRs, blocking these receptors is a possible therapy. However, these channels are involved in many cellular pathways so this approach will likely have

many side effects. Nevertheless, blocking RyR with dantrolene has been studied extensively [60, 74, 97, 98]. The stabilizing effect of dantrolene inhibits aberrant activation of the channel and prevents excessive Ca²⁺ release from intracellular stores. Pharmacological inhibitors of SACE (dantrolene), of calcium-activated potassium channels (apamin), and of calcium-dependent phosphatase calcineurin (FK506) are able to rescue structural plasticity defects in neurons from AD mouse models. Incubation with dantrolene or apamin also rescues LTP defects in hippocampal slices from AD mouse models [99]. Dantrolene treatment fully normalizes ER Ca²⁺ signaling within somatic and dendritic compartments in early and late stage AD mice in hippocampal slices. The elevated RyR2 levels in AD mice are restored to control levels with dantrolene treatment, as are synaptic transmission, and synaptic plasticity. A β deposition within the cortex and hippocampus is also reduced in dantrolene-treated AD mice [100].

Preventing the changes in calcium can also alter the defining pathologies in AD. Dantrolene-induced lowering of RyR-mediated Ca²⁺ release leads to the reduction of both intracellular and extracellular A β load in neuroblastoma cells as well as in primary cultured neurons derived from Tg2576 mice [98]. There are several studies showing that dantrolene treatment is effective *in vivo*. A β deposition within the cortex and hippocampus is also reduced in dantrolene-treated AD mice [100]. Dantrolene significantly improves cognition in a murine model of AD and is associated with a reduction in amyloid plaque burden [98, 100, 101]. However, other studies show that there may be undesirable effects. Long term feeding increases plaque formation and leads to loss of synaptic markers [52]. The difficulty is that dantrolene lacks specificity and may be more directed to muscle ryanodine receptors [102].

A genetic approach has also been used to evaluate the importance of RyR3 in AD pathology. The deletion of RyR3 increased hippocampal neuronal network excitability and accelerated AD pathology, leading to mushroom spine loss and increased amyloid accumulation. In contrast, deletion of RyR3 in older APPS1 mice (≥ 6 mo) rescued network excitability and mushroom spine loss, reduced amyloid plaque load and reduced the number of spontaneous seizures. The results suggest that in young AD neurons, RyR3 protects AD neurons from synaptic and network dysfunction, while in older AD neurons, increased RyR3 activity contributes to pathology. These results imply that blockade of RyR3 may be beneficial for those in the later stages of the disease, but RyR activators may be beneficial when used prior to disease onset or in its initial stages [103].

An alternative approach is based on manipulating the STIM2 that is so critical in regulation of internal calcium stores. The Transient Receptor Potential Canonical 6 (TRPC6) and ORAI2 channels form a STIM2-regulated

SACE Ca^{2+} channel complex in hippocampal mushroom spines. A known TRPC6 activator, hyperforin, and a novel SACE positive modulator, NSN21778, can stimulate activity of SACE in the spines and rescue mushroom spine loss in both presenilin and APP knock-in mouse models of AD. Further, the NSN21778 rescues hippocampal long-term potentiation impairment in APP knock-in mouse model. Thus, the STIM2-regulated TRPC6/ORAI2 SACE channel complex in dendritic mushroom spines is a potential new therapeutic target for the treatment of memory loss in AD and that NSN21778 is a potential candidate molecule for therapeutic intervention [104].

Conclusions

Abnormalities in calcium regulation are central to AD pathophysiology—a calcinist view of AD. Although the changes have been known for decades, the targets have not translated to the clinic. Calcium regulation including the interaction of mitochondria to ER and to SACE is very cell-type specific. The initial AD-related changes were all discovered in fibroblasts. Much of the remainder of the modeling has been done in cell lines or mouse or rat neurons. The relation of any of these results to what happens in human neurons or other brain cell types is unknown. This question can now be addressed with the use of neurons derived from fibroblasts. Similar methods could be used to explore these dynamics in other cell types. Stem cell derived neurons provide a better model for exploring detailed mechanistic studies which will be required to determine the clinical implications of our findings.

