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Intracellular calcium signalling in Alzheimer's disease

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Abstract

More than two decades ago, dysregulation of the intracellular Ca^{2+} homeostasis was suggested to underlie the development of Alzheimer's disease (AD). This hypothesis was tested in numerous *in vitro* studies, which revealed multiple Ca^{2+} signalling pathways able to contribute to AD pathology. It remained, however, unclear whether these pathways are also activated *in vivo*, in cells involved in signal processing in the living brain. Here we review recent data analysing intracellular Ca^{2+} signalling *in vivo* in the context of previous *in vitro* findings. We particularly focus on the processes taking place in the immediate vicinity of amyloid plaques and on their possible role for AD-mediated brain dysfunction.

Keywords: hyperactivity • plaque vicinity • calcium dyshomeostasis • seizure • *in vivo* calcium imaging • two-photon microscopy

Introduction

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder, characterized by distinct neuropathological lesions. These include extracellular deposits of β amyloid (Aβ) in senile plaques, accumulation of intraneuronal neurofibrillary tangles and profound neuronal death (reviewed in [1–3]). Clinical symptoms include the inability to encode new memories as well as cognitive and behavioural impairments [4]. Most cases of the disease are sporadic, with advancing age being the major risk factor for developing AD. The prevalence of AD rises exponentially with age from approximately 1% at 65 years to 40% after the age of 90 [5–7]. Furthermore, individuals harbouring the ε4 allele of apolipoprotein E have an increased risk for developing sporadic, late-onset AD [8, 9]. A small fraction of AD patients, however, have an inherited autosomal dominant form of the disease. These hereditary AD forms are characterized by an earlier onset and are typically caused by mutations in genes encoding human amyloid precursor protein (APP) or presenilin 1 (PS1) and presenilin 2 (PS2) [1, 3, 10]. The presenilins are the part of the γ-secretase complex involved in the synthesis of Aβ, which is derived from APP by sequential enzymatic cleavage by β-APP cleaving enzyme and γ-secretase complex [10, 11].

Expressed in transgenic mice, APP and presenilins with familial mutations allow various aspects of AD neuropathology to be modelled. The mutant mice develop senile plaques and neurofibrillary tangles, exhibit dysregulation of the intracellular Ca^{2+} homeostasis, brain inflammatory response and memory impairment. However, they do not recapitulate the widespread neuronal loss seen in humans [12].

Accumulation of Aβ plays a crucial role in the genesis of AD [2, 3, 13]. Among the three forms of Aβ ($Aβ_{38}$, $Aβ_{40}$, $Aβ_{42}$), $Aβ_{42}$ seems to be the most important for the pathogenesis of the disease because it more easily aggregates into oligomers and amyloid fibrils [14]. Mounting evidence suggests that the soluble oligomers (presumably dimers and trimers) are the neurotoxic species in AD [13]. Indeed, naturally secreted small Aβ oligomers have been shown to inhibit long-term potentiation (LTP, [15]), the

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electrophysiological correlate of learning and memory [13, 16] and to induce a loss of hippocampal synapses [17–19]. Moreover, similar effects were caused by oligomers extracted from the cerebral cortex of AD patients. In wild-type rodents, human oligomers inhibited LTP, enhanced long-term depression, reduced dendritic spine density and interfered with the memory of a learned behaviour [20]. Interestingly, the soluble Aβ dodecamer (Aβ*56, [21]) also seems to impair memory. Thus, young rats injected intracranially with Aβ*56 purified from the brains of old AD mouse mutants showed a reduced performance in the Morris water maze test (a common test for spatial learning).

As a matter of course, formation of Aβ oligomers is abetted by Aβ accumulation within the brain. Interestingly, mutations associated with inherited forms of AD promote both accumulation and oligomerization of Aβ. Thus, familial APP mutations that flank or occur within the Aβ region alter the amount or aggregation properties of Aβ, whereas mutations within presenilins were found to increase the $A\beta_{42}/A\beta_{40}$ ratio [10, 13]. Another source of Aβ accumulation within the brain is the imbalance between its production and clearance caused by impaired degradation of Aβ (reviewed in [22, 23]). Aβ is cleaved by several proteases, including neprilysin [24, 25], insulin-degrading enzyme [25], endothelin-converting enzyme [26], plasmin [27] and cathepsin B [28]. Neprilysin [25, 29] and cathepsin B [28] overexpression in transgenic mice reduces total Aβ levels and plaque deposition, whereas their pharmacological blockade or genetic ablation increases Aβ load [28, 30, 31]. The activity/expression of neprilysin is down-regulated with aging and at the early stage of AD [28, 32–34], suggesting that decreased activity of Aβ-degrading enzymes may contribute to the sporadic form of the disease.

In this paper we are going to review recent findings regarding the mechanisms of AD. In particular, we will focus on the role of calcium (Ca^{2+}) signalling as well as the regulation of the intracellular Ca^{2+} homeostasis in AD, discussing data obtained in various AD mouse models, both *in vitro* and *in vivo*.

Dysregulation of Ca^{2+} homeostasis in AD

Numerous studies suggest that besides Aβ accumulation, dysregulation of the intracellular Ca^{2+} homeostasis might act as an important progenitor of AD. The Ca^{2+} hypothesis of AD is supported by the fact that many AD-related genes (*e.g.* those encoding APP, PS1 and PS2) are also involved in Ca^{2+} signalling [35]. The role of disturbed Ca^{2+} homeostasis as a proximal cause of brain aging and neurodegenerative processes like AD was postulated by Khachaturian more than 20 years ago [36, 37]. However, it is still debated whether the disturbed intracellular Ca^{2+} homeostasis is the cause or the result of altered Aβ and tau production [38]. Recent data reviewed below suggest a complex mutually potentiating interaction between Aβ accumulation and Ca^{2+} dyshomeostasis.

Aβ accumulation causes Ca^{2+} dyshomeostasis

In vitro studies have revealed several mechanisms by which Aβ can increase intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). In lipid bilayers, for example Aβ causes the formation of cation-selective ion pores through which Ca^{2+} passes into the cytosol [39, 40]. The formation of the pores by Aβ oligomers is enhanced by the presence of phosphatidylserine, one of the earliest signs of apoptosis, on the cell surface [41]. Thus, an already suffering neuron with increased phosphatidylserine on the cell surface might show an enhanced vulnerability towards Aβ oligomers. Interestingly, specific blockage of ion-conducting Aβ-channels with the peptide NA-7 eliminates any signs of Aβ-induced apoptosis in cell culture models [42].

Studies in human neuroblastoma cells have revealed that oligomeric Aβ can non-selectively increase Ca^{2+} permeability of cellular membranes, thus increasing both Ca^{2+} influx from the extracellular space and Ca^{2+} leakage from the intracellular Ca^{2+} stores [43]. Furthermore, Aβ can interact with endogenous Ca^{2+}-permeable channels in the cell membrane, thus increasing NMDA receptor-dependent Ca^{2+} influx [44] or causing free radical-mediated potentiation of Ca^{2+} entry through voltage-gated Ca^{2+} channels [45]. The finding that the blocker of L-type Ca^{2+} channels nimodipine reduces Aβ-mediated cell death [45] has suggested that neuronal death is directly related to Aβ-induced Ca^{2+} influx through voltage-gated Ca^{2+} channels. Other Ca^{2+}-permeable channels, however, are inhibited by Aβ. It blocks, for example presynaptic P/Q-type voltage-gated Ca^{2+} channels [46] as well as Ca^{2+}-permeable α_7-containing nicotinic acetylcholine receptor-channels [47].

Membrane-associated oxidative stress represents another mechanism by which Aβ can impair intracellular Ca^{2+} homeostasis. Reactive oxygen species (H_2O_2 and hydroxyl radicals), generated during formation of Aβ oligomers, could attack cell membranes and initiate lipoperoxidation [48]. Membrane lipid peroxidation results in the generation of neurotoxic lipid aldehydes (such as 4-hydroxynonenal), which impair the function of membrane proteins involved in ion transport. The latter include ATPases (*e.g.* Na^+/K^+-ATPase and Ca^{2+}-ATPase) and glutamate and glucose transporters [49, 50]. The Aβ-mediated impairment of ion-motive ATPases was observed in both primary neuron cultures and synaptosomes from adult postmortem hippocampus [49]. It results in membrane depolarization and opening of NMDA receptor-channels as well as voltage-gated Ca^{2+} channels (see above), while impaired Ca^{2+}-ATPase activity reduces the ability of the cell to extrude Ca^{2+} [49]. Impairment of glutamate transport results in increased extracellular glutamate and overstimulation of glutamate receptors, whereas the impairment of glucose transport causes ATP depletion and decreased activity of ion-motive ATPases [50]. Both processes might further increase intracellular Ca^{2+} levels (reviewed in [51]).

