PERSPECTIVE

Role of presynaptic calcium stores for neural network dysfunction in Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia representing a major problem for public health. In 2017 there were an estimated 50 million patients worldwide and this number is expected to almost double every 20 years, reaching 75 million in 2030 and 131.5 million in 2050 (https://www.alz.co.uk/research/statistics). Clinically there are two forms of the disease: the sporadic form (also called late onset AD, LOAD) and the familial form (FAD). LOAD is the most common form. Its prevalence increases with advancing age from 1% in the 65–70 years old cohort to more that 30% after the age of 85. It is characterized by moderate to extreme severity with the advancing age being the main risk factor for LOAD. Familiar AD represents some 5–10% of all AD cases. FAD is linked to mutations in a specific set of genes, most often in the genes encoding amyloid precursor protein (APP) and the presenilins (PS) 1 and 2. Interestingly, the vast majority of AD related mutations are located on PS1 (Steiner et al., 2008; Mattson, 2010) thus identifying this protein as one of the main targets for FAD-modifying therapies. Here we address the role of AD-related presenilin mutations for Ca^{2+} dyshomeostasis and *in vivo* neural network dysfunction in AD.

The role of presenilins: Presenilins are transmembrane proteins that harbor the catalytic site of the γ-secretase complex, which mediates the intramembranous cleavage of many type I membrane proteins, including APP (Steiner et al., 2008). Numerous FAD-associated presenilin mutations were shown to affect the cleavage specificity of γ-secretase, thus increasing the production of the aggregation-prone and neurotoxic variant of amyloid β peptide $Aβ_{42}$ (Steiner et al., 2008). Gradual accumulation of Aβ plays the central role in the so called "amyloid hypothesis" of AD (Selkoe, 2002; Selkoe and Hardy, 2016). Consistently, over the last decades therapeutic strategies mainly focused on decreasing Aβ levels inside the brain by either downregulating its production/accumulation or upregulating its clearance. So far, however, the results obtained were rather disappointing. In several trials using antibodies against Aβ, however, post hoc analyses hinted towards a reduction of cognitive decline in patients with mild, but not moderate, form of AD (Selkoe and Hardy, 2016).

Besides playing an important role in the γ-secretase complex, presenilins also have other functions, mostly related to the intracellular Ca^{2+} homeostasis (Hermes et al., 2010; Mattson, 2010; Briggs et al., 2017; Popugaeva et al., 2017). Accordingly, PS mutations were reported to modify intracellular Ca^{2+} signaling in various experimental AD models. As illustrated in **Figure 1**, multiple *in vitro* studies identified IP_3 receptors (IP_3Rs), ryanodine receptors (RyRs), sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCAs) as well as a membrane-associated enzyme Phospholipase C (PLC) as potential interaction partners of presenilins. Multiple AD-associated PS1 and PS2 mutations (*e.g.*, PS1-M146V, PS2-N141I and PS2-M239V) were shown to potentiate the IP_3-mediated Ca^{2+} release from the intracellular Ca^{2+} stores, likely through an enhancement of the open probability of the IP_3 receptors (reviewed in (Hermes et al., 2010; Briggs et al., 2017; Popugaeva et al., 2017). Similarly, mutant presenilins were reported to upregulate the expression levels of RyRs, and to increase RyR-mediated Ca^{2+} release from the intracellular Ca^{2+} stores. Studies in brain slices derived from PS1-M146V KI mice and 3xTg-AD mice have documented a substantial increase in the amplitude of RyR-mediated Ca^{2+} signals across neuronal subcompartments, with particularly large RyR-mediated Ca^{2+} signals in dendrites and dendritic spines (Chakroborty and Stutzmann, 2014; Briggs et al., 2017). In addition, FAD-linked PS mutations were reported to cause an increase in the basal activity of PLC (Hermes et al., 2010). *In vitro* studies have also pointed towards colocalization and physical interaction between PS and SERCA pumps (**Figure 1**). Depletion of PS1 and PS2 resulted in diminished SERCA function, whereas the expression of mutated presenilin (*e.g.*, PS1-M146V) increased SERCA activity, thus resulting in stronger accumulation of Ca^{2+} within the intracellular stores. Moreover, the work of Bezprozvanny's group (reviewed in Popugaeva et al., 2017) has suggested that presenilins might themselves represent the Ca^{2+} leak channels of the endoplasmic reticulum and that the AD-related PS mutations, including M146V, L166P, A246E, E273A, G384A, and P436Q, impair their leak channel function, again leading to the overfilling of the intracellular Ca^{2+} stores. Such overfilling, in turn, impairs the function of store-operated Ca^{2+} channels (SOCC) as well as associated STIM and Orai proteins and is believed to play the central role in the AD-related Ca^{2+} dyshomeostasis, which represents one of the main functional hallmarks of AD (Hermes et al., 2010; Briggs et al., 2017; Popugaeva et al., 2017).