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References

1. Querfurth HW, Selkoe DJ (1994) Calcium Ionophore Increases amyloid beta. Peptide production by cultured cells. *Biochemistry* 33:4550–4561
2. Calingasan NY, Uchida K, Gibson GE (1999) Protein-bound acrolein. *J Neurochem* 72:751–756
3. Castellani RJ, Lee H-G, Zhu X, Nunomura A, Perry G, Smith MA (2006) Neuropathology of Alzheimer disease: pathognomonic but not pathogenic. *Acta Neuropathol* 111:503–509
4. Cecchi C, Fiorillo C, Sorbi S, Latorraca S, Nacmias B, Bagnoli S, Nassi P, Liguri G (2002) Oxidative stress and reduced antioxidant defenses in peripheral cells from familial Alzheimer's patients. *Free Radical Biol Med* 33:1372–1379
5. Cecchi C, Latorraca S, Sorbi S, Iantomasi T, Favilli F, Vincenzini MT, Liguri G (1999) Glutathione level is altered in lymphoblasts from patients with familial Alzheimer's disease. *Neurosci Lett* 275:152–154
6. Eckert A, Schindowski K, Leutner S, Luckhaus C, Touchet N, Czech C, Müller WE (2001) Alzheimer's disease-like

- alterations in peripheral cells from presenilin-1 transgenic mice. *Neurobiol Dis* 8:331–342
7. O'Connor T, Sadleir KR, Maus E, Velliquette RA, Zhao J, Cole SL, Eimer WA, Hitt B, Bembinster LA, Lammich S, Lichtenthaler SF, Hébert SS, De Strooper B, Haass C, Bennett DA, Vassar R (2008) Phosphorylation of the translation initiation factor eIF2 α increases BACE1 levels and promotes amyloidogenesis. *Neuron* 60:988–1009
8. Liu F, Shi J, Tanimukai H, Gu J, Gu J, Grundke-Iqbal I, Iqbal K, Gong C-X (2009) Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. *Brain* 132:1820–1832
9. Decuyper J-P, Monaco G, Missiaen L, De Smedt H, Parys JB, Bultynck G (2011) IP3 receptors, mitochondria, and Ca^{2+} signaling: implications for aging. *J Aging Res* 2011:20
10. Sammels E, Parys JB, Missiaen L, De Smedt H, Bultynck G (2010) Intracellular Ca^{2+} storage in health and disease: a dynamic equilibrium. *Cell Calcium* 47:297–314
11. Chakroborty S, Kim J, Schneider C, Jacobson C, Molgó J, Stutzmann GE (2012) Early presynaptic and postsynaptic calcium signaling abnormalities mask underlying synaptic depression in presymptomatic Alzheimer's disease mice. *J Neurosci* 32:8341–8353
12. Foskett JK, White C, Cheung K-H, Mak D-OD (2007) Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol Rev* 87:593–658
13. Corbett EF, Michalak M (2000) Calcium, a signaling molecule in the endoplasmic reticulum? *Trends Biochem Sci* 25:307–311
14. Green KN, Demuro A, Akbari Y, Hitt BD, Smith IF, Parker I, LaFerla FM (2008) SERCA pump activity is physiologically regulated by presenilin and regulates amyloid β production. *J Cell Biol* 181:1107–1116
15. Baba-Aissa F, Raeymaekers L, Wuytack F, Dode L, Casteels R (1998) Distribution and isoform diversity of the organellar Ca^{2+} pumps in the brain. *Mol Chem Neuropathol* 33:199–208
16. Kang S, Dahl R, Hsieh W, Shin A, Zsebo KM, Buettner C, Hajar RJ, Lebeche D (2016) Small molecular allosteric activator of the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) attenuates diabetes and metabolic disorders. *J Biol Chem* 291:5185–5198
17. Adachi T (2010) Modulation of vascular sarco/endoplasmic reticulum calcium ATPase in cardiovascular pathophysiology. In: Paul MV (ed) *Advances in pharmacology*. Academic Press, New York, pp 165–195
18. Schlossmann J, Ammendola A, Ashman K, Zong X, Huber A, Neubauer G, Wang G-X, Allescher H-D, Korth M, Wilm M, Hofmann F, Ruth P (2000) Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase I[β]. *Nature* 404:197–201
19. Smith IF, Hitt B, Green KN, Oddo S, LaFerla FM (2005) Enhanced caffeine-induced Ca^{2+} release in the 3xTg-AD mouse model of Alzheimer's disease. *J Neurochem* 94:1711–1718
20. Cheung K-H, Shineman D, Müller M, Cárdenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VMY, Foskett JK (2008) Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron* 58:871–883
21. Rybalchenko V, Hwang S-Y, Rybalchenko N, Koulen P (2008) The cytosolic N-terminus of presenilin-1 potentiates mouse ryanodine receptor single channel activity. *Int J Biochem Cell Biol* 40:84–97
22. Cheung K-H, Mei L, Daniel Mak D-O, Hayashi I, Iwatsubo T, Kang DE, Foskett JK (2010) Gain-of-function enhancement of InsP(3) receptor modal gating by familial Alzheimer's