Not only Aβ itself but also other products of APP metabolism may affect the intracellular Ca^{2+} homeostasis. During APP processing, the amyloidogenic carboxy-terminal fragment is cleaved by γ-secretase, liberating the APP intracellular domain (AICD). AICD, too, was shown to influence intracellular Ca^{2+} signalling [52]. In mouse embryonic fibroblasts, inositol-triphosphate (IP_3)-mediated Ca^{2+} release from the intracellular Ca^{2+} stores was significantly reduced both in the absence of presenilins ($PS1^{-/-}PS2^{-/-}$ mice, γ-secretase inactive) and in the absence of APP ($APP^{-/-}$ mice). Importantly, this functional deficit was rescued only by cotransfecting fibroblasts with cDNA encoding either AICD itself, or AICD-containing parts of APP. Based on the finding that AICD can form a transcriptionally active complex [53], the authors suggested that AICD may affect Ca^{2+} signalling by regulating the expression of genes involved in Ca^{2+} homeostasis. This latter statement, however, caused controversial discussions within the AD community (for details see [54, 55]). On the other hand, the activation of the non-amyloidogenic secretory pathway (*e.g.* cutting APP by α-secretase) results in the generation of sAPPα. This protein is neuroprotective because it activates K^+ channels *via* cGMP, thus causing membrane hyperpolarization and reducing Ca^{2+} influx [56, 57].

Ca^{2+} dyshomeostasis increases Aβ production

Several *in vitro* studies have shown that increased intracellular Ca^{2+} levels can trigger Aβ formation and aggregation to protofibrils, implicating Ca^{2+} dyshomeostasis as a possible causal factor in sporadic forms of AD (reviewed in [38]).

Initial evidence that APP processing is regulated by intracellular Ca^{2+} came from studies in non-neural cells (HEK-293) overexpressing human APP [58, 59]. These early studies have shown that a global increase in the cytosolic free Ca^{2+} concentration caused by Ca^{2+} ionophore A23187 enhances Aβ production. Aβ levels were also increased when stimulating ryanodine receptors (Ca^{2+} release channels of endoplasmic reticulum [ER]) by caffeine [59]. Thus, Ca^{2+} release from intracellular Ca^{2+} stores can also contribute to the genesis of Aβ. Thapsigargin, a compound that inhibits uptake of Ca^{2+} into ER by sarco-/endoplasmic reticulum calcium ATPases (SERCAs), thereby causing an increase in $[Ca^{2+}]_i$, augmented the caffeine-stimulated release of Aβ [59]. In CHO cells overexpressing human APP, Buxbaum *et al.* [60] observed a more complex effect of thapsigargin: the formation of Aβ was stimulated at lower concentrations (10 nM) only and was inhibited at higher concentrations (20 nM). Later studies on neuronal cell cultures supported the finding that Ca^{2+} dyshomeostasis can influence APP processing. A depolarization-induced increase in $[Ca^{2+}]_i$, for example specifically induced the production of large amounts of intraneuronal Aβ_{42}, causing neuronal death [61]. In contrast to non-neural cells [59], however, it was found that Ca^{2+} release from the intracellular Ca^{2+} stores was not sufficient to induce generation of Aβ in neurons [61].

Very recently, Dreses-Werringloer *et al.* [62] identified a novel transmembrane glycoprotein with Ca^{2+} channel properties, which they called calcium homeostasis modulator 1 (*CALHM1*). Surprisingly, Ca^{2+} influx *via* CALHM1 decreased the total amount of extracellular Aβ. On the contrary, the *CALHM1* polymorphism P86L increases Aβ levels by interfering with CALHM1-mediated Ca^{2+} permeability. These results are in contrast to the previous studies, which have shown that increased transmembrane Ca^{2+} influx enhances Aβ production (see above). The authors have also suggested that the *CALHM1* polymorphism P86L is associated with an increased risk for late-onset AD [62]. This observation, however, was not confirmed in several recent studies analysing the potential association between AD risk and *CALHM1* polymorphism in independent datasets of AD patients and control individuals [63, 64]. Thus, the role of *CALHM1* as a risk factor in AD remains unclear.

Taken together, the *in vitro* data strongly suggest that Aβ accumulation causes Ca^{2+} dyshomeostasis and, *vice versa*, Ca^{2+} dysregulation causes Aβ overproduction. Furthermore, increases in $[Ca^{2+}]_i$ may trigger Ca^{2+}-activated kinases, which mediate the phosphorylation of tau [65, 66] thereby facilitating the development of neurofibrillary tangles. These neuropathological changes may worsen disease symptoms and ultimately may lead to neuronal death. The 'chicken or the egg' conundrum (*i.e.* whether Ca^{2+} acts upstream or downstream of Aβ), however, is very difficult to resolve, since altered Ca^{2+} homeostasis affects the metabolism of the AD-related pathological proteins (Aβ and tau) and, conversely, the accumulation of these proteins further disturbs the Ca^{2+} metabolism.

Presenilins and Ca^{2+} homeostasis

Presenilins play a double role in the pathogenesis of AD. First, presenilins are the part of the γ-secretase complex, which generates Aβ through APP cleavage (see above). Familial AD-linked presenilin mutations were shown to elevate the concentration of the aggregation-prone form of Aβ (Aβ_{42}) in expense of Aβ_{40} (reviewed in [10]). As a consequence, the presenilin-mediated elevation of the Aβ_{42}/Aβ_{40}-ratio activates Aβ-dependent mechanisms of Ca^{2+} dyshomeostasis (see section 'Aβ accumulation causes Ca^{2+} dyshomeostasis'). Secondly, presenilins can directly alter intracellular Ca^{2+} homeostasis. They interact with three key components of the Ca^{2+} signalling cascade: IP_3 receptors (reviewed in [67, 68]), ryanodine receptors [69–71] and SERCA pumps (Fig. 1, [72]). Already early studies in fibroblasts from AD patients [73, 74] have revealed the altered properties of IP_3 receptor-mediated Ca^{2+} release from the intracellular Ca^{2+} stores. Subsequently, potentiation of IP_3-mediated Ca^{2+} signals by presenilin mutations has been documented in different experimental systems ranging from *Xenopus* oocytes to cells from transgenic animals [75, 76]. While the above studies have suggested that exaggerated $[Ca^{2+}]_i$ responses in cells expressing mutant presenilins are caused by overfilling of intracellular Ca^{2+} stores (discussed in [35, 67]), Cheung *et al.* [68] recently discovered a mechanism that can account for potentiated IP_3-mediated Ca^{2+} signalling in the

