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REVIEW

Expansion of the calcium hypothesis of brain aging and Alzheimer's disease: minding the store

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Key words: CICR, hippocampus; imaging; IP₃; L-type Ca²⁺ channels; ryanodine receptor.

Summary

Evidence accumulated over more than two decades has implicated Ca²⁺ dysregulation in brain aging and Alzheimer's disease (AD), giving rise to the Ca²⁺ hypothesis of brain aging and dementia. Electrophysiological, imaging, and behavioral studies in hippocampal or cortical neurons of rodents and rabbits have revealed aging-related increases in the slow afterhyperpolarization, Ca²⁺ spikes and currents, Ca²⁺ transients, and L-type voltage-gated Ca²⁺ channel (L-VGCC) activity. Several of these changes have been associated with age-related deficits in learning or memory. Consequently, one version of the Ca²⁺ hypothesis has been that increased L-VGCC activity drives many of the other Ca²⁺-related biomarkers of hippocampal aging. In addition, other studies have reported aging- or AD model-related alterations in Ca²⁺ release from ryanodine receptors (RyR) on intracellular stores. The Ca²⁺-sensitive RyR channels amplify plasmalemmal Ca²⁺ influx by the mechanism of Ca²⁺-induced Ca²⁺ release (CICR). Considerable evidence indicates that a preferred functional link is present between L-VGCCs and RyRs which operate in series in heart and some brain cells. Here, we review studies implicating RyRs in altered Ca²⁺ regulation in cell toxicity, aging, and AD. A recent study from our laboratory showed that increased CICR plays a necessary role in the emergence of Ca²⁺-related biomarkers of aging. Consequently, we propose an expanded L-VGCC/Ca²⁺ hypothesis, in which aging/pathological changes occur in both L-type Ca²⁺ channels and RyRs, and interact to abnormally amplify Ca²⁺ transients. In turn, the increased transients result in dysregulation of multiple Ca²⁺-dependent processes and, through somewhat different pathways, in accelerated functional decline during aging and AD.

Introduction

It has been over 20 years since it was initially proposed that altered Ca²⁺ regulation might play a role in brain aging and Alzheimer's disease (AD) (Landfield, 1983, 1987; Khachaturian, 1984, 1989; Gibson & Peterson, 1987; Disterhoft *et al.*, 1994). In brain neurons from aging rodents and rabbits, as compared to neurons from younger animals, Ca²⁺ influx associated with action potentials induces a larger Ca²⁺-dependent afterhyperpolarization (AHP) (Landfield & Pitler, 1984; Kerr *et al.*, 1989; Moyer *et al.*, 1992; Potier *et al.*, 1992; Disterhoft *et al.*, 1996, 2004; Stutzmann *et al.*, 2006) and impairs short-term synaptic plasticity (Landfield *et al.*, 1986; Thibault *et al.*, 2001). Furthermore, these findings have been reinforced by studies showing that pharmacologically isolated Ca²⁺ action potentials (Pitler & Landfield, 1990; Disterhoft *et al.*, 1996), whole-cell Ca²⁺ currents (Campbell *et al.*, 1996), and Ca²⁺ transients during repetitive spike trains also are larger in hippocampal neurons from aged animals (Thibault *et al.*, 2001; Hemond & Jaffe, 2005). Conversely, Ca²⁺ influx via ligand-gated N-methyl-D-aspartate (NMDA) receptor channels appears reduced in aged animals (Barnes *et al.*, 1997; Magnusson, 1998; Shankar *et al.*, 1998).

Our studies on this general Ca²⁺ dysregulation hypothesis have focused primarily on apparent excess Ca²⁺ influx via voltage-gated Ca²⁺ channels (VGCC) (Landfield, 1996; Thibault *et al.*, 1998). Studies of the L-type VGCC (L-VGCC) antagonist suggested that the aging-related increase in Ca²⁺-mediated responses might depend on greater activity through L-VGCC (Moyer *et al.*, 1992; Campbell *et al.*, 1996). Increased L-VGCC activity with aging was confirmed directly by single channel recording in partially dissociated hippocampal slices (Thibault & Landfield, 1996). Moreover, changes in L-VGCCs appear to be functionally relevant, as L-VGCC antagonists improve learning and memory in aged animals (Deyo *et al.*, 1989; Disterhoft *et al.*, 2004) and some AD patients (Forette *et al.*, 2002). Furthermore, the increase in L-VGCC density is positively correlated with cognitive impairment in aged animals (Thibault & Landfield, 1996).

In addition to the accumulating evidence of increased Ca²⁺ influx through L-VGCCs, there is also recent evidence that altered function of intracellular organelles might play a critical role in Ca²⁺ regulation during aging or AD (Toescu & Verkhratsky, 2003). In particular, changes in intracellular Ca²⁺ release from the endoplasmic reticulum (ER) appear likely to contribute to brain Ca²⁺ dyshomeostasis, and have been associated with

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changes in [Ca²⁺]. Therefore, in this review, we summarize several lines of evidence implicating altered release from intracellular stores in aging and AD, and attempt to integrate this evidence with the role of Ca²⁺ influx in aging-related Ca²⁺ dysregulation.

Interactions between L-VGCCs and Ca²⁺-induced Ca²⁺ release from the endoplasmic/sarcoplasmic reticulum

Several comprehensive reviews have recently considered mechanisms associated with Ca²⁺ sequestration and release by the ER in both peripheral cells (Bootman *et al.*, 2001; Berridge, 2002; Carafoli, 2002; Fill & Copello, 2002) and in neurons (Paschen & Mengesdorf, 2005; Verkhratsky, 2005). Accordingly, only the points most relevant to ER function in brain aging are briefly recapitulated here. Two distinct intracellular Ca²⁺ release channels are present in several types of muscle and brain cells, the inositol 1,4,5-trisphosphate receptor (IP₃R) and the ryanodine receptor (RyR), each having multiple isoforms in different tissues. These receptor channels function to amplify or trigger Ca²⁺ rises initiated by either plasmalemmal Ca²⁺ influx or ligand binding, thereby inducing Ca²⁺ signaling cascades. Amplification is achieved through either the actions of Ca²⁺-induced Ca²⁺ release (CICR), provided by RyR, or actions of IP₃-induced Ca²⁺ release (IICR) through IP₃Rs.

Originally described in skeletal and cardiac muscle cells, RyRs in the membrane of the sarcoplasmic reticulum are an integral and essential Ca²⁺ source for excitation-contraction coupling (Endo, 1977; Fill *et al.*, 1989; Takeshima *et al.*, 1989; Meissner, 1994). Furthermore, an apparent direct physical interaction, which favors alignment between L-VGCCs and RyRs, enables L-VGCCs to function as a preferred source of extracellularly derived Ca²⁺ in triggering CICR from RyRs and amplifying Ca²⁺ transients (Lu *et al.*, 1994; Cheng *et al.*, 1996; Wang *et al.*, 2001). In the brain, similar Ca²⁺ amplification functions of RyRs have been identified, again mediated in part by a close juxtaposition to L-VGCCs (Chavis *et al.*, 1996; Empson & Galione, 1997; Borde *et al.*, 2000; Fagni *et al.*, 2000; Sukhareva *et al.*, 2002).

The other major source of intracellular Ca²⁺ occurs in response to stimulation of IP₃Rs by IP₃ generated from activation of a number of metabotropic G-protein-coupled receptors. In some cases IP₃Rs can also trigger Ca²⁺-sensitive K⁺ channels and hyperpolarize neurons (Sawada *et al.*, 1987; Fink *et al.*, 1988; Furuichi *et al.*, 1989; Zhang *et al.*, 1990; Berridge, 1993; Khodakhah & Ogden, 1995; Irving & Collingridge, 1998; Taylor *et al.*, 1999; Jochenning *et al.*, 2002; Rossi & Taylor, 2004). Moreover, IP₃Rs are also sensitive to Ca²⁺ concentrations (Bezprozvanny *et al.*, 1991; Missiaen *et al.*, 1992; Tsukioka *et al.*, 1994; Hagar *et al.*, 1998) and, depending on the cell type studied, it appears that IP₃R may also be favorably aligned with L-VGCCs or metabotropic glutamate receptors (mGluR), through interactions with the scaffold protein Homer 1a (Tu *et al.*, 1998; Fagni *et al.*, 2000; Yamamoto *et al.*, 2005).

Release of Ca²⁺ from these two intracellular channels is regulated in part by the Ca²⁺ concentration gradient present

between luminal ER Ca²⁺ and cytoplasmic Ca²⁺ (Alonso *et al.*, 1999; Kiryushko *et al.*, 2002; Solovyova *et al.*, 2002) and is, thus, also dependent on the Ca²⁺-refilling function of sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPases (SERCA). Sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPases maintain the relatively high levels of Ca²⁺ in the ER (hundreds of μM) that serve CICR, and IICR, and, in the process, contribute to the control and reduction of cytosolic Ca²⁺ (Thastrup *et al.*, 1990; MacLennan *et al.*, 1997; Mogami *et al.*, 1998; Meldolesi, 2001; Berridge, 2002; Verkhratsky, 2004).