- disease-linked presenilin mutants in human cells and mouse neurons. *Sci Signaling* 3:ra22
23. Del Prete D, Checler F, Chami M (2014) Ryanodine receptors: physiological function and deregulation in Alzheimer disease. *Mol Neurodegener* 9:1–15
 24. Kemmerling U, Muñoz P, Müller M, Sánchez G, Aylwin ML, Klann E, Carrasco MA, Hidalgo C (2007) Calcium release by ryanodine receptors mediates hydrogen peroxide-induced activation of ERK and CREB phosphorylation in N2a cells and hippocampal neurons. *Cell Calcium* 41:491–502
 25. Zhang H, Wu L, Pchitskaya E, Zakharova O, Saito T, Saido T, Bezprozvanny I (2015) Neuronal store-operated calcium entry and mushroom spine loss in amyloid precursor protein knock-in mouse model of Alzheimer's disease. *J Neurosci* 35:13275–13286
 26. Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL (2006) Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem* 281:20661–20665
 27. Liou J, Kim ML, Do Heo W, Jones JT, Myers JW, Ferrell JE Jr, Meyer T (2005) STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol* 15:1235–1241
 28. Tong BC-K, Lee CS-K, Cheng W-H, Lai K-O, Kevin Foskett J, Cheung K-H (2016) Familial Alzheimer's disease-associated presenilin 1 mutants promote γ -secretase cleavage of STIM1 to impair store-operated Ca²⁺. *Sci Signaling* 9:ra89
 29. Sun S, Zhang H, Liu J, Popugayeva E, Xu N-J, Feske S, White Iii Charles L, Bezprozvanny I (2014) Reduced synaptic STIM2 expression and impaired store-operated calcium entry cause destabilization of mature spines in mutant presenilin mice. *Neuron* 82:79–93
 30. Murakoshi H, Yasuda R (2012) Postsynaptic signaling during plasticity of dendritic spines. *Trends Neurosci* 35:135–143
 31. Lam M, Dubyak G, Chen L, Nuñez G, Miesfeld RL, Distelhorst CW (1994) Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca²⁺ fluxes. *Proc Natl Acad Sci USA* 91:6569–6573
 32. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, Korsmeyer SJ (2005) Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci USA* 102:105–110
 33. Chen R, Valencia I, Zhong F, McColl KS, Roderick HL, Bootman MD, Berridge MJ, Conway SJ, Holmes AB, Mignery GA, Vezel P, Distelhorst CW (2004) Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. *J Cell Biol* 166:193–203
 34. Tsujimoto Y, Shimizu S (2000) VDAC regulation by the Bcl-2 family of proteins. *Cell Death Differ* 7:1174
 35. Paillusson S, Stoica R, Gomez-Suaga P, Lau DHW, Mueller S, Miller T, Miller CCJ (2016) There's something wrong with my MAM; the ER-mitochondria axis and neurodegenerative diseases. *Trends Neurosci* 39:146–157
 36. Area-Gomez E, del Carmen Lara Castillo M, Tambini MD, Guardia-Laguarta C, de Groof AJC, Madra M, Ikenouchi J, Umeda M, Bird TD, Sturley SL, Schon EA (2012) Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO J* 31:4106–4123
 37. Hedskog L, Pinho CM, Filadi R, Rönnbäck A, Hertwig L, Wiehager B, Larssen P, Gellhaar S, Sandebring A, Westerlund M, Graff C, Winblad B, Galter D, Behbahani H, Pizzo P, Glaser E, Ankarcrona M (2013) Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models. *Proc Natl Acad Sci* 110:7916–7921