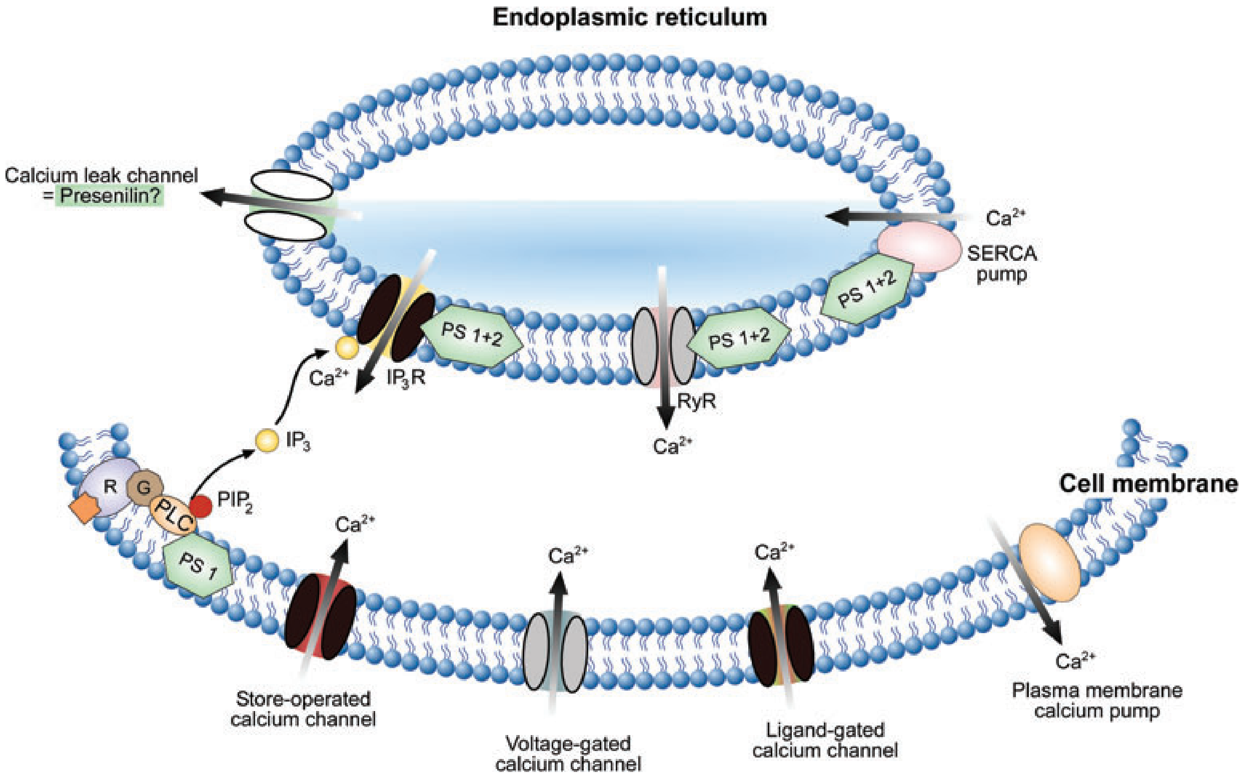

Fig. 1 Presenilin-mediated regulation of the intracellular Ca^{2+} homeostasis. A scheme illustrating the mechanisms underlying intracellular regulation of $[Ca^{2+}]_i$. Ca^{2+} ions enter the cytosol through ligand-gated, voltage-gated or store-operated Ca^{2+} channels in the cell membrane. In addition, they are released from the intracellular Ca^{2+} stores of the ER *via* IP_3 receptor channels (IP_3R) or ryanodine receptor-channels (RyR). IP_3 is produced from phosphatidylinositol-4,5-bisphosphate (PIP_2) by PLC in response to the activation of metabotropic receptors (R). Intracellular Ca^{2+} levels (30–100 nM at rest, [130]) are controlled by plasma membrane Ca^{2+} pumps and by SERCAs. Presenilins are located both within the plasma membrane and the ER membrane and interact with many important elements of the Ca^{2+} signalling cascade (as indicated). Familiar AD mutations in presenilins (*i*) potentiate PLC activity, (*ii*) increase Ca^{2+} release through both IP_3 and ryanodine receptors and (*iii*) modulate activity of SERCA pumps. In addition, presenilins may function as Ca^{2+} leak channels of the ER (indicated). AD-related mutations in presenilins have been shown to render these leak channels non-functional, thereby causing an overload of the ER Ca^{2+} stores.

absence of elevated Ca^{2+} within the ER. The authors have shown that presenilins can physically interact with the IP_3 receptor-channel and thereby stimulate its gating activity (Fig. 1). Expression of two different presenilins with familial mutations (PS1 (M146L) and PS2 (N141I)) sensitized the IP_3 receptor-channel to IP_3 and enhanced IP_3-mediated Ca^{2+} release from the intracellular Ca^{2+} stores. In addition to potentiating agonist-induced Ca^{2+} transients, this sensitization enabled Ca^{2+} release even at very low resting concentrations of IP_3, causing continuous IP_3 receptor-mediated 'leak' of Ca^{2+} from the ER.

As shown by studies in human SH-SY5Y neuroblastoma cells, presenilins may also regulate IP_3 production by influencing basal activity of the IP_3-producing enzyme phospholipase C (PLC) (Fig. 1, [77]). Familial PS1 mutations (PS1-ΔE9 and PS1 M146V) enhance PLC activity, thereby increasing IP_3 levels within the cell which, in turn, mediate enhanced Ca^{2+} release from the intracellular Ca^{2+} stores [77, 78].

In addition to interaction with the IP_3 receptor-mediated signalling, PS1 and PS2 were shown to interact with the ryanodine receptor at the cytoplasmic side of the ER membrane. For example, they increase Ca^{2+} flux through brain-type ryanodine receptor-channels incorporated into lipid bilayers (Fig. 1, [70, 71]). As documented in a recent study [79], this interaction seems to be important for the regulation of neurotransmitter release in the hippocampus. Using a genetic approach for selective inactivation of presenilins both in presynaptic and in postsynaptic neurons of the Schaeffer-collateral pathway, Zhang *et al.* demonstrated that presynaptic inactivation of presenilins impairs synaptic glutamate release and LTP. The underlying mechanism involves ryanodine receptor-mediated Ca^{2+}-induced Ca^{2+} release from the ER. Presenilins seem to control this Ca^{2+} release, thereby modulating the release probability of neurotransmitters.

Along with Ca^{2+} release channels, Ca^{2+} pumps are the key component of the Ca^{2+} regulatory system. Since many presenilin mutations lead to enhanced filling of Ca^{2+} stores, presenilins might also regulate Ca^{2+} pumps. Indeed, using gain-of-function and loss-of-function approaches in both mammalian cell culture and *Xenopus* oocyte models, Green *et al.* [72] have demonstrated that presenilins physically associate with SERCA pumps and are necessary for their proper function (Fig. 1). Furthermore, modulating SERCA activity in CHO cells altered the amount of Aβ produced by these cells [72]. These data suggest that dysregulation of SERCA pumps (caused by AD-relevant presenilin mutations) may contribute to the pathogenesis of AD.

Presenilins not only modulate the function of the other Ca^{2+} regulating proteins, but also seem to form Ca^{2+}-permeable ion channels themselves (Fig. 1). In a recent study, Tu *et al.* [80] report that in planar lipid bilayers, wild-type presenilins form low-conductance Ca^{2+}-permeable channels, which account for ~80% of passive Ca^{2+} leak from the ER. Notably, this ability of presenilins is disrupted by two AD-relevant presenilin mutations, PS1-M146V and PS2-N141I [80]. These results were confirmed by a subsequent study demonstrating that out of 6 familial AD mutations tested, five mutations abolished Ca^{2+} leak function of presenilins [81]. The consequence of an impaired Ca^{2+} leak from the ER would be again an overfilling of the intracellular Ca^{2+} stores [82] as well as deficits in capacitative calcium entry [83, 84].

It has to be mentioned that ER is not the only Ca^{2+} store within the cell. Intracellular Ca^{2+} is also buffered by mitochondria (for review see [85]). However, mitochondrial dysfunction in AD seems to be linked to Aβ rather than to presenilins and has recently been discussed in several review articles (see, *e.g.* [86, 87]). We would like to refer the reader to those papers for further details on this issue.

Taken together, *in vitro* data suggest that presenilins control the functional state of the intracellular Ca^{2+} stores through interaction with both SERCAs and multiple Ca^{2+} release mechanisms. These complex interactions might explain why presenilin mutations have such widespread effects on intracellular Ca^{2+} signalling. However, it remains unclear to which extent these processes also occur *in vivo*, in the intact brain tissue.

