

## PERSPECTIVE

# Beta-amyloid pore linked to controlled calcium influx into the cell: A new paradigm for Alzheimer's Disease

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**Abstract**

Despite tremendous worldwide efforts, clinical trials assessing Alzheimer's disease (AD)-related therapeutics have been relentlessly unsuccessful. Hence, there is an urgent need to challenge old hypotheses with novel paradigms. An emerging concept is that the amyloid-beta ( $A\beta$ ) peptide, which was until recently deemed a major player in the cause of AD, may instead modulate synaptic plasticity and protect against excitotoxicity. The link between  $A\beta$ -mediated synaptic plasticity and  $A\beta$  trafficking is central for understanding AD pathogenesis and remains a perplexing relationship. The crossover between  $A\beta$  pathological and physiological roles is subtle and remains controversial. Based on existing literature, as a signaling molecule,  $A\beta$  is proposed to modulate its own turnover and synaptic plasticity through what is currently believed to be the cause of AD: the transient formation of pore-like oligomers. A change of perspective regarding how  $A\beta$  pores exert a protective function will unavoidably revolutionize the entire field of anti-amyloid drug development.

**KEYWORDS**

aging, Alzheimer's disease, beta-amyloid pore, calcium, cholesterol dyshomeostasis, endocytic trafficking, excitotoxicity, synaptic plasticity

## 1 | $A\beta$ PHYSIOLOGICAL FUNCTION

The insurgence of Alzheimer's disease (AD) is associated with the accumulation and aggregation of the amyloid-beta ( $A\beta$ ) peptide produced along the amyloidogenic pathway by the sequential cleavage of the amyloid precursor protein (APP) by  $\beta$ -secretase and  $\gamma$ -secretase in endosomal compartments (Figure 1).<sup>1</sup> The primary product is  $A\beta_{40}$  (40 residues in length), whereas a small portion is an  $A\beta_{42}$  variant, which is more hydrophobic and prone to fibrillation.<sup>2</sup> Upon accumulation,  $A\beta$  peptides can self-assemble into organized macrostructures such as fibrils, followed by insoluble plaques that deposit in specific regions of the AD brain.

$A\beta$  plays a beneficial role in several physiological functions, including the regulation of synaptic function and facilitation of neuronal growth and survival.<sup>3</sup> Malinow's and Holtzman's groups demonstrated that

increased synaptic activity enhances  $A\beta$  secretion, while reduced activity inhibits it.<sup>4,5</sup> In physiological conditions, picomolar concentrations of  $A\beta$  increase hippocampal long-term potentiation (LTP), whereas nanomolar concentrations inhibit it.<sup>6</sup> Therefore,  $A\beta$  may serve as a feedback mechanism to prevent synaptic hyperactivation and excitotoxicity.

There are meaningful analogies between AD, autism, and Down syndrome (DS), reinforcing the notion of an  $A\beta$ -mediated physiological regulation of synaptic plasticity.

LTP, a form of synaptic plasticity probably implicated in learning and memory, is impaired in DS as in AD.<sup>7</sup> With progressive aging, 50% or more DS individuals will develop AD-type pathology. In individuals with DS, APP overexpression possibly leads to increased  $A\beta$  production in the brain. The therapeutic reduction of  $A\beta$  levels can relieve some behavioral deficits typical of the DS phenotype.

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Conversely, in autism spectrum disorders (ASD), LTP is enhanced<sup>8</sup>: the core pathology is hyper-reactivity and hyper-plasticity of local neuronal circuits, confirmed to be debilitating.<sup>9</sup> Interestingly, both A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> levels were significantly low in patients with severe autism.<sup>10</sup>

Intriguingly, aberrations in synaptic activity occur in parallel with A $\beta$  expression. In DS, A $\beta$  overexpression may downregulate synaptic plasticity, while in autism, synaptic hyperactivity may not be rescued owing to low A $\beta$  expression. In DS, A $\beta$  overproduction does not necessarily lead to A $\beta$  accumulation and plaque appearance: aging is a crucial co-player.

Thus, the overproduction of A $\beta$  peptides may represent the cell's primary effort to maintain homeostasis in response to adverse conditions mediated via increased neuronal excitability.