 38. Hurst S, Hoek J, Sheu S-S (2016) Mitochondrial Ca²⁺ and regulation of the permeability transition pore. *J Bioenerg Biomembr* 1–21. doi:10.1007/s10863-016-9672-x
 39. Rizzuto R, Brini M, Murgia M, Pozzan T (1993) Microdomains with high Ca²⁺ close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* 262:744–747
 40. Hajnóczky G, Csordás G, Yi M (2002) Old players in a new role: mitochondria-associated membranes, VDAC, and ryanodine receptors as contributors to calcium signal propagation from endoplasmic reticulum to the mitochondria. *Cell Calcium* 32:363–377
 41. Denton RM (2009) Regulation of mitochondrial dehydrogenases by calcium ions. *Biochim Biophys Acta (BBA)* 1787:1309–1316
 42. Balaban RS (2009) The role of Ca²⁺ signaling in the coordination of mitochondrial ATP production with cardiac work. *Biochim Biophys Acta (BBA)* 1787:1334–1341
 43. Müller M, Cheung K-H, Foskett JK (2010) Enhanced ROS generation mediated by Alzheimer's disease presenilin regulation of InsP3R Ca²⁺ signaling. *Antioxidants Redox Signaling* 14:1225–1235
 44. Khachaturian ZS (1994) Calcium hypothesis of Alzheimer's disease and brain aging. *Ann N Y Acad Sci* 747:1–11
 45. Gibson GE, Peterson C (1987) Calcium and the aging nervous system. *Neurobiol Aging* 8:329–343
 46. Peterson C, Gibson GE, Blass JP (1985) Altered calcium uptake in cultured skin fibroblasts from patients with Alzheimer's disease. *N Engl J Med* 312:1063–1065
 47. Gibson GE, Nielsen P, Sherman KA, Blass JP (1987) Diminished mitogen-induced calcium uptake by lymphocytes from Alzheimer patients. *Biol Psychiatry* 22:1079–1086
 48. Leissring MA, Akbari Y, Fanger CM, Cahalan MD, Mattson MP, LaFerla FM (2000) Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J Cell Biol* 149:793–798
 49. Gibson G, Perrino P, Diemel GA (1986) In vivo brain calcium homeostasis during aging. *Mech Ageing Dev* 37:1–12
 50. Stutzmann GE, Caccamo A, LaFerla FM, Parker I (2004) Dysregulated IP3 signaling in cortical neurons of knock-in mice expressing an Alzheimer's-linked mutation in presenilin1 results in exaggerated Ca²⁺ signals and altered membrane excitability. *J Neurosci* 24:508–513
 51. Chakroborty S, Goussakov I, Miller MB, Stutzmann GE (2009) Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3xTg-AD mice. *J Neurosci* 29:9458–9470
 52. Zhang H, Sun S, Herreman A, De Strooper B, Bezprozvanny I (2010) Role of presenilins in neuronal calcium homeostasis. *J Neurosci* 30:8566–8580
 53. Emilsson L, Saetre P, Jazin E (2006) Alzheimer's disease: mRNA expression profiles of multiple patients show alterations of genes involved with calcium signaling. *Neurobiol Dis* 21:618–625
 54. Annaert W, De Strooper B (1999) Presenilins: molecular switches between proteolysis and signal transduction. *Trends Neurosci* 22:439–443
 55. De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K, Van Leuven F (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391:387–390
 56. Li H, Wolfe MS, Selkoe DJ (2009) Toward structural elucidation of the γ -secretase complex. *Structure* 17:326–334
 57. Gibson GE, Vestling M, Zhang H, Szolosi S, Alkon D, Lannfelt L, Gandy S, Cowburn RF (1997) Abnormalities in Alzheimer's disease fibroblasts bearing the APP670/671 mutation. *Neurobiol Aging* 18:573–580

58. Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL (1994) Internal Ca^{2+} mobilization is altered in fibroblasts from patients with Alzheimer disease. *Proc Natl Acad Sci* 91:534–538
59. Leissring MA, Paul BA, Parker I, Cotman CW, LaFerla FM (1999) Alzheimer's presenilin-1 mutation potentiates inositol 1,4,5-trisphosphate-mediated calcium signaling in *Xenopus*. *J Neurochem* 72:1061–1068
60. Stutzmann GE, Smith I, Caccamo A, Oddo S, LaFerla FM, Parker I (2006) Enhanced ryanodine receptor recruitment contributes to Ca^{2+} Disruptions in young, adult, and aged Alzheimer's disease mice. *J Neurosci* 26:5180–5189
61. Herms J, Schneider I, Dewachter I, Caluwaerts N, Kretschmar H, Van Leuven F (2003) Capacitive calcium entry is directly attenuated by mutant presenilin-1, independent of the expression of the amyloid precursor protein. *J Biol Chem* 278:2484–2489
62. Etcheberrigaray R, Hirashima N, Nee L, Prince J, Govoni S, Racchi M, Tanzi RE, Alkon DL (1998) Calcium responses in fibroblasts from asymptomatic members of Alzheimer's disease families. *Neurobiol Dis* 5:37–45
63. Leissring MA, LaFerla FM, Callamaras N, Parker I (2001) Subcellular mechanisms of presenilin-mediated enhancement of calcium signaling. *Neurobiol Dis* 8:469–478
64. Hirashima N, Etcheberrigaray R, Bergamaschi S, Racchi M, Battaini F, Binetti G, Govoni S, Alkon DL (1996) Calcium responses in human fibroblasts: a diagnostic molecular profile for Alzheimer's disease. *Neurobiol Aging* 17:549–555
65. Stutzmann GE (2005) Calcium dysregulation, IP3 signaling, and Alzheimer's disease. *Neuroscientist* 11:110–115
66. Stutzmann GE (2007) The pathogenesis of Alzheimers disease: is it a lifelong "calciumopathy"? *Neuroscientist* 13:546–559
67. Shilling D, Müller M, Takano H, Daniel Mak D-O, Abel T, Coulter DA, Foskett JK (2014) Suppression of InsP_3 receptor-mediated Ca^{2+} signaling alleviates mutant presenilin-linked familial Alzheimer's disease pathogenesis. *J Neurosci* 34:6910–6923
68. LaFerla FM (2002) Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nat Rev Neurosci* 3:862–872
69. Smith IF, Green KN, LaFerla FM (2005) Calcium dysregulation in Alzheimer's disease: recent advances gained from genetically modified animals. *Cell Calcium* 38:427–437
70. Yoo AS, Cheng I, Chung S, Grenfell TZ, Lee H, Pack-Chung E, Handler M, Shen J, Xia W, Tesco G, Saunders AJ, Ding K, Frosch MP, Tanzi RE, Kim T-W (2000) Presenilin-mediated modulation of capacitative calcium entry. *Neuron* 27:561–572
71. Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP (2000) Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. *J Biol Chem* 275:18195–18200
72. Goussakov I, Chakraborty S, Stutzmann GE (2011) Generation of dendritic Ca^{2+} oscillations as a consequence of altered ryanodine receptor function in AD neurons. *Channels (Austin)* 5:9–13
73. Goussakov I, Miller MB, Stutzmann GE (2010) NMDA-mediated Ca^{2+} influx drives aberrant ryanodine receptor activation in dendrites of young Alzheimer's disease mice. *J Neurosci* 30:12128–12137
74. Bruno AM, Huang JY, Bennett DA, Marr RA, Hastings ML, Stutzmann GE (2012) Altered ryanodine receptor expression in mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* 33(1001):e1001–e1006
75. Hayrapetyan V, Rybalchenko V, Rybalchenko N, Koulen P (2008) The N-terminus of presenilin-2 increases single channel activity of brain ryanodine receptors through direct protein–protein interaction. *Cell Calcium* 44:507–518