Dysregulation of Ca^{2+} homeostasis *in vivo*

Recently, three *in vivo* studies have investigated Ca^{2+} dynamics in the brains of AD mouse mutants. Using an adenoviral-based expression of the genetically encoded Ca^{2+} indicator Yellow Cameleon 3.6, Kuchibhotla *et al.* [88] observed increased resting intracellular Ca^{2+} levels in dendrites and dendritic spines of APP_{Swe}/PS1-ΔE9 (APP/PS1) transgenic mice. This 'Ca^{2+} overload' was extremely evident in close proximity to amyloid plaques. Within 25 μm of a plaque, more than 30% of all neurites exhibited strongly elevated intracellular Ca^{2+} levels (yellow-red neurites in Fig. 2). Interestingly, the Ca^{2+} overload observed in APP/PS1 mice was associated with morphological neuritic alterations. These were mediated, at least in part, by activation of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin [88]. These data support previous studies from the Bacskai/Hymans group [89] identifying plaque vicinity as a noxious factor in AD. However, the exact mechanism by which amyloid plaques affect $[Ca^{2+}]_i$ in spines and neurites remains unclear.

Notably, in the absence of human APP mutations, mutant PS1 transgenic mice (PS1- ΔE9 or PS1M146V) did not exhibit any neuritic Ca^{2+} overload, suggesting that these mutations alone are not sufficient to induce Ca^{2+} overload *in vivo* [88]. This result is surprising in view of several *in vitro* studies pointing to a key role of presenilins in Ca^{2+} dyshomeostasis (see above).

Consistent with data from Kuchibhotla *et al.* [88], our Ca^{2+} imaging study on double-transgenic mice overexpressing APP_{Swe} and mutant PS1 (G384A) revealed a profound functional impairment in 50% of layer 2/3 neurons [90]. Almost half of these neurons (21% of the total population) were 'hyperactive', *i.e.* displayed increased frequencies of spontaneous Ca^{2+} transients. The other 29% of cells were 'silent', showing no Ca^{2+} transients over a 6-min.-long recording period (Fig. 2). Interestingly, the hyperactive cells were found only in close proximity to plaques ($<$60 μm from the plaque border; yellow-red neurons in Fig. 2). In contrast to our initial expectation that Ca^{2+} transients in hyperactive cells would result from spontaneous Ca^{2+} release from overfilled intracellular Ca^{2+} stores (see above), these Ca^{2+} transients were tetrodotoxin-sensitive and thus caused by action potential firing. Further analyses have shown that these Ca^{2+} transients are of synaptic origin because they are completely and reversibly blocked by the glutamate receptor blockers CNQX (6-cyano-5-nitroquinoxaline-2,3-dione) and APV (D,L-2-amino-5-phosphonovaleric acid). It remains, however, to be established whether and to what extent the increased frequency of Ca^{2+} transients in hyperactive cells causes the Ca^{2+} overload observed by Kuchibhotla *et al.* [88].

In the latest *in vivo* Ca^{2+} imaging study, Bacskai and colleagues quantitatively determined resting $[Ca^{2+}]_i$ in astrocytes of APP/PS1 mice and observed a global astrocytic response to plaque deposition [91]. Compared to wild-type mice, $[Ca^{2+}]_i$ was globally increased in the astrocytic network of 6- to 8-month-old mutant mice (yellow-green astrocytes in Fig. 2). Furthermore, astrocytes in mutant mice exhibited a significant increase in spontaneous activity (see also [92]). Sometimes this activity was correlated over long distances (up to 200 μm), thus forming an intercellular Ca^{2+} wave. While the increase in $[Ca^{2+}]_i$ and in the frequency of astrocytic Ca^{2+} transients was independent of plaque proximity, the pacemaker-astrocytes initiating the waves (yellow-red astrocytes in Fig. 2) were located 24.8 ± 7.8 μm from the three-dimensionally nearest amyloid plaque. These data suggest that plaques or plaque-associated bioactive species trigger these Ca^{2+} waves. Notably, increased astrocyte activity was not affected by blocking neuronal activity with tetrodotoxin. Therefore, increased astrocytic activity is not a simple reflection of neuronal hyperactivity in the plaque vicinity.

Taken together, these studies reveal a complex and widespread pattern of dysregulation of the intracellular Ca^{2+} homeostasis

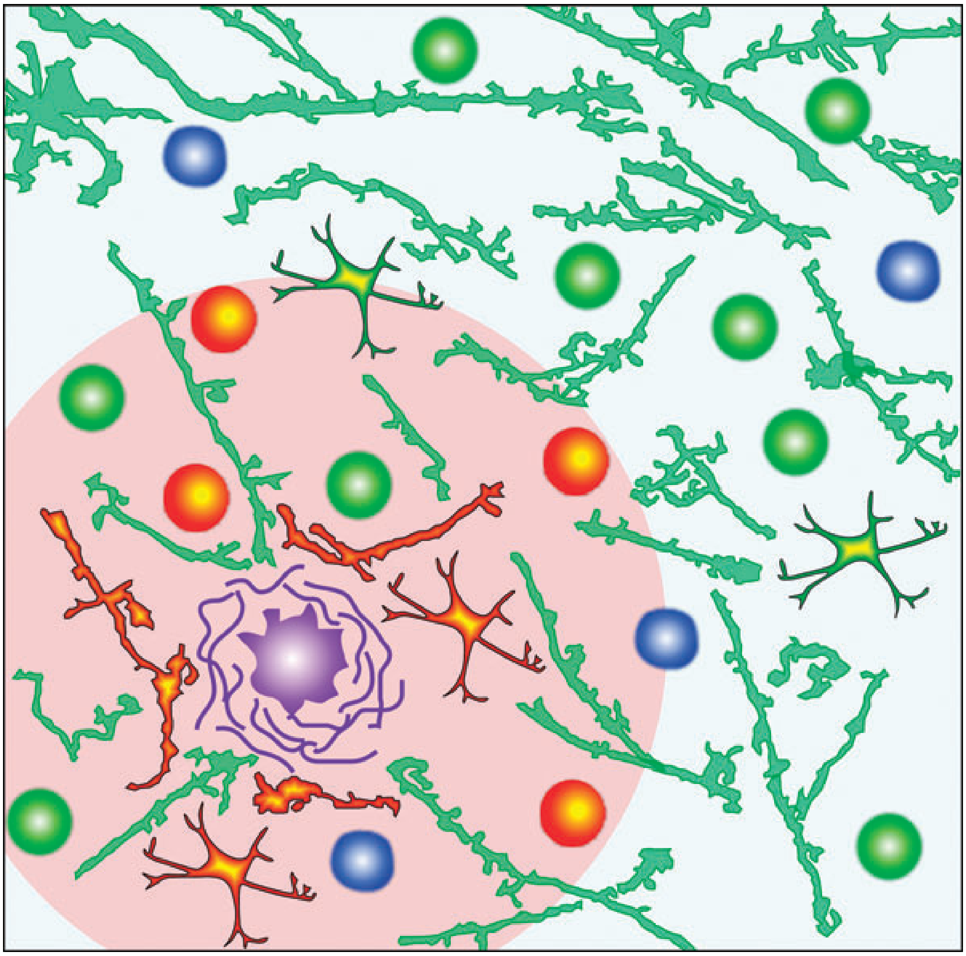

Fig. 2 Pathological changes in brain parenchyma in the vicinity of amyloid plaques. Schematic drawing illustrating an amyloid plaque (purple) surrounded by neurons (circles), dendrites (branches) and astrocytes (branched cells). Three different types of neurons are illustrated: normally active neurons (white-green), hyperactive neurons (yellow-red) and silent neurons (white-blue; [90]). Dendritic branches with an increased intracellular Ca^{2+} concentration are coloured yellow-red while the ones with normal resting $[Ca^{2+}]_i$ appear green [88]. The pacemaker astrocytes initiating propagating $[Ca^{2+}]_i$ waves are shown in yellow-red and the rest of the astrocytes (having an increased resting $[Ca^{2+}]_i$) are shown in yellow-green [91]. Note that most of the changes in the intracellular Ca^{2+} homeostasis are restricted to the immediate plaque vicinity (an area situated less than 60 μm from the plaque border, coloured in pink).

in vivo, involving several cellular/synaptic mechanisms in two different cell types. Some of these pathological changes are condensed in the plaque vicinity while others, like those in the astrocytic network, are diffusely distributed all over the brain.