Synaptic dysfunction in AD: Postmortem studies in AD patients identified the degree of synaptic/spine loss as the best neuropathological correlate of the patient's cognitive impairment (Selkoe and Hardy, 2016). Consistently, several *in vitro* animal studies have shown that soluble Aβ oligomers inhibit inter-synaptic vesicle trafficking and activity-dependent rapid synaptogenesis, selectively depress glutamatergic synaptic transmission, reduce expression of AMPA and promote endocytosis of NMDA receptors (Briggs et al., 2017). Importantly, Aβ oligomers isolated directly from brains of AD patients caused an impairment of long-term potentiation (LTP) and an enhancement of long-term depression (LTD) in mouse hippocampal slices. Moreover, an injection of these oligomers into the lateral ventricle disrupted the memory of a learned behavior in normal rats (summarized in Selkoe and Hardy, 2016).

An impaired synaptic transmission was also observed in predepositing 3xTg-AD mice. A suggested cause of the impairment is an activation of hyperpolarizing Ca^{2+}-dependent SK2 K^+ channels, activated by excess of Ca^{2+} released from the overfilled intracellular Ca^{2+} stores (Briggs et al., 2017). Additional evidence suggests that FAD-mediated impairment of synaptic plasticity can occur in the absence of amyloid and tau pathologies. *In vitro* studies in PS1-M146V KI mice, for example, have demonstrated an impairment of both the early and the late phases of LTP (Mattson, 2010). Taken together, these data substantiate the so called "synaptic failure hypothesis" of AD (Selkoe, 2002).

Neural network hyperactivity in AD: In contrast to what might have being assumed based on the synaptic failure hypothesis of AD, a growing number of studies in humans and mouse models of AD identify neuronal network hyperactivity as an emerging functional hallmark of AD. Indeed, aberrant cortical network activity is observed in AD patients with the sporadic as well as the familiar forms of the disease, in particular those carrying a mutation in the presenilin genes (reviewed in Palop and Mucke, 2009, 2016). In patients, the aberrant forms of neural network hyperactivity include subclinical epileptiform activity (spikes and sharp waves) as well as clinically apparent seizures. According to recent reports, the incidence of seizures is roughly 7–8-fold higher in AD patients compared to the age-matched control group and severe AD cases are more likely to develop unprovoked seizures (Palop and Mucke, 2009, 2016). Moreover, in AD patients evaluated longitudinally, epileptiform activity was associated with a faster cognitive decline. Whereas previously epileptiform activity was considered as an epiphenomenon of the end-stage neurodegeneration, resent data suggest that neuronal hyperactivity can occur early during disease progression, even 4–7 years before the disease diagnosis is made (Palop and Mucke, 2016). Interestingly, 42% of young (58–68 years old) patients with the sporadic form of AD as well as non-demented carriers of the apolipoprotein E4, a known genetic risk factor of sporadic AD, also develop subclinical epileptiform activity (Palop and Mucke, 2009; Lerdkrai et al., 2018).