Dysregulated Ca²⁺ and ER function in models of ischemia and toxicity

Although cell culture models of Ca²⁺-dependent cell death are generally not viewed as clear models of brain aging, or even AD, they are often employed in studies of ischemic events. These events increase in frequency with advancing age, and it is also possible that neuronal vulnerability from such events increases with aging. Therefore, examining the role of Ca²⁺ release from intracellular stores in cell death models may help elucidate implications of aging-related alterations in intracellular release. In particular, delayed toxicity after exposure to high glutamate (GLU) in cell culture (excitotoxicity) is a common model used to mimic a wide range of neurological insults, including anoxia/ischemia, head and spinal cord trauma, and even chronic neurodegenerative diseases such as AD. Dysregulated Ca²⁺ homeostasis and altered Ca²⁺ influx through NMDA receptors were identified as primary contributors to neuronal cell death early in the study of excitotoxicity (Rothman & Olney, 1986; Choi *et al.*, 1987; Wahl *et al.*, 1989; Regan & Choi, 1991; Randall & Thayer, 1992; Dubinsky, 1993; Lu *et al.*, 1994; Marks *et al.*, 1996; Tymianski & Tator, 1996; Toescu, 1998; Lee *et al.*, 1999; Limbrick *et al.*, 2001; Lipton, 2004). In excitotoxicity models, Ca²⁺ dysregulation is frequently manifested as an irreversible Ca²⁺ rise or slowed Ca²⁺ clearance, and is ultimately associated with neuronal death.

Several investigations of excitotoxicity have focused on a potential role of the ER in sustained Ca²⁺ elevations. These studies have found that blocking CICR with high concentrations of ryanodine, which lock RyRs in a low conductance state (Bezprozvanny *et al.*, 1991; Coronado *et al.*, 1994; Humerickhouse *et al.*, 1994), or irreversibly inhibiting SERCA function and passively emptying ER stores with thapsigargin prior to GLU exposure, reduces sustained Ca²⁺ plateaus, as well as other indices associated with neuronal cell death (e.g. lactate dehydrogenase (LDH) release) (Frandsen & Schousboe, 1991; Segal & Manor, 1992; Leski *et al.*, 1999; Clodfelter *et al.*, 2002). Similar results have been noted in models of stroke and ischemia, particularly in astrocyte preparations (Duffy & MacVicar, 1996; Kuwabara *et al.*, 1996; Verkhratsky *et al.*, 1998; Aley *et al.*, 2006). Somewhat paradoxically, while short-term ER Ca²⁺ depletion prior to an insult appears protective against necrotic (excitotoxic) cell death, long-term depletion of ER Ca²⁺ induces apoptosis, as indicated by elevations of apoptotic markers, stress responses and disturbance

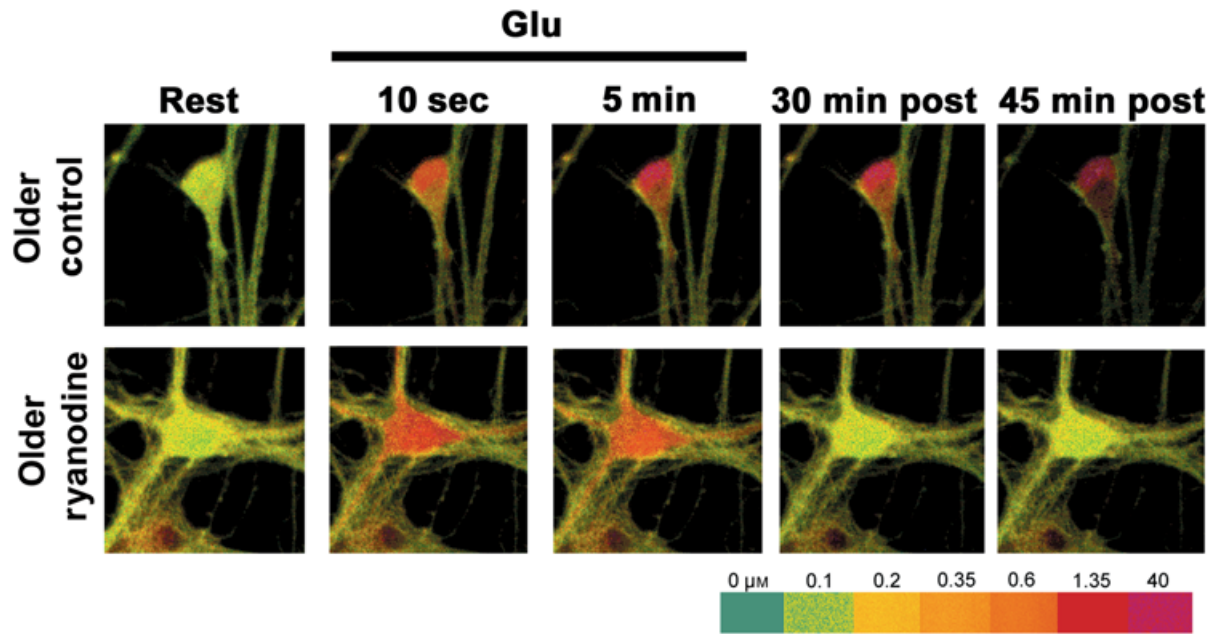


Fig. 1 Ryanodine protection of older cultured hippocampal neurons from excitotoxicity. Following a glutamate insult, older cultured neurons exhibit a sustained [Ca²⁺]_i elevation leading to cell death. Confocal indo-1 Ca²⁺ imaging shows ryanodine facilitated the recovery (decline) of the Ca²⁺ plateau and protected older neurons following glutamate insult (modified from Clodfelter *et al.* copyright 2002 with permission from Elsevier).

in protein synthesis, and/or massive cell death (Doutheil *et al.*, 1999; Mengesdorf *et al.*, 2001; Verkhatsky & Petersen, 2002; Paschen, 2003; Verkhatsky & Toescu, 2003; Lindholm *et al.*, 2006).

Thus, excessive release of Ca²⁺ from the ER may play an important role in excitotoxicity. Moreover, evidence suggests that such excessive release may be dependent on the relative maturity of the cells. It is well established that embryonic cortical/hippocampal neurons become increasingly vulnerable to GLU toxicity after a few weeks in culture (Choi, 1992; Toescu & Verkhatsky, 2000), an age in culture that coincides with the emergence of sustained Ca²⁺ plateaus following GLU insult (Attucci *et al.*, 2002). Interestingly, ryanodine is particularly effective in reversing the Ca²⁺ plateau and in providing neuroprotection in older cultures (Fig. 1) (Clodfelter *et al.*, 2002). Moreover, recent evidence suggests that the lethal Ca²⁺ plateau may be maintained by sustained Ca²⁺ influx via depolarized NMDA receptors (Norris *et al.*, 2006). Together, these data indicate that the plateau may be sustained by CICR. Although age in culture is clearly not equivalent to brain aging, it is associated with increasing vulnerability and Ca²⁺ influx, which may model some aspects of normal aging (Porter *et al.*, 1997). Conceivably therefore, if Ca²⁺ release from ER is altered with aging, this alteration may develop in parallel with altered Ca²⁺ influx (Clodfelter *et al.*, 2002).

Ca²⁺ release from ER in models of AD

With the increasing development of transgenic (Tg) mouse models of AD, numerous studies testing the view that altered Ca²⁺ homeostasis might play a role in AD have recently emerged.

Initial studies in fibroblasts from AD patients (Gibson *et al.*, 1996) or in cells bearing the human presenilin 1 (PS1) AD mutation (Begley *et al.*, 1999; Guo *et al.*, 1999; Leissring *et al.*, 1999; LaFerla, 2002; Stutzmann, 2005) found evidence of abnormal Ca²⁺ release through IP₃R pathways (Leissring *et al.*, 1999). Interestingly, abnormal IP₃-mediated Ca²⁺ elevations in fibroblasts have also been seen in asymptomatic members of AD families (Etcheberrigaray *et al.*, 1998).

Several studies also have implicated RyRs as being responsible for enhanced intracellular release in PS1 mutated animals (Chan *et al.*, 2000; Mattson *et al.*, 2000; Schneider *et al.*, 2001; Popescu & Ankarcrona, 2004; Stutzmann *et al.*, 2006). Smith and colleagues (2005) examined cultured cortical neurons from mice bearing a transgene containing three AD-related mutations (3xTg mice), which develop both plaques and tangles, and observed an increase in RyR expression along with greater Ca²⁺ efflux from the stores in response to caffeine (an agonist at the RyR) (Smith *et al.*, 2005). A recent study combining electrophysiological and Ca²⁺ imaging methods in cortical slice neurons from Tg mice bearing the PS1 mutation alone, or the 3xTg transgene, or nontransgenic control animals, assessed the effects of aging vs. those of the PS1 mutation on ER release (Stutzmann *et al.*, 2006). This study found that the PS1 mutation is a critical calciopathic mutation and that increased RyR expression is likely a major factor in the AD mutation-mediated enhancement of ER release. Although photolysis of IP₃ was shown to evoke larger Ca²⁺ transients and Ca²⁺-dependent hyperpolarizations in Tg mice, the increase in IP₃ effects was mediated by CICR from RyRs, triggered in response to IICR. However, some puzzling results also were seen. The enhanced IP₃-mediated Ca²⁺ release and resulting

hyperpolarization was larger in Tgs than in non-Tgs at all ages and did not change with aging in any Tg or non-Tg model. Conversely, the AHP induced by trains of spikes and VGCC activation increased with aging in all three model strains but did not differ between Tg and non-Tg mice (Stutzmann *et al.*, 2006).