76. Huang H-M, Chen H-L, Gibson GE (2014) Interactions of endoplasmic reticulum and mitochondria Ca^{2+} stores with capacitative calcium entry. *Metab Brain Dis* 29:1083–1093
77. Bojarski L, Pomorski P, Szybinska A, Drab M, Skibinska-Kijek A, Gruszczynska-Biegala J, Kuznicki J (2009) Presenilin-dependent expression of STIM proteins and dysregulation of capacitative Ca^{2+} entry in familial Alzheimer's disease. *Biochim Biophys Acta (BBA)* 1793:1050–1057
78. Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee S-F, Hao Y-H, Serneels L, De Strooper B, Yu G, Bezprozvanny I (2006) Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* 126:981–993
79. Nelson O, Tu H, Lei T, Bentahir M, de Strooper B, Bezprozvanny I (2007) Familial Alzheimer disease-linked mutations specifically disrupt Ca^{2+} leak function of presenilin 1. *J Clin Invest* 117:1230–1239
80. Nelson O, Supnet C, Tolia A, Horr  K, De Strooper B, Bezprozvanny I (2011) Mutagenesis mapping of the presenilin 1 calcium leak conductance pore. *J Biol Chem* 286:22339–22347
81. Bezprozvanny I (2013) Presenilins and calcium signaling: systems biology to the rescue. *Sci Signaling* 6:pe24
82. Shilling D, Mak D-OD, Kang DE, Foskett JK (2012) Lack of evidence for presenilins as endoplasmic reticulum Ca^{2+} leak channels. *J Biol Chem* 287:10933–10944
83. Li X, Dang S, Yan C, Gong X, Wang J, Shi Y (2013) Structure of a presenilin family intramembrane aspartate protease. *Nature* 493:56–61
84. Theobald DL (2016) Presenilin adopts the CIC channel fold. *Protein Sci* 25:1363–1365
85. Bandara S, Malmersj  S, Meyer T (2013) Regulators of calcium homeostasis identified by inference of kinetic model parameters from live single cells perturbed by siRNA. *Sci Signaling* 6:ra56
86. Huang H-M, Chen H-L, Xu H, Gibson GE (2005) Modification of endoplasmic reticulum Ca^{2+} stores by select oxidants produces changes reminiscent of those in cells from patients with Alzheimer disease. *Free Radical Biol Med* 39:979–989
87. Grover AK, Samson SE, Misquitta CM (1997) Sarco(endoplasmic reticulum Ca^{2+} pump isoform SERCA3 is more resistant than SERCA2b to peroxide. *Am J Physiol* 273:C420–C425
88. Grover AK, Kwan C-Y, Samson SE (2003) Effects of peroxynitrite on sarco/endoplasmic reticulum Ca^{2+} pump isoforms SERCA2b and SERCA3a. *Am J Physiol* 285:C1537–C1543
89. Gibson GE, Chen H-L, Xu H, Qiu L, Xu Z, Denton TT, Shi Q (2012) Deficits in the mitochondrial enzyme α -ketoglutarate dehydrogenase lead to Alzheimer's disease-like calcium dysregulation. *Neurobiol Aging* 33(1121):e1113–e1121, e1124
90. Chen H, Denton TT, Xu H, Calingasan N, Beal MF, Gibson GE (2016) Reductions in the mitochondrial enzyme α -ketoglutarate dehydrogenase complex in neurodegenerative disease: beneficial or detrimental? *J Neurochem* 139:823–838
91. Parent A, Linden DJ, Sisodia SS, Borchelt DR (1999) Synaptic transmission and hippocampal long-term potentiation in transgenic mice expressing FAD-linked presenilin 1. *Neurobiol Dis* 6:56–62
92. Zhang C, Wu B, Beglopoulos V, Wines-Samuels M, Zhang D, Dragatsis I, Sudhof TC, Shen J (2009) Presenilins are essential for regulating neurotransmitter release. *Nature* 460:632–636
93. Gibson G, Huang H-M (2004) Mitochondrial enzymes and endoplasmic reticulum calcium stores as targets of oxidative stress in neurodegenerative diseases. *J Bioenerg Biomembr* 36:335–340
94. Mattson MP, Guo Q (1997) Cell and molecular neurobiology of presenilins: a role for the endoplasmic reticulum in the pathogenesis of Alzheimer's disease? *J Neurosci Res* 50:505–513