Studies in transgenic mouse models of AD (hereafter referred to as "AD mice") have also provided ample evidence for amyloidosis-induced network hyperactivity and seizures (see Supplementary Table 1 in Palop and Mucke, 2016). Multiple mouse lines (over)expressing AD-related human mutations in APP alone or together with mutations in tau protein and/or presenilins are reported to show either neuronal network hyperactivity detectable by *in vivo* Ca^{2+} imaging (Busche et al., 2008; Lerdkrai et al., 2018) or spontaneous epileptiform discharges ("spikes") as well as seizures detectable by EEG recordings (Palop and Mucke, 2016). In concordance with human data, neuronal hyperactivity was also observed in ApoE4-KI mice as well as in PS45 presenilin mutant mice (PSEN1 (G384A)), developing neither amyloid plaques nor neuroinflammation (Palop and Mucke, 2016; Lerdkrai et al., 2018). Moreover, in PS45 mice neuronal hyperactivity was evident even in young adult (6–7-month-old) animals (Lerdkrai et al., 2018), in line with the notion that neuronal hyperactivity emerges during early stages of the disease.

Furthermore, our recent *in vivo* data suggest that neuronal hyperactivity develops also during healthy brain ageing, albeit to a lesser extent (Lerdkrai et al., 2018). Interestingly, the ageing-related neuronal hyperactivity is prominent already in 10–14 months old mice. Such animals are not yet considered old and their age roughly corresponds to humans in their fourth or fifth decade of life. Furthermore, neurons are not the only cell type in the brain getting hyperactive with ageing. Similar trend was previously observed *in vivo* for cortical microglia (Brawek et al., 2014). Thus, during normal ageing the brain of middle-aged mice (and possibly also humans) reaches a different set point with more active neurons and microglia. Such conditions render the

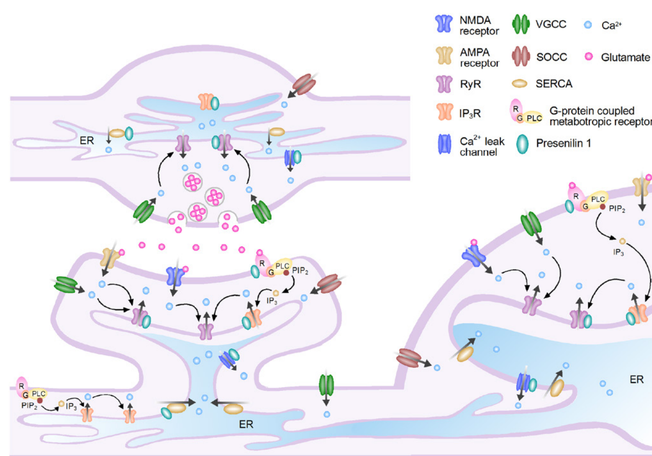

Figure 1 Main components regulating store-operated Ca^{2+} signaling in pre- and postsynaptic neuronal compartments.
Schematic representation of the key players regulating store-operated Ca^{2+} signaling as well as their interactions with presenilins. Arrows indicate the direction of ion flux. ER: Endoplasmic reticulum; RyR: ryanodine receptor; IP_3R: inositol trisphosphate receptor; IP_3: inositol 1,4,5-trisphosphate; PIP2: phosphatidylinositol 4,5-bisphosphate; SERCA: sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; NMDA: N-methyl-D-aspartate; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; VGCC: voltage-gated Ca^{2+} channels; PLC: phospholipase C; SOCC: store-operated Ca^{2+} entry channel.

brain vulnerable to both neuroinflammation and seizure development, thus alleviating the development of AD.