While little is known regarding underlying mechanisms, it appears that altered CICR, perhaps in combination with IICR, confer some of the phenotypes of disrupted Ca²⁺ homeostasis in neurons from 3×Tg mice. Still, other sources and mechanisms likely also contribute. The PS1 mutation (which, alone, does not induce amyloid plaques), in combination with amyloid precursor protein (APP) mutations, increases Aβ production (Mullan & Crawford, 1993; Price & Sisodia, 1994; Tanzi *et al.*, 1996; Holcomb *et al.*, 1998; Selkoe, 1998). Some studies have found that Aβ production can exacerbate Ca²⁺ responses to NMDA or GLU exposure (Mattson, 1997). Furthermore, Aβ toxicity has been attributed, in part, to effects on VGCCs (Davidson *et al.*, 1994; Weiss *et al.*, 1994; Ueda *et al.*, 1997; Ramsden *et al.*, 2002; Bobich *et al.*, 2004; Webster *et al.*, 2006), which could trigger CICR from IP₃Rs or RyRs (Koizumi *et al.*, 1998; Ferreira *et al.*, 2004). However, APP proteolysis (γ-secretase activity) alone does not appear sufficient, because the PS1 mutation (rather than other more amyloidogenic mutations) must be present for the Ca²⁺ dysregulation to occur (Stutzmann *et al.*, 2006). A possible alternative mechanism suggests that presenilins form Ca²⁺ leak channels in ER membranes of mouse fibroblasts, independently of γ-secretase activity. Mutations in presenilin interfere with this leak function, and result in greater Ca²⁺ filling and release from ER (Tu *et al.*, 2006). Furthermore, a gene microarray study conducted in autopsied hippocampal tissue from human AD patients (Blalock *et al.*, 2004) found that multiple genes encoding proteins involved in ER receptor function, or in protein folding and chaperoning, which are also mediated in part by the ER, were down-regulated in incipient AD. These widespread changes may reflect ER membrane/receptor instability in sporadic AD as well.

In addition, it should be noted that effects of PS1 mutations on Ca²⁺ dysregulation have been observed to occur via other processes, including capacitative Ca²⁺ entry (Yoo *et al.*, 2000; Smith *et al.*, 2002; Herms *et al.*, 2003; Zatti *et al.*, 2006), changes in mitochondrial potential (Begley *et al.*, 1999; Ankarcona & Hultenby, 2002; Chan *et al.*, 2002; Behbahani *et al.*, 2006), and L-VGCCs (Cook *et al.*, 2005). Clearly therefore additional work will be needed to resolve the relative contributions of the different sources to the Ca²⁺ dysregulation seen in various models of neurodegenerative diseases.

Neuronal ER release in normal aging

Electrophysiological markers of brain aging have been extensively characterized in the hippocampal formation (Landfield & Pitler, 1984; Moyer *et al.*, 1992; Barnes, 1994; Thibault *et al.*, 1998; Norris *et al.*, 1998; Disterhoft *et al.*, 2004; Burke & Barnes, 2006), a region well-established to be important for memory processes and highly vulnerable to deleterious/degenerative changes with aging. Many of the consistent biomarkers of aging, such as the

slow AHP (sAHP), are Ca²⁺-dependent or Ca²⁺-mediated. However, it is important to assess the degree to which the ER contributes to the established biomarkers of aging. Both CICR and IICR pools exist within the ER of hippocampal CA1 and CA3 pyramidal neurons. The amount of Ca²⁺ released via CICR and IICR depends on binding of intracellular ligands including Ca²⁺, cyclic ADP ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP) or IP₃ (Verkhatsky, 2005), and also depends on the Ca²⁺ sequestering capacity of the ER, which determines ER Ca²⁺ content ([Ca²⁺]_l) (Verma *et al.*, 1992; Murayama & Ogawa, 1996; Dawson, 1997; Garaschuk *et al.*, 1997). Solovyova and colleagues using a dual indicator loading technique (low affinity indicator for imaging Ca²⁺ in the ER, and high affinity indicator for imaging Ca²⁺ in the cytosol) were able to show that the resting [Ca²⁺]_l in sensory neurons is in the range of 200–300 μM, and high concentrations of IP₃ or caffeine result in approximately a 40% decrease in luminal Ca²⁺ (Solovyova *et al.*, 2002). Depolarization induced [Ca²⁺]_l release was less effective, ranging from 5 to 30 μM. Other techniques for imaging Ca²⁺ within the ER include the use of aequorin or cameleons. However, there are limitations with these techniques, as the Ca²⁺ reporting proteins must be genetically engineered and selectively targeted to the ER (Miyawaki *et al.*, 1997; Alonso *et al.*, 1998; Solovyova & Verkhatsky, 2002). In addition, they require long incubation times for transfecting and loading and, thus, preclude their use in acute brain slices.

Consequently, there have been only a handful of studies in neurons examining the effects of aging on ER Ca²⁺ concentration and release, or on RyR expression. Studies focusing on measures of ER Ca²⁺ content have generally relied on the use of single wavelength indicators to measure changes in [Ca²⁺]_i transients activated by caffeine, and have found varying results, depending on the experimental approach or preparation. In an early study, no net change in ER Ca²⁺ release with aging was reported in synaptosomes from the whole brain (Martinez-Serrano *et al.*, 1992). More recently, acute dissociation of several brain regions (cerebellar, basal forebrain, and hippocampal neurons) from aged animals found that CICR magnitude was reduced and that Ca²⁺ transients recovered more slowly (Verkhatsky *et al.*, 1994; Kirischuk & Verkhatsky, 1996; Murchison & Griffith, 1999; Xiong *et al.*, 2002; Alshuaib *et al.*, 2006). In studies focusing on RyR expression, no clear pattern or consistent changes have been seen in neurons of normal aging rats and mice. Two studies reported no change in brain RyR expression during aging (Martini *et al.*, 1994; Stutzmann *et al.*, 2006), although a recent study of peripheral neurons found a transient elevation in protein levels (RyR3) in mid-aged rats (Vanterpool *et al.*, 2006).

Another approach to the investigation of the possible role of the ER in brain aging is to examine the effects of aging on Ca²⁺-dependent processes that are modulated, in part, by intracellular Ca²⁺ release. In CA1 neurons, postsynaptic injection of IP₃ or of RyR inhibitors prevents the induction of long-term potentiation and attenuates paired-pulse facilitation (Wang & Kelly, 1997). Similarly, bath application of thapsigargin or cyclopiazonic

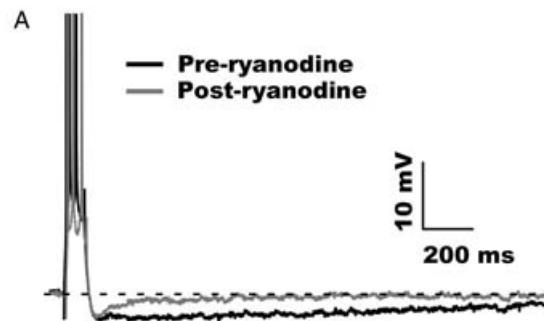
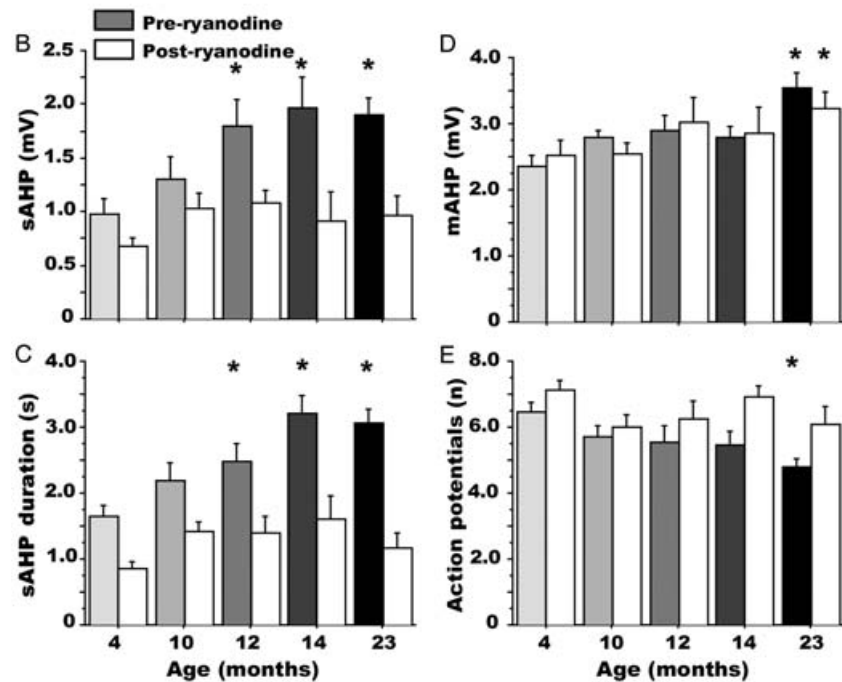