AD-mediated hyperactivity and synaptic network dysfunction

The observation that amyloid plaques *in vivo* are surrounded by hyperactive cells [90] came as a surprise because of the wealth of data showing that AD is associated with synaptic dismantling and 'synaptic failure' [93]. Already early histological studies of brain tissue from AD patients were able to reveal neuronal loss, shrinkage of dendritic trees and a decrease in the density of synapses [94–98]. Further studies in mutant mice have shown that elevated Aβ levels result in spine loss [17, 99–101], reduction in the number of excitatory synapses [102] and depressed glutamatergic synaptic transmission [103, 104] accompanied by glutamate receptor endocytosis [104, 105]. In cultured cortical neurons, application of Aβ induced internalization of NMDA receptors [105], whereas Aβ overexpression in organotypic hippocampal slices promoted AMPA receptor removal followed by the loss of dendritic spines and NMDA responses [104]. In double knock-in mice carrying human mutations in the genes for APP and PS1, only AMPA receptors were down-regulated, while NMDA receptors remained unaffected [106]. In agreement with these data, electrophysiological studies of triple transgenic mice harbouring $PS1_{M146V}$, APP_{Swe} and tau_{P301L} [107] revealed deficits in LTP. The Aβ-mediated LTP blockade was also observed *in vivo* [16]. This elegant study demonstrated that inhibition of hippocampal LTP is attributable specifically to oligomers, not monomers or fibrils, of naturally secreted human Aβ. In view of the cumulative evidence described above, one might expect that accumulation of Aβ over the course of the disease decreases overall activity of neuronal networks.

Surprisingly, electrophysiological studies performed by Palop *et al.* [108] in freely moving hAPP transgenic mice revealed an increase in neuronal activity. By means of prolonged video-EEG monitoring, the authors observed generalized, sharp synchronous discharges in the cortex and in the hippocampus of mutant mice. Interestingly, some of the hippocampal discharges remained focal, with minimal spread to the neocortex [108]. Subsequently, similar epileptiform activity was observed in other AD mouse models, including Tg2576, hAPP/PS1 and APdE9 mice [109, 110].

Our data, obtained in the cortex of APP_{Swe}/PS1 mice, additionally revealed a population of cells with augmented neuronal activity. Compared to control mice, the fraction of such hyperactive neurons in AD-mutants increased 16-fold [90]. Since application of the $GABA_A$ agonist diazepam reduced activity of hyperactive neurons to normal levels, and application of the $GABA_A$ antagonist gabazine was less effective in hyperactive cells compared to normal ones, our results support the hypothesis that hyperactivity is caused, at least in part, by impaired synaptic inhibition. The fact that some neurons become hyperactive and others silent [90] suggests that anatomical remodelling of both excitatory and inhibitory synapses underlies the changes in neuronal activity in AD. This conclusion is supported by the data of Palop *et al.* [108], showing that spontaneous epileptiform activity in cortical and hippocampal networks is accompanied by GABAergic sprouting in the dentate gyrus. Such abnormal sprouting, this time of excitatory entorhinal axons, was also observed by Phinney *et al.* [111] within the hippocampus and the thalamus of APP23 mice [112].

According to recent data, increased synaptic transmission enhances APP endocytosis *in vivo via* clathrin-mediated recycling of synaptic vesicles [113]. This, in turn, increases Aβ generation and its release into the brain interstitial fluid. Thus, neuronal hyperactivity is very likely to result in enhanced Aβ production and increased conversion of soluble Aβ into oligomers and/or plaques. This process may sustain a vicious cycle by further increasing neuronal hyperactivity.

Neuronal hyperactivity: implications for humans

Several studies have shown that AD patients are more susceptible to epileptic seizures than the control population. It is estimated that 7–21% of patients with sporadic AD will develop at least one clinically apparent seizure, which is about 5 to 10 times the risk for non-demented aged people [109, 114]. Patients with the early-onset (familiar) form of the disease, in particular those carrying a mutation in the presenilin genes, have a dramatically higher risk for developing seizures. Snider *et al.* [115], for example, have analysed 18 families with three or more family members exhibiting very early-onset AD (40 years or younger; 106 individuals in total). Out of these 18 families, 12 were analysed with respect to epileptic seizures. The latter were found in 75% of all cases, clearly indicating a close relation between the early-onset AD and seizure activity. Furthermore, the risk of developing seizures is substantially increased in patients with Down syndrome (who have three copies of the APP gene) [116]. Epileptiform activity has also been reported in non-demented carriers of the apolipoprotein E4, a known genetic risk factor of sporadic AD [117].

In many additional cases, neuronal hyperactivity may occur in a milder form (*e.g.* as interictal epileptiform discharges), thus remaining clinically undetected. Indeed, many clinical symptoms of AD, such as amnestic wandering, agitation, disorientation and, in particular, episodic fluctuations in functionality could be explained by sporadic hyperactivity of respective neuronal networks. Consistent with this hypothesis, a recent fMRI study in humans has reported the failure of task-induced inactivation of default network activity and hippocampal hyperactivation in asymptomatic and minimally impaired older individuals [118]. As revealed by simultaneous Pittsburg compound B imaging, this hyperactivity was associated with significant accumulation of fibrillar amyloid β-protein in the examined brain regions.

Taken together, data obtained in AD patients and mutant mice point towards a strong association between neuronal hyperactivity and AD. This suggests that neuronal (and glial) hyperactivity may represent a mechanism causally related to AD-mediated cognitive impairments.

Plaque vicinity

Although accumulation of cerebral amyloid plaques is the major hallmark of AD, the key question – how exactly do amyloid plaques impair brain function in AD patients – has not been answered. The principal argument against amyloid plaques as the primary toxic species stems from studies showing that the number of amyloid plaques does not correlate well with the severity of cognitive impairments. Indeed, several studies identified patients who had no overt symptoms of dementia antemortem, but post-mortem were found to have many plaque deposits [119, 120]. Other studies indicate that the severity of the cognitive decline in AD patients is better correlated with the concentration of soluble Aβ oligomers than with the density of amyloid plaques [121, 122]. *In vivo* studies in mouse models of AD also confirmed that plaque formation is not necessary for learning and memory deficits [123, 124], thus suggesting that soluble Aβ oligomers (and not plaques) are the primary toxic species (reviewed in [2]).

Recent data, however, seem to challenge this hypothesis. Thus, in transgenic mouse models, loss of dendritic spines, shaft atrophy of dendrites and the development of large axonal varicosities were only observed inside and within 15–50 μm of amyloid plaques [99]. Furthermore, repeated two-photon imaging *in vivo* has shown that plaque deposition precedes neuritic deformation. Indeed, dystrophic neurites become visible only 3 to 4 days after the first appearance of a new plaque [89]. Further studies have shown that (*i*) increased resting Ca^{2+} concentration in neurites [88], (*ii*) synaptically driven neuronal hyperactivity [90], (*iii*) initiation of intercellular Ca^{2+} waves in astrocytes [91] and (*iv*) the already mentioned loss of dendritic spines [101] and excitatory synapses [102] are predominantly observed in close proximity to amyloid plaques (Fig. 2). Furthermore, the *in vivo* data in humans mentioned above [118] show a strong correlation between plaque-associated amyloid deposition and dysfunction of neural systems supporting the formation of new memories. Taken together, these recent *in vivo* data establish plaques as a critical mediator of cellular/network pathology in AD.

The mechanisms of this plaque-mediated toxicity remain unclear. On the one hand, the concentration of diffusible Aβ oligomers is increased within and in the immediate vicinity of amyloid plaques (about 6.5 μm from the edge of the plaque, [102]). The oligomer-rich volume is 180% larger than the volume of the dense core of the plaque and corresponds to the area with severe decrease in synapse density. Based on this observation, Koffie *et al.* [102] have suggested that senile plaques act as a potent local reservoir of oligomeric Aβ, which in turn acts as a toxic moiety to synapses in the cortex.

However, Aβ oligomers are not the only potentially toxic species in the plaque vicinity. As revealed by histological studies [125, 126], glial cells, namely activated astrocytes and microglia (the major immunocompetent cells in the brain), cluster around sites of amyloid deposition. These cells produce a wide variety of potentially neurotoxic substances, such as reactive oxygen and nitrogen species, inflammatory cytokines (*e.g.* interleukins, tumour necrosis factor-α and transforming growth factor-β), prostaglandins, complement system proteins and other inflammatory mediators [127]. Each of these factors may, alone or in concert, contribute to cellular/network dysfunction in the plaque vicinity.

