

Amyloid Goes Global

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Abstract

The brains of patients with Alzheimer's disease (AD) contain abundant plaques composed of amyloid β ($A\beta$) peptides. It has been assumed that amyloid plaques and soluble $A\beta$ oligomers induce neuronal pathology in AD; however, the mechanism by which amyloid mediates pathological effects is not clearly understood. In vivo calcium (Ca^{2+}) imaging and array tomography studies with AD mouse models are providing new insights into the changes that occur in brain structure and function as a result of amyloid plaque accumulation. The unexpected lesson from these studies is that amyloid plaques result in both localized and global changes in brain function. The amyloid-induced effects include "short-range" changes in neuronal Ca^{2+} levels, "medium-range" changes in neuronal activity and synaptic density, and "long-range" changes in Ca^{2+} signaling in astrocytes and induction of intracellular Ca^{2+} waves spreading through a network of astrocytes. These results have potential implications for understanding synaptic and neuronal network dysfunction in AD brains.

The dominant idea in the field of Alzheimer's disease (AD) is the "amyloid hypothesis", which states that increased production of amyloidogenic A β 42 peptide (or an increase in the ratio of A β 42 to A β 40) is a major cause of neuronal and synaptic loss in AD [1]. Both A β 40 and A β 42 peptides are generated following proteolytic cleavage of A β -precursor protein (APP). APP is a single transmembrane domain protein that is cleaved initially at the extracellular domain by β -secretase and subsequently within the transmembrane domain by γ -secretase. The A β 40 peptide contains the APP sequence between the β -secretase and γ -secretase cleavage sites. The site of γ -secretase cleavage can occasionally shift by two amino acids towards the APP C-terminus, adding two extra hydrophobic amino acids (isoleucine and alanine) and increasing the propensity of the resulting A β 42 peptide to aggregate. The increase in A β 42:A β 40 ratio leads to formation of amyloid oligomers and plaques, which have been postulated to induce neuronal dysfunction and death [1]. The experimental support for the amyloid hypothesis comes from the accumulation of amyloid plaques in the brains of AD patients, as well as the familial AD (FAD) cases resulting from missense mutations in APP or in presenilins (PS), the catalytic subunits of γ -secretase. It has been speculated that formation of soluble A β oligomers and insoluble amyloid plaques drives the pathological processes in the AD brains. Based on these assumptions, therapies that prevent the formation of amyloid plaques or facilitate amyloid clearance have been the main focus of AD drug development [2], although the key question about the amyloid hypothesis – how exactly does amyloid impair brain function? – has not been answered. Several *in vivo* Ca²⁺ imaging studies performed in transgenic mouse models that overexpress both the mutated human APP and PS1 proteins found in FAD (*APP/PS1* mice) provide a first look at changes in brain function resulting from accumulation of amyloid plaques. The unexpected lesson from these studies is that amyloid plaques result in both localized and global changes in brain function.

The pathogenic actions of A β oligomers have been assumed to be highly localized, i.e., at the level of individual synapses or spines. Indeed, local effects of A β oligomers facilitating AMPA (amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor internalization [3,4] and NMDA (N-methyl-D-aspartate) receptor trafficking and function [5-7] have been described. *In vivo* Ca²⁺ imaging experiments performed on transgenic mice expressing KM670/671NL (Swedish) mutated APP alone (*APP*) or with a PS1 mutant lacking exon 9 (*APP/PS1- Δ E9*), approximately 35% of neurites located in the immediate vicinity (< 25 μ m) of the A β plaques exhibited elevated resting Ca²⁺ concentrations and displayed abnormal morphology, such as lack of dendritic spines [8]. Because A β oligomers can form Ca²⁺-permeable pores in the plasma membrane [9], the most likely explanation for these results is that the high concentration of A β oligomers increased Ca²⁺ permeability in the area surrounding amyloid plaques. These studies provide strong evidence for local (< 25 μ m) pathogenic effects of amyloid, which result in destabilization of intracellular neuronal Ca²⁺ signaling and impaired synaptic function (Fig 1).

Another in vivo Ca^{2+} imaging study performed on transgenic mice expressing mutated APP and human PS1 with a G384A point mutation (*APP/PS1-G384A*) reported that in the area close to the plaques, the fraction of hyperactive neurons (which showed spontaneous Ca^{2+} transients at much higher frequencies than usual) increased by 16-fold, and the fraction of silent neurons (which showed no spontaneous Ca^{2+} transients over 6 minute long recording sessions) increased by 3-fold when compared to wild-type mice [10]. These medium-range effects of amyloid ($< 60 \mu\text{m}$) have been linked to impaired local synaptic inhibition, which resulted in hyperactivity of disinhibited neurons [10] (Fig 1). Similar hyperactivity has been previously described in electrophysiological studies of *APP* transgenic mice [11]. It has been speculated that the loss of inhibition is due to local anatomical remodeling of synaptic contacts in the vicinity of amyloid plaque, which occurs as a response to amyloid-induced activation of microglia.