Fig. 2 Ryanodine reduces the slow afterhyperpolarization (AHP) in an age-dependent manner. (A) Representative example of the blocking effect of 20 μM ryanodine on the AHP of a 23-month-old rat CA1 neuron. (B) Age dependence of slow AHP (sAHP) amplitude, before and following ryanodine application. (C) Age dependence of slow AHP duration, pre- and postryanodine. (D) Age dependence of medium AHP (mAHP) amplitude, pre- and postryanodine. (E) Age-dependence measures of spike-frequency accommodation, pre- and postryanodine. * indicates a significant difference from the 4-month-old group ($P < 0.05$). Note that aging changes in sAHP markers emerge at 12 months of age (preryanodine group), and ryanodine completely eliminates the aging effects (B and C), indicating a selective blockade of the aging-related increase in Ca²⁺-induced Ca²⁺ release (CICR). The initial mAHP is not modulated by CICR (A) and its age dependence was not altered by ryanodine (D). Action potential accommodation changes generally followed the sAHP pattern, but the aging effect at 12 months was not significant in this subset of cells (mean \pm SEM) (from Gant *et al.* copyright 2006 with permission from the Society for Neuroscience).



acid (blockers of SERCA) prevents the induction of long-term depression in both single neurons and in field potential measures (Reyes & Stanton, 1996). High concentrations of ryanodine also selectively reduce the sAHP and spike-frequency accommodation (Borde *et al.*, 2000; Shah & Haylett, 2000). While examining the effect of aging on long-term depression induction (Norris *et al.*, 1998), Foster and colleagues recently reported that cyclopiazonic acid, thapsigargin or ryanodine (agents that reduce CICR) all prevented long-term depression in aged neurons (Kumar & Foster, 2005). However, long-term potentiation, which tends to be decreased with aging (Burke & Barnes, 2006), was enhanced by high ryanodine concentrations in aged slices (Kumar & Foster, 2004). Ca²⁺-dependent processes mediated largely by IICR and mGluRs activation also have been shown to change with aging. Compared to younger animals, type 1 mGluR activation results in a reduced phosphoinositide turnover in aged rats, perhaps mediated by a reduction in phospholipase C activity (Nicolle *et al.*, 1999). Similarly, protein kinase C (PKC) was also reported to show reduced activity in aging neurons (Araki *et al.*, 1994; Pascale *et al.*, 1998).

Thus, the evidence on the nature of altered CICR or IICR in neurons of normally aging mammals is somewhat inconsistent, perhaps reflecting the type of preparation, cell or brain region specificity, or the difficulty in imaging Ca²⁺ and its sources within the intact hippocampal slice (Brown & Jaffe, 1994). Recently therefore we sought to systematically test the contributions of CICR to aging changes in one of the brain regions studied most extensively in relation to aging (hippocampus). Specifically, we tested the key prediction that, if increased CICR plays a major role in normal brain aging, then blocking it with high concentration ryanodine should reduce the aging differences in multiple Ca²⁺ biomarkers of aging.

More broadly, in fact, several other important tenets of the overall Ca²⁺ hypothesis have, for some time, required adequate testing. These tenets and predictions include: (i) if a common mechanism of Ca²⁺ dysregulation underlies many aspects of brain aging, then multiple Ca²⁺-dependent biomarkers of aging in the hippocampus should emerge at approximately the same age in adulthood; and (ii) if Ca²⁺ dysregulation is a major factor in cognitive decline then Ca²⁺ biomarkers should precede or

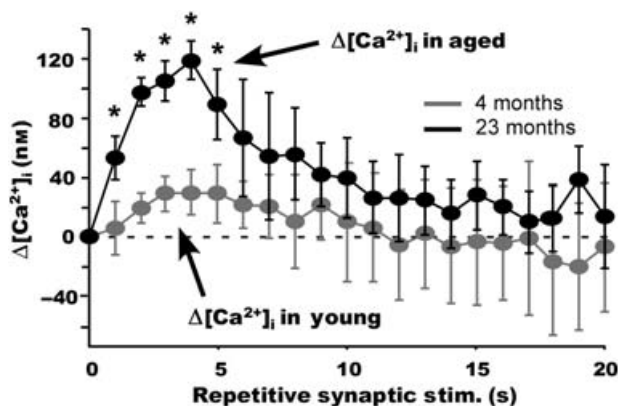


Fig. 3 Ryanodine-sensitive component of the $[\text{Ca}^{2+}]_i$ rise during repetitive synaptic stimulation. Ca^{2+} -induced Ca^{2+} release (CICR) contribution to the $[\text{Ca}^{2+}]_i$ rise was determined by subtracting $[\text{Ca}^{2+}]_i$ measures following ryanodine from those before ryanodine application ($\Delta[\text{Ca}^{2+}]_i$), in neurons from 4- and 23-month-old animals during 20-s trains of 7 Hz suprathreshold synaptic stimulation. Values shown represent only the (CICR) component of the Ca^{2+} response that was blocked by ryanodine. Note that the ryanodine-sensitive component of $[\text{Ca}^{2+}]_i$ is significantly greater in aged rat neurons and contributes to the Ca^{2+} response primarily during the first 5 s of stimulation. * indicates a significant difference from the 4-month-old group ($P < 0.05$). (mean \pm SEM).

coincide with the earliest age of cognitive impairment, which in some studies of rats has been as early as 12-months old (approximately mid-life). To test these predictions and the involvement of CICR on the emergence of Ca^{2+} -related biomarkers, we recently conducted an extensive age course study combining electrophysiological and Ca^{2+} imaging techniques in hippocampal slices from male rats. Animals at five age points were used to identify the age of onset for three Ca^{2+} -mediated markers of aging, the sAHP, spike accommodation, and the synaptically activated Ca^{2+} transient. A subset of hippocampal slices received a high dose of ryanodine to block the contribution of CICR to the overall Ca^{2+} response. In this study, we also employed the least invasive procedures available (sharp intracellular electrodes instead of patch clamping electrodes, nondissociated slices) to minimize interactions of preparation trauma and age.

Results were consistent with the above predictions. That is, ryanodine essentially eliminated aging differences in the three markers (e.g. the sAHP, Fig. 2), and the three biomarkers were first detectable simultaneously and at 12 months of age (Fig. 2), an age range early enough to account for cognitive decline. The ryanodine-sensitive component of the Ca^{2+} response (i.e. CICR) during a 20-s train of synaptic spikes appears to be minimal in young neurons compared to aged neurons and, notably, CICR contributed most to the $[\text{Ca}^{2+}]_i$ elevation during the first few seconds of the train (Fig. 3). This rapid 'booster' action of CICR on Ca^{2+} responses is consistent with its strong effect on the AHP (Fig. 2) (Gant *et al.*, 2006).

Thus, results of this large study provide considerable support for the proposition that in the hippocampus, an aging-related increase in CICR is necessary, from the onset, for the development of aging changes in several Ca^{2+} -related processes. Moreover, the findings may help to resolve some of the contradictions in

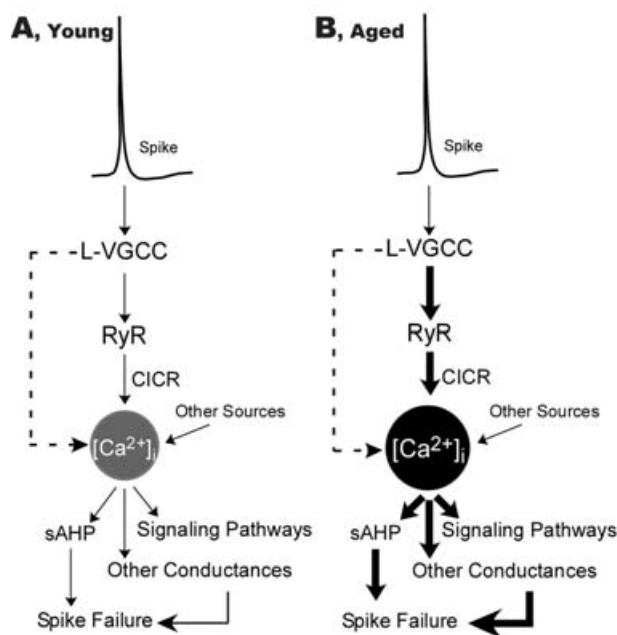


Fig. 4 Schematic model of alterations in L-type voltage-gated Ca^{2+} channels (L-VGCC) and Ca^{2+} -induced Ca^{2+} release (CICR) that drive other Ca^{2+} -related hippocampal biomarkers of aging. With aging, increased L-VGCC activity and enhanced CICR operate in series, amplifying the impact of Ca^{2+} influx on multiple Ca^{2+} -dependent functions. The thickness of arrows schematically represents the activity of Ca^{2+} flux or signaling pathways in aged rat neurons (B) relative to young (A). These pathways are increased at several stages despite equivalent spike amplitudes and durations. Dashed arrows indicate a possible direct parallel contribution of L-VGCCs to $[\text{Ca}^{2+}]_i$. (From Gant *et al.* copyright 2006 with permission from the Society for Neuroscience).

the literature by elucidating the conditions under which the contributions of CICR are most prominent. However, one apparent paradox is that similar kinds of evidence support a critical role for L-VGCCs in aging-related Ca^{2+} dysregulation (Thibault *et al.*, 1998; Disterhoft *et al.*, 2004). Nevertheless, these two lines of evidence are not necessarily contradictory, given that L-VGCCs and RyRs appear to operate in series in many cell types. In this view, then, Ca^{2+} influx via L-VGCCs may be the preferred source for triggering elevated CICR in aging. Together, the data suggest that aging changes in both types of channel may be part of the same pathway of dysregulation, in turn, suggesting the utility of expanding this version of the Ca^{2+} hypothesis to incorporate the results on Ca^{2+} release from intracellular stores (Fig. 4).