Interestingly, a recent *in vivo* study has shown that acute application of lipopolysaccharides (the major toxins of gram-negative bacteria inducing inflammatory response in the central nervous system (for review see [128])) induces aberrant neuronal activity in the rat somatosensory cortex [129]. Notably, lipopolysaccharide-induced pathology manifested itself in a potentiation of somatosensory evoked potentials as well as epileptiform discharges and seizures. This aberrant cortical excitability was prevented by an interleukin-1 receptor antagonist, suggesting that interleukin-1, released by activated microglia, provokes neuronal hyperactivity.

Thus, an amyloid plaque, surrounded by synchronized hyperactive neurons and pacemaker astrocytes, represents a grain of hyperactivity within the brain parenchyma (Fig. 2). Such grains of hyperactivity may trigger a variety of pathological activities, including paradoxically increased fMRI signals [118], interictal epileptiform discharges and epileptic seizures.

Conclusions

Recent high-resolution analyses of cortical function in mouse models of AD revealed a marked dysregulation (mainly potentiation) of intracellular calcium homeostasis *in vivo*. This is reflected in (*i*) increased resting Ca^{2+} levels in neurons and astrocytes and (*ii*) increased frequency of spontaneous Ca^{2+} waves in neighbouring hyperactive neurons and in astrocytic networks. Notably, many of these pathological changes are either restricted to or governed from the immediate vicinity of amyloid plaques. Taken together, these new data identify hyperactive neurons and glia themselves as well as the 'hyperactive' plaque vicinity as important vicious species in AD.

Does the 'hyperactive' plaque vicinity play a major causal role in AD pathology? Cumulative evidence suggests that this may be the case, although more direct experiments are needed to definitively answer this question. The good news, however, is that this question is relatively easy to address because plaque vicinity can be targeted pharmacologically (*e.g.* by the drugs recognizing a fibrillar form of Aβ).

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References

1. **Selkoe DJ.** Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev*. 2001; 81: 741–66.
2. **Haass C, Selkoe DJ.** Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nature Rev Mol Cell Biol*. 2007; 8: 101–12.
3. **Hardy J, Selkoe DJ.** Medicine–The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002; 297: 353–6.
4. **Alzheimer A.** Über eigenartige Krankheitsfälle des späteren Alters. *Z ges Neurol Psychiatr.* 1911; 4: 356–85.
5. **Bachman DL, Wolf PA, Linn R, *et al.*** Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. *Neurology*. 1992; 42: 115–9.
6. **Kukull WA, Bowen JD.** Dementia epidemiology. *Med Clin North Am.* 2002; 86: 573–90.
7. **Evans DA, Funkenstein HH, Albert MS, *et al.*** Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *JAMA*. 1989; 262: 2551–6.
8. **Cedazo-Minguez A.** Apolipoprotein E and Alzheimer's disease: molecular mechanisms and therapeutic opportunities. *J Cell Mol Med.* 2007; 11: 1227–38.
9. **Corder EH, Saunders AM, Strittmatter WJ, *et al.*** Gene dose of apolipoprotein-E type-4 allele and the risk of Alzheimers-disease in late-onset families. *Science*. 1993; 261: 921–3.

10. **Haass C.** Take five–BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *EMBO J.* 2004; 23: 483–8.
11. **Steiner H, Haass C.** Intramembrane proteolysis by presenilins. *Nat Rev Mol Cell Biol.* 2000; 1: 217–24.
12. **Morrissette DA, Parachikova A, Green KN, *et al.*** Relevance of transgenic mouse models to human alzheimer disease. *J Biol Chem.* 2009; 284: 6033–7.
13. **Walsh DM, Selkoe DJ.** A beta oligomers – a decade of discovery. *J Neurochem.* 2007; 101: 1172–84.
14. **Chen YR, Glabe CG.** Distinct early folding and aggregation properties of Alzheimer amyloid-beta peptides A beta 40 and A beta 42 – stable trimer or tetramer formation by A beta 42. *J Biol Chem.* 2006; 281: 24414–22.
15. **Bliss TV, Lomo T.** Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol.* 1973; 232: 331–56.
16. **Walsh DM, Klyubin I, Fadeeva JV, *et al.*** Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature.* 2002; 416: 535–9.
17. **Shankar GM, Bloodgood BL, Townsend M, *et al.*** Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J Neurosci.* 2007; 27: 2866–75.
18. **Lacor PN, Buniel MC, Furlow PW, *et al.*** Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci.* 2007; 27: 796–807.
19. **Lacor PN, Buniel MC, Chang L, *et al.*** Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci.* 2004; 24: 10191–200.
20. **Shankar GM, Li S, Mehta TH, *et al.*** Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med.* 2008; 14: 837–42.
21. **Lesne S, Koh MT, Kotilinek L, *et al.*** A specific amyloid-beta protein assembly in the brain impairs memory. *Nature.* 2006; 440: 352–7.
22. **Selkoe DJ.** Clearing the brain's amyloid cobwebs. *Neuron.* 2001; 32: 177–80.
23. **Tanzi RE, Moir RD, Wagner SL.** Clearance of Alzheimer's Abeta peptide: the many roads to perdition. *Neuron.* 2004; 43: 605–8.
24. **Iwata N, Tsubuki S, Takaki Y, *et al.*** Identification of the major A beta(1–42)-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat Med.* 2000; 6: 143–50.
25. **Leissring MA, Farris W, Chang AY, *et al.*** Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron.* 2003; 40: 1087–93.
26. **Choi DS, Wang D, Yu GQ, *et al.*** PKC epsilon increases endothelin converting enzyme activity and reduces amyloid plaque pathology in transgenic mice. *Proc Natl Acad Sci USA.* 2006; 103: 8215–20.
27. **Tucker HM, Kihiko M, Caldwell JN, *et al.*** The plasmin system is induced by and degrades amyloid-beta aggregates. *J Neurosci.* 2000; 20: 3937–46.
28. **Mueller-Steiner S, Zhou Y, Arai H, *et al.*** Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. *Neuron.* 2006; 51: 703–14.
29. **Meilandt WJ, Cisse M, Ho K, *et al.*** Neprilysin overexpression inhibits plaque formation but fails to reduce pathogenic A beta oligomers and associated cognitive deficits in human amyloid precursor protein transgenic mice. *J Neurosci.* 2009; 29: 1977–86.
30. **Mouri A, Zou LB, Iwata N, *et al.*** Inhibition of neprilysin by thiorphan (i.c.v.) causes an accumulation of amyloid beta and impairment of learning and memory. *Behav Brain Res.* 2006; 168: 83–91.
31. **Huang SM, Mouri A, Kokubo H, *et al.*** Neprilysin-sensitive synapse-associated amyloid-beta peptide oligomers impair neuronal plasticity and cognitive function. *J Biol Chem.* 2006; 281: 17941–51.
32. **Iwata N, Takaki Y, Fukami S, *et al.*** Region-specific reduction of A beta-degrading endopeptidase, neprilysin, in mouse hippocampus upon aging. *J Neurosci Res.* 2002; 70: 493–500.
33. **Caccamo A, Oddo S, Sugarman MC, *et al.*** Age- and region-dependent alterations in A beta-degrading enzymes: implications for A beta-induced disorders. *Neurobiol Aging.* 2005; 26: 645–54.
34. **Hellström-Lindahl E, Ravid R, Nordberg A.** Age-dependent decline of neprilysin in Alzheimer's disease and normal brain: inverse correlation with A beta levels. *Neurobiol Aging.* 2008; 29: 210–21.
35. **LaFerla FM.** Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nature Rev Neurosci.* 2002; 3: 862–72.
36. **Khachaturian ZS.** Calcium, membranes, aging, and Alzheimer's disease. Introduction and overview. *Ann N Y Acad Sci.* 1989; 568: 1–4.
37. **Khachaturian ZS.** Calcium hypothesis of Alzheimer's disease and brain aging. *Ann N Y Acad Sci.* 1994; 747: 1–11.
38. **Green KN, LaFerla FM.** Linking calcium to A beta and Alzheimer's disease. *Neuron.* 2008; 59: 190–4.
39. **Arispe N, Rojas E, Pollard HB.** Alzheimer-disease amyloid beta-protein forms calcium channels in bilayer-membranes–blockade by tromethamine and aluminum. *Proc Natl Acad Sci USA.* 1993; 90: 567–71.
40. **Glabe CG.** Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. *Neurobiol Aging.* 2006; 27: 570–5.
41. **Lee G, Pollard HB, Arispe N.** Annexin 5 and apolipoprotein E2 protect against Alzheimer's amyloid-beta-peptide cytotoxicity by competitive inhibition at a common phosphatidylserine interaction site. *Peptides.* 2002; 23: 1249–63.
42. **Arispe N, Diaz JC, Simakova O.** A beta ion channels. Prospects for treating Alzheimer's disease with A beta channel blockers. *Biochim Biophys Acta.* 2007; 1768: 1952–65.
43. **Demuro A, Mina E, Kayed R, *et al.*** Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem.* 2005; 280: 17294–300.
44. **De Felice FG, Velasco PT, Lambert MP, *et al.*** Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem.* 2007; 282: 11590–601.
45. **Ueda K, Shinohara S, Yagami T, *et al.*** Amyloid beta protein potentiates Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels: a possible involvement of free radicals. *J Neurochem.* 1997; 68: 265–71.
46. **Nimmrich V, Grimm C, Draguhn A, *et al.*** Amyloid beta oligomers (A beta(1–42) globulomer) suppress spontaneous synaptic activity by inhibition of P/Q-type calcium currents. *J Neurosci.* 2008; 28: 788–97.