Local actions of amyloid are further supported by a morphological analysis of brains from *APP/PS1-ΔE9* transgenic mice [12]. Using array tomography analysis of ultrathin (70 nm) fixed sections from *APP/PS1-ΔE9* mouse brains labeled with $\text{A}\beta$ oligomer-specific antibodies [13], these authors observed a halo of $\text{A}\beta$ oligomers extending a short range (6.5 μm) from the edge of the dense amyloid plaque [12] (Fig 1). Quantitative analysis revealed a reduction in the density of excitatory synapses (as visualized by staining with antibodies to postsynaptic density protein of apparent molecular weight 95 kDa or PSD-95) when compared to age-matched wild-type mice. Although the reduction in synaptic density was greatest (60%) in the immediate vicinity of the amyloid plaque, it remained significant at distances as far as 50 μm from the plaque. The spatial scale of synaptic changes observed by array tomography analysis is consistent with that of previously described changes in spontaneous neuronal excitability [10]. In both cases, the effects of the plaques are detected in the “middle range” of 50-60 μm (Fig 1).

The latest in vivo Ca^{2+} imaging study [14] indicates that pathological effects of amyloid can spread even farther in the brain. These authors studied Ca^{2+} dynamics in astrocytes from *APP/PS1-ΔE9* transgenic mice. They found that resting Ca^{2+} concentrations were globally elevated in the astrocytic network in the brains of *APP/PS1-ΔE9* mice. In addition, there was a significant (4-fold) increase in the fraction of spontaneously active astrocytes in the brains of *APP/PS1-ΔE9* mice, and the spontaneous Ca^{2+} signals in *APP/PS1-ΔE9* astrocytes were also 50% higher in amplitude than those in wild-type astrocytes. The effects on resting Ca^{2+} levels and spontaneous activity of astrocytes were observed throughout the brain and did not depend on proximity to the plaques. The increase in astrocytic Ca^{2+} activity was not blocked by tetrodotoxin (TTX) and appeared to result from direct effects of $\text{A}\beta$ oligomers on the astrocytic network rather than mediated by changes in neuronal activity. In addition, astrocytic Ca^{2+} signals could be correlated to the occurrence of intracellular Ca^{2+} waves (ICW) spreading through the astrocytic network [14]. This spread of ICW was observed at distances reaching as far as 200 μm from the plaques (Fig 1). The mechanism involved in plaque effects on the astrocytic Ca^{2+} signals appear to involve stimulation of initiator astrocytes within 25 μm of the

plaque, followed by long-range ICW propagation through the astrocytic network [14]. Although some of the effects observed by the authors were reported to be global, the study was performed in 6-8 month old mice with dense plaques, meaning that the average astrocyte-to-plaque distance was 50 μm [14]. Additional imaging studies with younger mice with a lower density of plaques in their brains will be needed to determine the upper limit for long-ranging effects of amyloid plaques on astrocytic Ca^{2+} signals.

The results of in vivo Ca^{2+} imaging and array tomography studies in *APP/PS1* mice [8,10,12,14] indicate that amyloid plaques have short-range (< 25 μm), medium-range (< 60 μm), and long-range (< 200 μm) effects on Ca^{2+} signaling and excitability (Fig 1). How do these findings relate to AD pathogenesis? AD has been proposed to result from synaptic failure due to local attack by $\text{A}\beta$ oligomers on synapses and dendritic spines [15]. However, it has been also suggested that AD symptoms may manifest because of neuronal network dysfunction due to localized synaptic loss and activation of microglia and astrocytes [16,17]. The results from the in vivo Ca^{2+} imaging and array tomography studies discussed here indicate that amyloid plaques may affect the properties of neuronal networks by acting both locally and at a distance. For example, long-range changes in astrocytic Ca^{2+} signals may affect glutamate clearance mechanisms, which in turn may affect neuronal network behavior. It will be interesting to determine if inhibition of ICWs in astrocytes of *APP/PS1* mice results in improved behavioral performance in memory tasks, as it would suggest that blocking Ca^{2+} waves in astrocytes may hold therapeutic potential for alleviating some of the symptoms in human AD patients or even in treating AD.

Fig. 1. “Short-range” (< 25 μm), “medium-range” (< 60 μm) and “long-range” (< 200 μm) effects of an amyloid plaque are shown. The presence of an amyloid plaque in brains of *APP/PS1* mice causes increases in resting Ca^{2+} concentrations in neurons within a short distance [8], increases in neuronal spontaneous activity and decreases in the density of excitatory synapses within a medium distance [10, 12], and spread of intracellular Ca^{2+} waves (ICW) through the astrocytic network over longer distances [14]. The resulting changes in neuronal and astrocytic properties likely lead to dysfunction within the neuronal network surrounding the amyloid plaques in the brains of patients with AD.

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