Conclusions and a new model of Ca^{2+} dysregulation in hippocampal aging

The work summarized above points to the following basic conclusions:

1 Extensive evidence supporting the hypothesis that Ca^{2+} dysregulation contributes in part to brain aging and AD that has accumulated for more than 20 years, some of it implicating a larger Ca^{2+} -dependent AHP and increased activity of L-type Ca^{2+}

channels in the functional and cognitive decline seen with normal aging in mammals.

2 Elevated Ca²⁺ release from RyRs appears to contribute importantly to cell death and vulnerability in several models of toxicity, which may have relevance to aging-associated ischemic events or other degenerative conditions.

3 Some types of AD mutations (e.g. presenilins), but not all, appear to alter RyR expression. Under some conditions, (e.g. IP₃ stimulation and consequent CICR), this can result in elevated intracellular Ca²⁺ release and greater hyperpolarization of cortical neurons from transgenic mice of all ages. Surprisingly, however, in the triple transgenic AD model, the aging-related increase in spike train-induced AHP did not differ from the aging change in the AHP seen in wild-type mice.

4 The observed contributions of altered CICR to Ca²⁺ dysregulation in neurons during normal aging have been somewhat inconsistent, apparently depending, in part, on cell type and preparation, regional localization and possibly species. However, our recent studies in hippocampal slices from rats of increasing age (five age points) indicate that elevated CICR, beginning at about 12 months of age, may be an important underlying factor in the emergence of multiple Ca²⁺-related biomarkers of brain aging in rats.

5 The apparent strong evidence linking both L-VGCCs and RyRs to dysregulated hippocampal Ca²⁺ homeostasis during aging, rather than being contradictory, may instead suggest an expanded model of the Ca²⁺ dysregulation pathway in brain aging and, perhaps in AD (as shown in Fig. 4). In this new model, L-VGCCs and RyRs operate in series and aging changes in both (or either) contribute to the aberrant amplification of Ca²⁺ transients.

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References

Aley PK, Murray HJ, Boyle JP, Pearson HA, Peers C (2006) Hypoxia stimulates Ca²⁺ release from intracellular stores in astrocytes via cyclic ADP ribose-mediated activation of ryanodine receptors. *Cell Calcium* **39**, 95–100.

Alonso MT, Barrero MJ, Carnicero E, Montero M, Garcia-Sancho J, Alvarez J (1998) Functional measurements of [Ca²⁺]_i in the endoplasmic reticulum using a herpes virus to deliver targeted aequorin. *Cell Calcium* **24**, 87–96.

Alonso MT, Barrero MJ, Michelena P, Carnicero E, Cuchillo I, Garcia AG, Garcia-Sancho J, Montero M, Alvarez J (1999) Ca²⁺-induced Ca²⁺ release in chromaffin cells seen from inside the ER with targeted aequorin. *J. Cell Biol.* **144**, 241–254.

Alshuaib WB, Cherian SP, Hasan MY, Fahim MA (2006) Modulation of neuronal [Ca²⁺]_i by caffeine is altered with aging. *Int. J. Dev. Neurosci.* **24**, 389–394.

Ankarcrona M, Hultenby K (2002) Presenilin-1 is located in rat mitochondria. *Biochem. Biophys. Res. Commun.* **295**, 766–770.

Araki T, Kato H, Kanai Y, Kogure K (1994) Age-dependent changes in second messenger and rolipram receptor systems in the gerbil brain. *J. Neural Transm. Gen. Sect.* **97**, 135–147.

Attucci S, Clodfelter GV, Thibault O, Staton J, Moroni F, Landfield PW, Porter NM (2002) Group I metabotropic glutamate receptor inhibition selectively blocks a prolonged Ca(2+) elevation associated with age-dependent excitotoxicity. *Neuroscience* **112**, 183–194.

Barnes CA (1994) Normal aging: regionally specific changes in hippocampal synaptic transmission. *Trends Neurosci.* **17**, 13–18.

Barnes CA, Rao G, Shen J (1997) Age-related decrease in the N-methyl-D-aspartateR-mediated excitatory postsynaptic potential in hippocampal region CA1. *Neurobiol. Aging* **18**, 445–452.

Begley JG, Duan W, Chan S, Duff K, Mattson MP (1999) Altered calcium homeostasis and mitochondrial dysfunction in cortical synaptic compartments of presenilin-1 mutant mice. *J. Neurochem.* **72**, 1030–1039.

Behbahani H, Shabalina IG, Wiehager B, Concha H, Hultenby K, Petrovic N, Nedergaard J, Winblad B, Cowburn RF, Ankarcrona M (2006) Differential role of Presenilin-1 and -2 on mitochondrial membrane potential and oxygen consumption in mouse embryonic fibroblasts. *J. Neurosci. Res.* **84**, 891–902.

Berridge MJ (1993) Inositol trisphosphate and calcium signalling. *Nature* **361**, 315–325.

Berridge MJ (2002) The endoplasmic reticulum: a multifunctional signaling organelle. *Cell Calcium* **32**, 235–249.

Bezprozvanny I, Watras J, Ehrlich BE (1991) Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* **351**, 751–754.

Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW (2004) Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc. Natl Acad. Sci. USA* **101**, 2173–2178.

Bobich JA, Zheng Q, Campbell A (2004) Incubation of nerve endings with a physiological concentration of Abeta1-42 activates CaV2.2(N-Type)-voltage operated calcium channels and acutely increases glutamate and noradrenaline release. *J. Alzheimers Dis.* **6**, 243–255.

Bootman MD, Collins TJ, Peppiatt CM, Prothero LS, MacKenzie L, De Smet P, Travers M, Tovey SC, Seo JT, Berridge MJ, Ciccolini F, Lipp P (2001) Calcium signalling – an overview. *Semin. Cell Dev. Biol.* **12**, 3–10.

Borde M, Bonansco C, de Sevilla F, Le Ray D, Buno W (2000) Voltage-clamp analysis of the potentiation of the slow Ca²⁺-activated K⁺ current in hippocampal pyramidal neurons. *Hippocampus* **10**, 198–206.

Brown TH, Jaffe DB (1994) Calcium imaging in hippocampal neurons using confocal microscopy. *Ann. N. Y. Acad. Sci.* **747**, 313–324.

Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. *Nat. Rev. Neurosci.* **7**, 30–40.

Campbell LW, Hao SY, Thibault O, Blalock EM, Landfield PW (1996) Aging changes in voltage-gated calcium currents in hippocampal CA1 neurons. *J. Neurosci.* **16**, 6286–6295.

Carafoli E (2002) Calcium signaling: a tale for all seasons. *Proc. Natl Acad. Sci. USA* **99**, 1115–1122.

Chan SL, Culmsee C, Haughey N, Klapper W, Mattson MP (2002) Presenilin-1 mutations sensitize neurons to DNA damage-induced death by a mechanism involving perturbed calcium homeostasis and activation of calpains and caspase-12. *Neurobiol. Dis.* **11**, 2–19.

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.