95. Reddy PH, Beal MF (2005) Are mitochondria critical in the pathogenesis of Alzheimer's disease? *Brain Res Rev* 49:618–632
96. Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G (2000) Oxidative stress in Alzheimer's disease. *Biochim Biophys Acta (BBA)* 1502:139–144
97. Kelliher M, Fastbom J, Cowburn RF, Bonkale W, Ohm TG, Ravid R, Sorrentino V, O'Neill C (1999) Alterations in the ryanodine receptor calcium release channel correlate with Alzheimer's disease neurofibrillary and β -amyloid pathologies. *Neuroscience* 92:499–513
98. Oulès B, Del Prete D, Greco B, Zhang X, Lauritzen I, Sevalle J, Moreno S, Trebak PP, Checler F, BF, Chami M (2012) Ryanodine receptor blockade reduces amyloid- β load and memory impairments in Tg2576 mouse model of Alzheimer disease. *J Neurosci* 32(34):11820–11834
99. Zhang H, Liu J, Sun S, Pchitskaya E, Popugaeva E, Bezprozvanny I (2015) Calcium signaling, excitability and synaptic plasticity defects in mouse model of Alzheimer's disease. *J Alzheimer's Dis* 45:561–580
100. Chakroborty S, Briggs C, Miller MB, Goussakov I, Schneider C, Kim J (2012) Stabilizing ER Ca^{2+} channel function as an early preventative strategy for Alzheimer's disease. *PLoS One* 7:274–279
101. Peng J, Liang G, Inan S, Wu Z, Joseph DJ, Meng Q, Peng Y, Eckenhoff MF, Wei H (2012) Dantrolene ameliorates cognitive decline and neuropathology in Alzheimer triple transgenic mice. *Neurosci Lett* 516:274–279
102. Popugaeva E, Bezprozvanny I (2013) Role of endoplasmic reticulum calcium signaling in the pathogenesis of Alzheimer disease. *Front Mol Neurosci* 6:29
103. Liu J, Supnet C, Sun S, Zhang H, Good L, Popugaeva E, Bezprozvanny I (2014) The role of ryanodine receptor type 3 in a mouse model of Alzheimer disease. *Channels (Austin)* 8:230–242
104. Zhang H, Sun S, Wu L, Pchitskaya E, Zakharova O, Fon Tacer K, Bezprozvanny I (2016) Store-operated calcium channel complex in postsynaptic spines: a new therapeutic target for Alzheimer's disease treatment. *J Neurosci* 36:11837–11850