47. **Liu QS, Kawai H, Berg DK.** beta-Amyloid peptide blocks the response of alpha 7-containing nicotinic receptors on hippocampal neurons. *Proc Natl Acad Sci USA*. 2001; 98: 4734–9.
48. **Hensley K, Carney JM, Mattson MP, *et al.*** A model for beta-amyloid aggregation and neurotoxicity based on free-radical generation by the peptide – relevance to alzheimer-disease. *Proc Natl Acad Sci USA*. 1994; 91: 3270–4.
49. **Mark RJ, Hensley K, Butterfield DA, Mattson MP.** Amyloid beta-peptide impairs ion-motive ATPase activities – evidence for a role in loss of neuronal Ca^{2+} homeostasis and cell-death. *J Neurosci*. 1995; 15: 6239–49.
50. **Mark RJ, Pang Z, Geddes JW, *et al.*** Amyloid beta-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation. *J Neurosci*. 1997; 17: 1046–54.
51. **Bezprozvanny I, Mattson MP.** Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci*. 2008; 31: 454–63.
52. **Leissring MA, Murphy MP, Mead TR, *et al.*** A physiologic signaling role for the gamma -secretase-derived intracellular fragment of APP. *Proc Natl Acad Sci USA*. 2002; 99: 4697–702.
53. **Cao X, Südhof TC.** A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science*. 2001; 293: 115–20.
54. **Hebert SS, Serneels L, Tolia A, *et al.*** Regulated intramembrane proteolysis of amyloid precursor protein and regulation of expression of putative target genes. *EMBO Rep*. 2006; 7: 739–45.
55. **Müller T, Meyer HE, Egensperger R, *et al.*** The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog Neurobiol*. 2008; 85: 393–406.
56. **Mattson MP, Cheng B, Culwell AR, *et al.*** Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron*. 1993; 10: 243–54.
57. **Furukawa K, Barger SW, Blalock EM, *et al.*** Activation of K+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. *Nature*. 1996; 379: 74–8.
58. **Querfurth HW, Selkoe DJ.** Calcium ionophore increases amyloid-beta peptide production by cultured-cells. *Biochemistry*. 1994; 33: 4550–61.
59. **Querfurth HW, Jiang JW, Geiger JD, *et al.*** Caffeine stimulates amyloid beta-peptide release from beta-amyloid precursor protein-transfected HEK293 cells. *J Neurochem*. 1997; 69: 1580–91.
60. **Buxbaum JD, Ruefli AA, Parker CA, *et al.*** Calcium regulates processing of the alzheimer amyloid protein-precursor in a protein-kinase C-independent manner. *Proc Natl Acad Sci USA*. 1994; 91: 4489–93.
61. **Pierrot N, Ghisdal P, Caumont AS, *et al.*** Intraneuronal amyloid-beta 1–42 production triggered by sustained increase of cytosolic calcium concentration induces neuronal death. *J Neurochem*. 2004; 88: 1140–50.
62. **Dreses-Werringloer U, Lambert JC, Vingtdeux V, *et al.*** A polymorphism in CALHM1 influences Ca^{2+} homeostasis, A beta levels, and Alzheimer's disease risk. *Cell*. 2008; 133: 1149–61.
63. **Bertram L, Schjeide BMM, Hooli B, *et al.*** No Association between CALHM1 and Alzheimer's disease risk. *Cell*. 2008; 135: 993–4.
64. **Sleegers K, Brouwers N, Bettens K, *et al.*** No association between CALHM1 and risk for Alzheimer dementia in a Belgian population. *Hum Mutat*. 2009; 30: E570–74.
65. **Avila J, Perez M, Lim F, *et al.*** Tau in neurodegenerative diseases: tau phosphorylation and assembly. *Neurotox Res*. 2004; 6: 477–82.
66. **Stutzmann GE.** The pathogenesis of Alzheimers disease – is it a lifelong "Calciumopathy"? *Neuroscientist*. 2007; 13: 546–59.
67. **Stutzmann GE.** Calcium dysregulation, IP3 signaling, and Alzheimer's disease. *Neuroscientist*. 2005; 11: 110–5.
68. **Cheung KH, Shineman D, Muller M, *et al.*** Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron*. 2008; 58: 871–83.
69. **Stutzmann GE, Smith I, Caccamo A, *et al.*** Enhanced ryanodine receptor recruitment contributes to Ca^{2+} disruptions in young, adult, and aged Alzheimer's disease mice. *J Neurosci*. 2006; 26: 5180–9.
70. **Rybalchenko V, Hwang SY, Rybalchenko N, *et al.*** The cytosolic N-terminus of presenilin-1 potentiates mouse ryanodine receptor single channel activity. *Int J Biochem Cell Biol*. 2008; 40: 84–97.
71. **Hayrapetyan V, Rybalchenko V, Rybalchenko N, *et al.*** The N-terminus of presenilin-2 increases single channel activity of brain ryanodine receptors through direct protein-protein interaction. *Cell Calcium*. 2008; 44: 507–18.
72. **Green KN, Demuro A, Akbari Y, *et al.*** SERCA pump activity is physiologically regulated by presenilin and regulates amyloid beta production. *J Cell Biol*. 2008; 181: 1107–16.
73. **Ito E, Oka K, Etcheberrigaray R, *et al.*** Internal Ca^{2+} mobilization is altered in fibroblasts from patients with Alzheimer disease. *Proc Natl Acad Sci USA*. 1994; 91: 534–8.
74. **Etcheberrigaray R, Hirashima N, Nee L, *et al.*** Calcium responses in fibroblasts from asymptomatic members of Alzheimer's disease families. *Neurobiol Dis*. 1998; 5: 37–45.
75. **Guo Q, Fu W, Sopher BL, *et al.*** Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nat Med*. 1999; 5: 101–6.
76. **Leissring MA, Paul BA, Parker I, *et al.*** Alzheimer's presenilin-1 mutation potentiates inositol 1,4,5-trisphosphate-mediated calcium signaling in *Xenopus oocytes*. *J Neurochem*. 1999; 72: 1061–8.
77. **Cedazo-Minguez A, Popescu BO, Ankarcrona M, *et al.*** The presenilin 1 deltaE9 mutation gives enhanced basal phospholipase C activity and a resultant increase in intracellular calcium concentrations. *J Biol Chem*. 2002; 277: 36646–55.
78. **Popescu BO, Cedazo-Minguez A, Benedikz E, *et al.*** Gamma-secretase activity of presenilin 1 regulates acetylcholine muscarinic receptor-mediated signal transduction. *J Biol Chem*. 2004; 279: 6455–64.
79. **Zhang C, Wu B, Beglopoulos V, *et al.*** Presenilins are essential for regulating neurotransmitter release. *Nature*. 2009; 460: 632–6.
80. **Tu H, Nelson O, Bezprozvanny A, *et al.*** Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell*. 2006; 126: 981–93.
81. **Nelson O, Tu H, Lei T, *et al.*** Familial Alzheimer disease-linked mutations specifically disrupt Ca^{2+} leak function of presenilin 1. *J Clin Invest*. 2007; 117: 1230–9.
82. **Garaschuk O, Yaari Y, Konnerth A.** Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurones. *J Physiol*. 1997; 502: 13–30.