- Chavis P, Fagni L, Lansman JB, Bockaert J (1996) Functional coupling between ryanodine receptors and L-type calcium channels in neurons. *Nature* **382**, 719–722.
- Cheng G, Liu BF, Yu Y, Diglio C, Kuo TH (1996) The exit from G(0) into the cell cycle requires and is controlled by sarco(endo)plasmic reticulum Ca²⁺ pump. *Arch. Biochem. Biophys.* **329**, 65–72.
- Choi DW (1992) Excitotoxic cell death. *J. Neurobiol.* **23**, 1261–1276.
- Choi DW, Maulucci-Gedde M, Kriegstein AR (1987) Glutamate neurotoxicity in cortical cell culture. *J. Neurosci.* **7**, 357–368.
- Clodfelter GV, Porter NM, Landfield PW, Thibault O (2002) Sustained Ca²⁺-induced Ca²⁺-release underlies the post-glutamate lethal Ca²⁺ plateau in older cultured hippocampal neurons. *Eur. J. Pharmacol.* **447**, 189–200.
- Cook DG, Li X, Cherry SD, Cantrell AR (2005) Presenilin 1 deficiency alters the activity of voltage-gated Ca²⁺ channels in cultured cortical neurons. *J. Neurophysiol.* **94**, 4421–4429.
- Coronado R, Morrissette J, Sukhareva M, Vaughan DM (1994) Structure and function of ryanodine receptors. *Am. J. Physiol.* **266**, C1485–C1504.
- Davidson RM, Shajenko L, Donta TS (1994) Amyloid β -peptide (A β P) potentiates a nimodipine-sensitive L-type barium conductance in N1E-115 neuroblastoma cells. *Brain Res.* **643**, 324–327.
- Dawson AP (1997) Calcium signalling: how do IP₃ receptors work? *Curr. Biol.* **7**, R544–R547.
- Deyo RA, Straube KT, Disterhoft JF (1989) Nimodipine facilitates associative learning in aging rabbits. *Science* **243**, 809–811.
- Disterhoft JF, Moyer JR Jr, Thompson LT (1994) The calcium rationale in aging and Alzheimer's disease. Evidence from an animal model of normal aging. *Ann. N. Y. Acad. Sci.* **747**, 382–406.
- Disterhoft JF, Thompson LT, Moyer JR Jr, Mogul DJ (1996) Calcium-dependent afterhyperpolarization and learning in young and aging hippocampus. *Life Sci.* **59**, 413–420.
- Disterhoft JF, Wu WW, Ohno M (2004) Biophysical alterations of hippocampal pyramidal neurons in learning, ageing and Alzheimer's disease. *Ageing Res. Rev.* **3**, 383–406.
- Douthel J, Treiman M, Oschlies U, Paschen W (1999) Recovery of neuronal protein synthesis after irreversible inhibition of the endoplasmic reticulum calcium pump. *Cell Calcium* **25**, 419–428.
- Dubinsky JM (1993) Intracellular calcium levels during the period of delayed excitotoxicity. *J. Neurosci.* **13**, 623–631.
- Duffy S, MacVicar BA (1996) *In vitro* ischemia promotes calcium influx and intracellular calcium release in hippocampal astrocytes. *J. Neurosci.* **16**, 71–81.
- Empson RM, Galione A (1997) Cyclic ADP-ribose enhances coupling between voltage-gated Ca²⁺ entry and intracellular Ca²⁺ release. *J. Biol. Chem.* **272**, 20967–20970.
- Endo M (1977) Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* **57**, 71–108.
- 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.
- Fagni L, Chavis P, Ango F, Bockaert J (2000) Complex interactions between mGluRs, intracellular Ca²⁺ stores and ion channels in neurons. *Trends Neurosci.* **23**, 80–88.
- Ferreiro E, Oliveira CR, Pereira C (2004) Involvement of endoplasmic reticulum Ca²⁺ release through ryanodine and inositol 1,4,5-triphosphate receptors in the neurotoxic effects induced by the amyloid- β peptide. *J. Neurosci. Res.* **76**, 872–880.
- Fill M, Copello JA (2002) Ryanodine receptor calcium release channels. *Physiol. Rev.* **82**, 893–922.
- Fill M, Ma JJ, Knudson CM, Imagawa T, Campbell KP, Coronado R (1989) Role of the ryanodine receptor of skeletal muscle in excitation-contraction coupling. *Ann. N. Y. Acad. Sci.* **560**, 155–162.
- Fink LA, Connor JA, Kaczmarek LK (1988) Inositol trisphosphate releases intracellularly stored calcium and modulates ion channels in molluscan neurons. *J. Neurosci.* **8**, 2544–2555.
- Forette F, Seux ML, Staessen JA, Thijs L, Babarskiene MR, Babeanu S, Bossini A, Fagard R, Gil-Extremera B, Laks T, Kopalava Z, Sarti C, Tuomilehto J, Vanhanen H, Webster J, Yodfat Y, Birkenhager WH (2002) The prevention of dementia with antihypertensive treatment: new evidence from the Systolic Hypertension in Europe (Syst-Eur) study. *Arch. Intern. Med.* **162**, 2046–2052.
- Frandsen A, Schousboe A (1991) Dantrolene prevents glutamate cytotoxicity and Ca²⁺ release from intracellular stores in cultured cerebral cortical neurons. *J. Neurochem.* **56**, 1075–1078.
- Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K (1989) Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. *Nature* **342**, 32–38.
- Gant JC, Sama MM, Landfield PW, Thibault O (2006) Early and simultaneous emergence of multiple hippocampal biomarkers of aging is mediated by Ca²⁺-induced Ca²⁺ release. *J. Neurosci.* **26**, 3482–3490.
- Garaschuk O, Yaari Y, Konnerth A (1997) Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurones. *J. Physiol.* **502** (Pt 1), 13–30.
- Gibson GE, Peterson C (1987) Calcium and the aging nervous system. *Neurobiol. Aging* **8**, 329–343.
- Gibson GE, Zhang H, Toral-Barza L, Szolosi S, Tofel-Greth B (1996) Calcium stores in cultured fibroblasts and their changes with Alzheimer's disease. *Biochim. Biophys. Acta* **1316**, 71–77.
- Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP (1999) Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nat. Med.* **5**, 101–106.
- Hagar RE, Burgstahler AD, Nathanson MH, Ehrlich BE (1998) Type III InsP₃ receptor channel stays open in the presence of increased calcium. *Nature* **396**, 81–84.
- Hemond P, Jaffe DB (2005) Caloric restriction prevents aging-associated changes in spike-mediated Ca²⁺ accumulation and the slow afterhyperpolarization in hippocampal CA1 pyramidal neurons. *Neuroscience* **135**, 413–420.
- Hermes J, Schneider I, Dewachter I, Caluwaerts N, Kretzschmar 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.
- Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med.* **4**, 97–100.
- Humerickhouse RA, Bidasee KR, Gerzon K, Emmick JT, Kwon S, Sutko JL, Ruest L, Besch HR Jr (1994) High affinity C10-Oeq ester derivatives of ryanodine. Activator-selective agonists of the sarcoplasmic reticulum calcium release channel. *J. Biol. Chem.* **269**, 30243–30253.
- Irving AJ, Collingridge GL (1998) A characterization of muscarinic receptor-mediated intracellular Ca²⁺ mobilization in cultured rat hippocampal neurones. *J. Physiol.* **511** (Pt 3), 747–759.
- Johanning FW, Zochowski M, Conway SJ, Holmes AB, Koulen P, Ehrlich BE (2002) Distinct intracellular calcium transients in neurites and somata integrate neuronal signals. *J. Neurosci.* **22**, 5344–5353.
- Kerr DS, Campbell LW, Hao SY, Landfield PW (1989) Corticosteroid modulation of hippocampal potentials: increased effect with aging. *Science* **245**, 1505–1509.
- Khachaturian ZS (1984) Scientific challenges and opportunities related to Alzheimer's disease. *Clin. Pharm.* **3**, 522–523.
- Khachaturian ZS (1989) The role of calcium regulation in brain aging: reexamination of a hypothesis. *Aging (Milano)* **1**, 17–34.