The role of presynaptic calcium stores for neuronal network hyperactivity: Intracellular Ca^{2+} stores are part of the endoplasmic reticulum, which in neurons is present both pre- and post-synaptically (**Figure 1**, see also Mattson, 2010; Briggs et al., 2017). The majority of *in vitro* data emphasized the involvement of the postsynaptic Ca^{2+} stores in the pathophysiology of AD. Thus, the postsynaptic Ca^{2+} stores in somata and dendrites of cortical and hippocampal neurons of AD mice were shown to release more Ca^{2+} in response to the application of IP_3R or RyR agonists and to strongly potentiate synaptic and NMDA-receptor mediated Ca^{2+} transients (reviewed in Mattson, 2010; Chakroborty and Stutzmann, 2014). Excessive RyR-mediated Ca^{2+} release was also observed in dendritic spines of AD mice and was suggested to dysregulates the maintenance of memory-associated mushroom spines via inhibition of the Ca^{2+} store-operated Ca^{2+} entry channels (SOCCs; **Figure 1**; Briggs et al., 2017; Popugaeva et al., 2017). Our recent *in vivo* data, however, revealed a rather minor contribution of store-mediated Ca^{2+} release both to somatic and synaptic Ca^{2+} signals in layer 2/3 cortical neurons (Lerdkrai et al., 2018). Although somatic RyR-mediated Ca^{2+} release signals were somewhat longer in AD compared to age-matched WT mice, we did not observe any increase in their amplitudes, much in contrast to more than 200–300% increase in amplitudes of RyR-mediated Ca^{2+} release signals *in vitro* (see above). Among cells with different activity patterns (*i.e.*, silent, normal and hyperactive; for details of cell classification see Busche et al., 2008), hyperactive cells showed the longest RyR-mediated Ca^{2+} release signals (Lerdkrai et al., 2018). Consistently, only in hyperactive cells spontaneous synaptically-driven dendritic Ca^{2+} transients showed a store-mediated component, which was blocked by emptying the intracellular stores. We concluded, therefore, that hyperactive cells are the only cells exhibiting AD-related overfilling of postsynaptic Ca^{2+} stores *in vivo*. Although for somatic and dendritic Ca^{2+} stores the degree of such overfilling is rather low, it cannot be excluded that somewhat larger dysfunction might be observed for Ca^{2+} stores in dendritic spines (**Figure 1**). Assuming that such dysfunction causes spine destabilization (see above), our recent data suggest that *in vivo* spine destabilization occurs in hyperactive cells only.

In contrast to what is known about the postsynaptic side, the role of presynaptic Ca^{2+} stores for synaptic and neural network dysfunction in AD is much less clear. Under physiological conditions presenilins seem to modulate the evoked glutamate release in a Ca^{2+} store-dependent manner, and RyRs of AD mice were shown to mediate an increase in frequency of spontaneous vesicle release from presynaptic terminals (Chakroborty and Stutzmann, 2014). This increase, however, was believed to deplete the pool of readily releasable vesicles and to cause a Ca^{2+}-dependent activation of SK2 K^+ channels (see above), both leading to weakening of synaptic transmission (Chakroborty and Stutzmann, 2014; Briggs et al., 2017). In contrast, our *in vivo* data suggest that an AD-related mutation in PSEN1 gene causes heightened presynaptic release of glutamate already in 6–7 months old mice, thus strongly contributing to AD-related neuronal hyperactivity. Consistently, emptying the Ca^{2+} stores in AD and presenilin mutant mice normalizes cortical neural network activity in these animals (Lerdkrai et al., 2018). Interestingly, ageing- or APP mutation-induced neuronal hyperactivity are not sensitive to store depletion. Together, these data suggest that a single allele of mutated PS1 is sufficient to induce an early and a profound neuronal hyperactivity, mainly caused by the dysfunction of presynaptic intracellular Ca^{2+} stores. This early hyperactivity is likely to enhance activity-dependent generation and release of amyloid β and tau as well as formation of amyloid plaques (Palop and Mucke, 2016). By this mechanism a single mutation in the PSEN1 gene can lead to early onset full-blown disease in humans. The validity of this hypothesis is also supported by the fact that drugs which either selectively dampen presynaptic release of neurotransmitters (*e.g.*, levetiracetam) or block RyRs releasing Ca^{2+} from the intracellular stores (*e.g.*, dantrolene), were recently shown to improve memory and cognition in mice and humans (reviewed in Palop and Mucke, 2016; Popugaeva et al., 2017; Lerdkrai et al., 2018).

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