83. **Parekh AB, Putney JW Jr.** Store-operated calcium channels. *Physiol Rev*. 2005; 85: 757–810.
84. **Parekh AB, Penner R.** Store depletion and calcium influx. *Physiol Rev*. 1997; 77: 901–30.
85. **Parekh AB.** Mitochondrial regulation of intracellular Ca^{2+} signaling: more than just simple Ca^{2+} buffers. *News Physiol Sci*. 2003; 18: 252–6.
86. **Celsi F, Pizzo P, Brini M, *et al.*** Mitochondria, calcium and cell death: a deadly triad in neurodegeneration. *Biochim Biophys Acta*. 2009; 1787: 335–44.
87. **Starkov AA, Beal FM.** Portal to Alzheimer's disease. *Nat Med*. 2008; 14: 1020–1.
88. **Kuchibhotla KV, Goldman ST, Lattarulo CR, *et al.*** Abeta plaques lead to aberrant regulation of calcium homeostasis *in vivo* resulting in structural and functional disruption of neuronal networks. *Neuron*. 2008; 59: 214–25.
89. **Meyer-Luehmann M, Spires-Jones TL, Prada C, *et al.*** Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature*. 2008; 451: 720–4.
90. **Busche MA, Eichhoff G, Adelsberger H, *et al.*** Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science*. 2008; 321: 1686–9.
91. **Kuchibhotla KV, Lattarulo CR, Hyman BT, *et al.*** Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. *Science*. 2009; 323: 1211–5.
92. **Takano T, Han X, Deane R, *et al.*** Two-photon imaging of astrocytic Ca^{2+} signaling and the microvasculature in experimental mice models of Alzheimer's disease. *Ann N Y Acad Sci*. 2007; 1097: 40–50.
93. **Selkoe DJ.** Alzheimer's disease is a synaptic failure. *Science*. 2002; 298: 789–91.
94. **Whitehouse PJ, Price DL, Struble RG, *et al.*** Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science*. 1982; 215: 1237–9.
95. **Buell SJ, Coleman PD.** Dendritic growth in the aged human brain and failure of growth in senile dementia. *Science*. 1979; 206: 854–6.
96. **Hamos JE, DeGennaro LJ, Drachman DA.** Synaptic loss in Alzheimer's disease and other dementias. *Neurology*. 1989; 39: 355–61.
97. **DeKosky ST, Scheff SW.** Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol*. 1990; 27: 457–64.
98. **Thal DR, Griffin WS, Braak H.** Parenchymal and vascular Abeta-deposition and its effects on the degeneration of neurons and cognition in Alzheimer's disease. *J Cell Mol Med*. 2008; 12: 1848–62.
99. **Tsai J, Grutzendler J, Duff K, *et al.*** Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. *Nat Neurosci*. 2004; 7: 1181–3.
100. **Moolman DL, Vitolo OV, Vonsattel JP, *et al.*** Dendrite and dendritic spine alterations in Alzheimer models. *J Neurocytol*. 2004; 33: 377–87.
101. **Spires TL, Meyer-Luehmann M, Stern EA, *et al.*** Dendritic spine abnormalities in amyloid precursor protein transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy. *J Neurosci*. 2005; 25: 7278–87.
102. **Koffie RM, Meyer-Luehmann M, Hashimoto T, *et al.*** Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci USA*. 2009; 106: 4012–7.
103. **Kamenetz F, Tomita T, Hsieh H, *et al.*** APP processing and synaptic function. *Neuron*. 2003; 37: 925–37.
104. **Hsieh H, Boehm J, Sato C, *et al.*** AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron*. 2006; 52: 831–43.
105. **Snyder EM, Nong Y, Almeida CG, *et al.*** Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci*. 2005; 8: 1051–8.
106. **Chang EH, Savage MJ, Flood DG, *et al.*** AMPA receptor downscaling at the onset of Alzheimer's disease pathology in double knockin mice. *Proc Natl Acad Sci USA*. 2006; 103: 3410–5.
107. **Oddo S, Caccamo A, Shepherd JD, *et al.*** Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron*. 2003; 39: 409–21.
108. **Palop JJ, Chin J, Roberson ED, *et al.*** Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron*. 2007; 55: 697–711.
109. **Palop JJ, Mucke L.** Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol*. 2009; 66: 435–40.
110. **Minkeviciene R, Rheims S, Dobszay MB, *et al.*** Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci*. 2009; 29: 3453–62.
111. **Phinney AL, Deller T, Stalder M, *et al.*** Cerebral amyloid induces aberrant axonal sprouting and ectopic terminal formation in amyloid precursor protein transgenic mice. *J Neurosci*. 1999; 19: 8552–9.
112. **Sturchler-Pierrat C, Abramowski D, Duke M, *et al.*** Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci USA*. 1997; 94: 13287–92.
113. **Cirrito JR, Kang JE, Lee J, *et al.*** Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. *Neuron*. 2008; 58: 42–51.
114. **Mendez M, Lim G.** Seizures in elderly patients with dementia: epidemiology and management. *Drug Aging*. 2003; 20: 791–803.
115. **Snider BJ, Norton J, Coats MA, *et al.*** Novel presenilin 1 mutation (S170F) causing Alzheimer disease with Lewy bodies in the third decade of life. *Arch Neurol*. 2005; 62: 1821–30.
116. **Menendez M.** Down syndrome, Alzheimer's disease and seizures. *Brain Dev*. 2005; 27: 246–52.
117. **Ponomareva NV, Korovaitseva GI, Rogaev EI.** EEG alterations in non-demented individuals related to apolipoprotein E genotype and to risk of Alzheimer disease. *Neurobiol Aging*. 2008; 29: 819–27.
118. **Sperling RA, Laviolette PS, O'Keefe K, *et al.*** Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron*. 2009; 63: 178–88.
119. **Arriagada PV, Marzloff K, Hyman BT.** Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology*. 1992; 42: 1681–8.
120. **Crystal HA, Dickson DW, Sliwinski MJ, *et al.*** Pathological markers associated with normal aging and dementia in the elderly. *Ann Neurol*. 1993; 34: 566–73.
121. **Naslund J, Haroutunian V, Mohs R, *et al.*** Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA*. 2000; 283: 1571–7.
122. **Lue LF, Kuo YM, Roher AE, *et al.*** Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol*. 1999; 155: 853–62.

123. **Koistinaho M, Ort M, Cimadevilla JM, *et al.*** Specific spatial learning deficits become severe with age in beta -amyloid precursor protein transgenic mice that harbor diffuse beta -amyloid deposits but do not form plaques. *Proc Natl Acad Sci USA*. 2001; 98: 14675–80.
124. **Westerman MA, Cooper-Blacketer D, Mariash A, *et al.*** The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci*. 2002; 22: 1858–67.
125. **Itagaki S, McGeer PL, Akiyama H, *et al.*** Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J Neuroimmunol*. 1989; 24: 173–82.
126. **Wegiel J, Wisniewski HM.** The complex of microglial cells and amyloid star in three-dimensional reconstruction. *Acta Neuropathol*. 1990; 81: 116–24.
127. **Streit WJ.** Microglia and Alzheimer's disease pathogenesis. *J Neurosci Res*. 2004; 77: 1–8.
128. **Alexander C, Rietschel ET.** Bacterial lipopolysaccharides and innate immunity. *J Endotoxin Res*. 2001; 7: 167–202.
129. **Rodgers KM, Hutchinson MR, Northcutt A, *et al.*** The cortical innate immune response increases local neuronal excitability leading to seizures. *Brain*. 2009; 132: 2478–86.
130. **Airaksinen MS, Eilers J, Garaschuk O, *et al.*** Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D28k gene. *Proc Natl Acad Sci USA*. 1997; 94: 1488–93.