- Khodakhah K, Ogden D (1995) Fast activation and inactivation of inositol trisphosphate-evoked Ca²⁺ release in rat cerebellar Purkinje neurones. *J. Physiol.* **487** (Pt 2), 343–358.
- Kirischuk S, Verkhratsky A (1996) Calcium homeostasis in aged neurones. *Life Sci.* **59**, 451–459.
- Kiryushko DV, Savtchenko LP, Verkhratsky AN, Korogod SM (2002) Theoretical estimation of the capacity of intracellular calcium stores in the Bergmann glial cell. *Pflügers Arch.* **443**, 643–651.
- Koizumi S, Ishiguro M, Ohsawa I, Morimoto T, Takamura C, Inoue K, Kohsaka S (1998) The effect of a secreted form of β -amyloid-precursor protein on intracellular Ca²⁺ increase in rat cultured hippocampal neurones. *Br. J. Pharmacol.* **123**, 1483–1489.
- Kumar A, Foster TC (2004) Enhanced long-term potentiation during aging is masked by processes involving intracellular calcium stores. *J. Neurophysiol.* **91**, 2437–2444.
- Kumar A, Foster TC (2005) Intracellular calcium stores contribute to increased susceptibility to LTD induction during aging. *Brain Res.* **1031**, 125–128.
- Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maeda Y, Kitagawa K, Imuta N, Kinoshita T, Stern DM, Yanagi H, Kamada T (1996) Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. *J. Biol. Chem.* **271**, 5025–5032.
- LaFerla FM (2002) Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nat. Rev. Neurosci.* **3**, 862–872.
- Landfield PW (1983) Mechanisms of altered neural function during aging. In: *Aging of the Brain* (Gipsen WH, Traber J, eds). Amsterdam, The Netherlands: Elsevier, pp. 51–57.
- Landfield PW (1987) 'Increased calcium-current' hypothesis of brain aging. *Neurobiol. Aging* **8**, 346–347.
- Landfield PW (1996) Aging-related increase in hippocampal calcium channels. *Life Sci.* **59**, 399–404.
- Landfield PW, Pitler TA (1984) Prolonged Ca²⁺-dependent afterhyperpolarizations in hippocampal neurons of aged rats. *Science* **226**, 1089–1092.
- Landfield PW, Pitler TA, Applegate MD (1986) The effects of high Mg²⁺-to-Ca²⁺ ratios on frequency potentiation in hippocampal slices of young and aged rats. *J. Neurophysiol.* **56**, 797–811.
- Lee JM, Zipfel GJ, Choi DW (1999) The changing landscape of ischaemic brain injury mechanisms. *Nature* **399**, A7–A14.
- 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* oocytes. *J. Neurochem.* **72**, 1061–1068.
- Leski ML, Valentine SL, Coyle JT (1999) L-type voltage-gated calcium channels modulate kainic acid neurotoxicity in cerebellar granule cells. *Brain Res.* **828**, 27–40.
- Limbrick DD Jr, Pal S, DeLorenzo RJ (2001) Hippocampal neurons exhibit both persistent Ca²⁺ influx and impairment of Ca²⁺ sequestration/extrusion mechanisms following excitotoxic glutamate exposure. *Brain Res.* **894**, 56–67.
- Lindholm D, Wootz H, Korhonen L (2006) ER stress and neurodegenerative diseases. *Cell Death Differ.* **13**, 385–392.
- Lipton SA (2004) Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. *NeuroRx* **1**, 101–110.
- Lu X, Xu L, Meissner G (1994) Activation of the skeletal muscle calcium release channel by a cytoplasmic loop of the dihydropyridine receptor. *J. Biol. Chem.* **269**, 6511–6516.
- MacLennan DH, Rice WJ, Green NM (1997) The mechanism of Ca²⁺ transport by sarco(endo)plasmic reticulum Ca²⁺-ATPases. *J. Biol. Chem.* **272**, 28815–28818.
- Magnusson KR (1998) The aging of the NMDA receptor complex. *Front. Biosci.* **3**, e70–80.
- Marks JD, Friedman JE, Haddad GG (1996) Vulnerability of CA1 neurons to glutamate is developmentally regulated. *Brain Res. Dev. Brain Res.* **97**, 194–206.
- Martinez-Serrano A, Blanco P, Satrustegui J (1992) Calcium binding to the cytosol and calcium extrusion mechanisms in intact synaptosomes and their alterations with aging. *J. Biol. Chem.* **267**, 4672–4679.
- Martini A, Battaini F, Govoni S, Volpe P (1994) Inositol 1,4,5-trisphosphate receptor and ryanodine receptor in the aging brain of Wistar rats. *Neurobiol. Aging* **15**, 203–206.
- Mattson MP (1997) Cellular actions of β -amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol. Rev.* **77**, 1081–1132.
- Mattson MP, Zhu H, Yu J, Kindy MS (2000) Presenilin-1 mutation increases neuronal vulnerability to focal ischemia *in vivo* and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. *J. Neurosci.* **20**, 1358–1364.
- Meissner G (1994) Ryanodine receptor/Ca²⁺ release channels and their regulation by endogenous effectors. *Annu. Rev. Physiol.* **56**, 485–508.
- Meldolesi J (2001) Rapidly exchanging Ca²⁺ stores in neurons: molecular, structural and functional properties. *Prog. Neurobiol.* **65**, 309–338.
- Mengesdorf T, Althausen S, Oberndorfer I, Paschen W (2001) Response of neurons to an irreversible inhibition of endoplasmic reticulum Ca(2+)-ATPase: relationship between global protein synthesis and expression and translation of individual genes. *Biochem. J.* **356**, 805–812.
- Missiaen L, De Smedt H, Droogmans G, Casteels R (1992) Luminal Ca²⁺ controls the activation of the inositol 1,4,5-trisphosphate receptor by cytosolic Ca²⁺. *J. Biol. Chem.* **267**, 22961–22966.
- Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, Tsien RY (1997) Fluorescent indicators for Ca²⁺ based on green fluorescent proteins and calmodulin. *Nature* **388**, 882–887.
- Mogami H, Tepikin AV, Petersen OH (1998) Termination of cytosolic Ca²⁺ signals: Ca²⁺ reuptake into intracellular stores is regulated by the free Ca²⁺ concentration in the store lumen. *EMBO J.* **17**, 435–442.
- Moyer JR Jr, Thompson LT, Black JP, Disterhoft JF (1992) Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. *J. Neurophysiol.* **68**, 2100–2109.
- Mullan M, Crawford F (1993) Genetic and molecular advances in Alzheimer's disease. *Trends Neurosci.* **16**, 398–403.
- Murayama T, Ogawa Y (1996) Properties of Ryr3 ryanodine receptor isoform in mammalian brain. *J. Biol. Chem.* **271**, 5079–5084.
- Murchison D, Griffith WH (1999) Age-related alterations in caffeine-sensitive calcium stores and mitochondrial buffering in rat basal forebrain. *Cell Calcium* **25**, 439–452.
- Nicolle MM, Colombo PJ, Gallagher M, McKinney M (1999) Metabotropic glutamate receptor-mediated hippocampal phosphoinositide turnover is blunted in spatial learning-impaired aged rats. *J. Neurosci.* **19**, 9604–9610.
- Norris CM, Blalock EM, Thibault O, Brewer LD, Clodfelter GV, Porter NM, Landfield PW (2006) Electrophysiological mechanisms of delayed excitotoxicity: positive feedback loop between NMDA receptor current and depolarization-mediated glutamate release. *J. Neurophysiol.* **96**, 2488–2500.
- Norris CM, Halpain S, Foster TC (1998) Reversal of age-related alterations in synaptic plasticity by blockade of L-type Ca²⁺ channels. *J. Neurosci.* **18**, 3171–3179.
- Pascale A, Govoni S, Battaini F (1998) Age-related alteration of PKC, a key enzyme in memory processes: physiological and pathological examples. *Mol. Neurobiol.* **16**, 49–62.
- Paschen W (2003) Endoplasmic reticulum: a primary target in various acute disorders and degenerative diseases of the brain. *Cell Calcium* **34**, 365–383.

- Paschen W, Mengesdorf T (2005) Cellular abnormalities linked to endoplasmic reticulum dysfunction in cerebrovascular disease – therapeutic potential. *Pharmacol. Ther.* **108**, 362–375.
- Pitler TA, Landfield PW (1990) Aging-related prolongation of calcium spike duration in rat hippocampal slice neurons. *Brain Res.* **508**, 1–6.
- Popescu BO, Ankarcrone M (2004) Mechanisms of cell death in Alzheimer's disease: role of presenilins. *J. Alzheimers Dis.* **6**, 123–128.
- Porter NM, Thibault O, Thibault V, Chen KC, Landfield PW (1997) Calcium channel density and hippocampal cell death with age in long-term culture. *J. Neurosci.* **17**, 5629–5639.
- Potier B, Rascol O, Jazat F, Lamour Y, Dutar P (1992) Alterations in the properties of hippocampal pyramidal neurons in the aged rat. *Neuroscience* **48**, 793–806.
- Price DL, Sisodia SS (1994) Cellular and molecular biology of Alzheimer's disease and animal models. *Annu. Rev. Med.* **45**, 435–446.
- Ramsden M, Henderson Z, Pearson HA (2002) Modulation of Ca²⁺ channel currents in primary cultures of rat cortical neurons by amyloid beta protein (1–40) is dependent on solubility status. *Brain Res.* **956**, 254–261.
- Randall RD, Thayer SA (1992) Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. *J. Neurosci.* **12**, 1882–1895.
- Regan RF, Choi DW (1991) Glutamate neurotoxicity in spinal cord cell culture. *Neuroscience* **43**, 585–591.
- Reyes M, Stanton PK (1996) Induction of hippocampal long-term depression requires release of Ca²⁺ from separate presynaptic and postsynaptic intracellular stores. *J. Neurosci.* **16**, 5951–5960.
- Rossi AM, Taylor CW (2004) Ca²⁺ regulation of inositol 1,4,5-trisphosphate receptors: can Ca²⁺ function without calmodulin? *Mol. Pharmacol.* **66**, 199–203.
- Rothman SM, Olney JW (1986) Glutamate and the pathophysiology of hypoxic – ischemic brain damage. *Ann. Neurol.* **19**, 105–111.
- Sawada M, Ichinose M, Maeno T (1987) Ionic mechanism of the outward current induced by intracellular injection of inositol trisphosphate into Aplysia neurons. *J. Neurosci.* **7**, 1470–1483.
- Schneider I, Reverse D, Dewachter I, Ris L, Caluwaerts N, Kuiperi C, Gillis M, Geerts H, Kretschmar H, Godaux E, Moechars D, Van Leuven F, Herms J (2001) Mutant presenilins disturb neuronal calcium homeostasis in the brain of transgenic mice, decreasing the threshold for excitotoxicity and facilitating long-term potentiation. *J. Biol. Chem.* **276**, 11539–11544.
- Segal M, Manor D (1992) Confocal microscopic imaging of [Ca²⁺]_i in cultured rat hippocampal neurons following exposure to N-methyl-D-aspartate. *J. Physiol.* **448**, 655–676.
- Selkoe DJ (1998) The cell biology of β -amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol.* **8**, 447–453.
- Shah M, Haylett DG (2000) Ca(2+) channels involved in the generation of the slow afterhyperpolarization in cultured rat hippocampal pyramidal neurons. *J. Neurophysiol.* **83**, 2554–2561.
- Shankar S, Teyler TJ, Robbins N (1998) Aging differentially alters forms of long-term potentiation in rat hippocampal area CA1. *J. Neurophysiol.* **79**, 334–341.
- Smith IF, Boyle JP, Vaughan PF, Pearson HA, Cowburn RF, Peers CS (2002) Ca(2+) stores and capacitative Ca(2+) entry in human neuroblastoma (SH-SY5Y) cells expressing a familial Alzheimer's disease presenilin-1 mutation. *Brain Res.* **949**, 105–111.
- Smith IF, Hitt B, Green KN, Oddo S, LaFerla FM (2005) Enhanced caffeine-induced Ca²⁺ release in the 3 \times Tg-AD mouse model of Alzheimer's disease. *J. Neurochem.* **94**, 1711–1718.
- Solovyova N, Verkhratsky A (2002) Monitoring of free calcium in the neuronal endoplasmic reticulum: an overview of modern approaches. *J. Neurosci. Methods* **122**, 1–12.
- Solovyova N, Veselovsky N, Toescu EC, Verkhratsky A (2002) Ca(2+) dynamics in the lumen of the endoplasmic reticulum in sensory neurons: direct visualization of Ca(2+)-induced Ca(2+) release triggered by physiological Ca(2+) entry. *EMBO J.* **21**, 622–630.
- Stutzmann GE (2005) Calcium dysregulation, IP3 signaling, and Alzheimer's disease. *Neuroscientist* **11**, 110–115.
- Stutzmann GE, Smith I, Caccamo A, Oddo S, Laferla FM, Parker I (2006) Enhanced ryanodine receptor recruitment contributes to Ca²⁺ disruptions in young, adult, and aged Alzheimer's disease mice. *J. Neurosci.* **26**, 5180–5189.
- Sukhareva M, Smith SV, Maric D, Barker JL (2002) Functional properties of ryanodine receptors in hippocampal neurons change during early differentiation in culture. *J. Neurophysiol.* **88**, 1077–1087.
- Takehima H, Nishimura S, Matsumoto T, Ishida H, Kangawa K, Minamino N, Matsuo H, Ueda M, Hanaoka M, Hirose T, Numa S (1989) Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor. *Nature* **339**, 439–445.
- Tanzi RE, Kovacs DM, Kim TW, Moir RD, Guenette SY, Wasco W (1996) The gene defects responsible for familial Alzheimer's disease. *Neurobiol. Dis.* **3**, 159–168.
- Taylor CP, Weber ML, Gaughan CL, Lehning EJ, LoPachin RM (1999) Oxygen/glucose deprivation in hippocampal slices: altered intraneuronal elemental composition predicts structural and functional damage. *J. Neurosci.* **19**, 619–629.
- Thastrup O, Cullen PJ, Drobak BK, Hanley MR, Dawson AP (1990) Thapsigargin, a tumor promoter, discharges intracellular Ca²⁺ stores by specific inhibition of the endoplasmic reticulum Ca²⁺-ATPase. *Proc. Natl Acad. Sci. USA* **87**, 2466–2470.
- Thibault O, Hadley R, Landfield PW (2001) Elevated postsynaptic [Ca²⁺]_i and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity. *J. Neurosci.* **21**, 9744–9756.
- Thibault O, Landfield PW (1996) Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* **272**, 1017–1020.
- Thibault O, Porter NM, Chen KC, Blalock EM, Kaminker PG, Clodfelter GV, Brewer LD, Landfield PW (1998) Calcium dysregulation in neuronal aging and Alzheimer's disease: history and new directions. *Cell Calcium* **24**, 417–433.
- Toescu EC (1998) Apoptosis and cell death in neuronal cells: where does Ca²⁺ fit in? *Cell Calcium* **24**, 387–403.
- Toescu EC, Verkhratsky A (2000) Neuronal ageing in long-term cultures: alterations of Ca²⁺ homeostasis. *Neuroreport* **11**, 3725–3729.
- Toescu EC, Verkhratsky A (2003) Neuronal ageing from an intraneuronal perspective: roles of endoplasmic reticulum and mitochondria. *Cell Calcium* **34**, 311–323.
- Tsukioka M, Iino M, Endo M (1994) pH dependence of inositol 1,4,5-trisphosphate-induced Ca²⁺ release in permeabilized smooth muscle cells of the guinea-pig. *J. Physiol.* **475**, 369–375.
- Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee SF, Hao YH, Serneels L, De Strooper B, Yu G, Bezprozvanny I (2006) Presenilins form ER Ca²⁺ leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* **126**, 981–993.
- Tu JC, Xiao B, Yuan JP, Lanahan AA, Loeffert K, Li M, Linden DJ, Worley PF (1998) Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. *Neuron* **21**, 717–726.
- Tymianski M, Tator CH (1996) Normal and abnormal calcium homeostasis in neurons: a basis for the pathophysiology of traumatic and ischemic central nervous system injury. *Neurosurgery* **38**, 1176–1195.
- Ueda K, Shinohara S, Yagami T, Asakura K, Kawasaki K (1997) Amyloid β protein potentiates Ca²⁺ influx through L-type voltage-sensitive Ca²⁺ channels: a possible involvement of free radicals. *J. Neurochem.* **68**, 265–271.
- Vanterpool CK, Vanterpool EA, Pearce WJ, Buchholz JN (2006) Advancing age alters the expression of the ryanodine receptor 3

- isoform in adult rat superior cervical ganglia. *J. Appl. Physiol.* **101**, 392–400.
- Verkhatsky A (2004) Endoplasmic reticulum calcium signaling in nerve cells. *Biol. Res.* **37**, 693–699.
- Verkhatsky A (2005) Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol. Rev.* **85**, 201–279.
- Verkhatsky A, Orkand RK, Kettenmann H (1998) Glial calcium: homeostasis and signaling function. *Physiol. Rev.* **78**, 99–141.
- Verkhatsky A, Petersen OH (2002) The endoplasmic reticulum as an integrating signalling organelle: from neuronal signalling to neuronal death. *Eur. J. Pharmacol.* **447**, 141–154.
- Verkhatsky A, Shmigol A, Kirischuk S, Pronchuk N, Kostyuk P (1994) Age-dependent changes in calcium currents and calcium homeostasis in mammalian neurons. *Ann. N. Y. Acad. Sci.* **747**, 365–381.
- Verkhatsky A, Toescu EC (2003) Endoplasmic reticulum Ca(2+) homeostasis and neuronal death. *J. Cell. Mol. Med.* **7**, 351–361.
- Verma A, Hirsch DJ, Snyder SH (1992) Calcium pools mobilized by calcium or inositol 1,4,5-trisphosphate are differentially localized in rat heart and brain. *Mol. Biol. Cell.* **3**, 621–631.
- Wahl P, Schousboe A, Honore T, Drejer J (1989) Glutamate-induced increase in intracellular Ca²⁺ in cerebral cortex neurons is transient in immature cells but permanent in mature cells. *J. Neurochem.* **53**, 1316–1319.
- Wang JH, Kelly PT (1997) Attenuation of paired-pulse facilitation associated with synaptic potentiation mediated by postsynaptic mechanisms. *J. Neurophysiol.* **78**, 2707–2716.
- Wang SQ, Song LS, Lakatta EG, Cheng H (2001) Ca²⁺ signalling between single L-type Ca²⁺ channels and ryanodine receptors in heart cells. *Nature* **410**, 592–596.
- Webster NJ, Ramsden M, Boyle JP, Pearson HA, Peers C (2006) Amyloid peptides mediate hypoxic increase of L-type Ca²⁺ channels in central neurones. *Neurobiol. Aging* **27**, 439–445.
- Weiss JH, Pike CJ, Cotman CW (1994) Ca²⁺ channel blockers attenuate β -amyloid peptide toxicity to cortical neurons in culture. *J. Neurochem.* **62**, 372–375.
- Xiong J, Verkhatsky A, Toescu EC (2002) Changes in mitochondrial status associated with altered Ca²⁺ homeostasis in aged cerebellar granule neurons in brain slices. *J. Neurosci.* **22**, 10761–10771.
- Yamamoto K, Sakagami Y, Sugiura S, Inokuchi K, Shimohama S, Kato N (2005) Homer 1a enhances spike-induced calcium influx via L-type calcium channels in neocortex pyramidal cells. *Eur. J. Neurosci.* **22**, 1338–1348.
- 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 TW (2000) Presenilin-mediated modulation of capacitative calcium entry. *Neuron* **27**, 561–572.
- Zatti G, Burgo A, Giacomello M, Barbiero L, Ghidoni R, Sinigaglia G, Florean C, Bagnoli S, Binetti G, Sorbi S, Pizzo P, Fasolato C (2006) Presenilin mutations linked to familial Alzheimer's disease reduce endoplasmic reticulum and Golgi apparatus calcium levels. *Cell Calcium* **39**, 539–550.
- Zhang ET, Hansen AJ, Wieloch T, Lauritzen M (1990) Influence of MK-801 on brain extracellular calcium and potassium activities in severe hypoglycemia. *J. Cereb. Blood Flow Metab.* **10**, 136–139.