It is worth noting that neuronal excitation also triggers the translation of tau protein. Similar to A $\beta$ , tau protein seems to be physiologically involved in synaptic plasticity. However, it has a prominent role in long-term depression. Besides A $\beta$  deposition into plaques, tau aggregation is another pathological marker for degeneration in AD.<sup>11</sup>

## 2 | A $\beta$ IN ACTION

In response to injury or disease, synapses modulate their strength and form new connections with other neurons. Synaptic activity is modulated by neurotransmitters released by presynaptic exocytosis of synaptic vesicles (SVs) that travel into inter-synaptic spaces through the microtubule-based axonal transport machinery.<sup>12</sup> Upon neuronal insults, an increase in intracellular calcium (Ca<sup>2+</sup>) mediated by A $\beta$ <sub>42</sub> activates the CaMKK-to-CaMKIV pathway to promote neuronal survival.<sup>13</sup> Ca<sup>2+</sup>/calmodulin-dependent protein kinase IV (CaMKIV) mediated phosphorylation of synapsin 9 dissociates the SV-synapsin-actin ternary complex, inhibiting, in turn, the transport of SVs.<sup>14</sup> Hence, by indirectly suppressing the inter-synaptic vesicle trafficking needed for rapid functional synapse formation and transmission, A $\beta$ <sub>42</sub> affords protection against neuronal injury.<sup>15</sup>

In early AD, synaptic plasticity is impaired, and synaptic density is reduced.<sup>16,17</sup>

During the last few decades, small A $\beta$  oligomers have emerged as primary neurotoxic species in AD. From a functional outlook, these oligomers are shaped as pores in the plasma membrane (PM)<sup>18,19</sup> and are proposed to allow dysregulated Ca<sup>2+</sup> entry in the cytoplasm of brain cells.<sup>20</sup> Intracellular Ca<sup>2+</sup> levels are highly regulated to precisely modulate neuronal functions, including membrane excitability, neurotransmitter release, and synaptogenesis. Ca<sup>2+</sup>-binding proteins (CaBP) contribute to the maintenance of Ca<sup>2+</sup> homeostasis within neurons. CaBP variants with different kinetics and buffering capacities for Ca<sup>2+</sup> are differentially expressed across the central nervous system. The selective susceptibility of cholinergic neurons to neurodegenerative insults correlates with a loss of the Ca<sup>2+</sup>-binding protein calbindin with age and reduced Ca<sup>2+</sup> buffering capacities.<sup>21</sup> Protracted Ca<sup>2+</sup> dysregulation leads to an accumulation of reactive oxygen species and, ultimately, apoptosis and neuronal death.<sup>22</sup>

Consistent with this view, Ca<sup>2+</sup> channel blockers have been evaluated to reverse A $\beta$ -induced deficits. However, at least three clinical studies emphasized that older individuals administering Ca<sup>2+</sup> channel

### RESEARCH IN CONTEXT

1. **Systematic review:** This perspective reviews literature data to propose a physiological role of the beta-amyloid (A $\beta$ ) pore in the regulation of synaptic plasticity.
2. **Interpretation:** The results of this investigation suggest that the A $\beta$ -pore might afford protection against neuronal injury by promoting Ca<sup>2+</sup> influx to supply the cell's physiological demands in response to hyperexcitation.
3. **Future directions:** Experimental strategies are proposed to inspect the hypothesized A $\beta$ -pore mediated synaptic regulation linked to Ca<sup>2+</sup> influx and A $\beta$ -trafficking in autism, Down syndrome, and Alzheimer's disease, where A $\beta$  is differently expressed.

blockers were more likely to experience cognitive decline than those using other agents.<sup>23,24,25</sup> These outcomes suggest that blocking Ca<sup>2+</sup> entry to restore its physiological intracellular concentration may aggravate the pathological scenario.

The physiological linkage between A $\beta$  and Ca<sup>2+</sup> in regulating synaptic activity could explain why blocking Ca<sup>2+</sup> influx through the cell membrane exacerbates the cognitive decline in older individuals. An intriguing question is whether the A $\beta$ -mediated influx of Ca<sup>2+</sup> associated with the activation of the CaMKK-to-CaMKIV pathway is permitted via A $\beta$ -pore-like oligomers.

Considering the critical role of Ca<sup>2+</sup> as a second messenger in many cellular processes (e.g., exocytosis of secretory vesicles), its experimentally observed permeation through A $\beta$ -pores may not be just coincidental.<sup>20</sup> Such an argument can be extended to other amyloidosis, where amyloid proteins (including prion, I $\beta$ let amyloid polypeptide, and  $\alpha$ -synuclein) forming Ca<sup>2+</sup>-permeable pores are considered to be implicated in the onset of spongiform encephalopathies, Type 2 diabetes mellitus, and Parkinson's disease, respectively.<sup>26,27</sup>

Experimental *in vivo* studies may help unravel the temporal relationship between synaptic plasticity, A $\beta$  accumulation, and Ca<sup>2+</sup> homeostasis.<sup>17,28</sup>

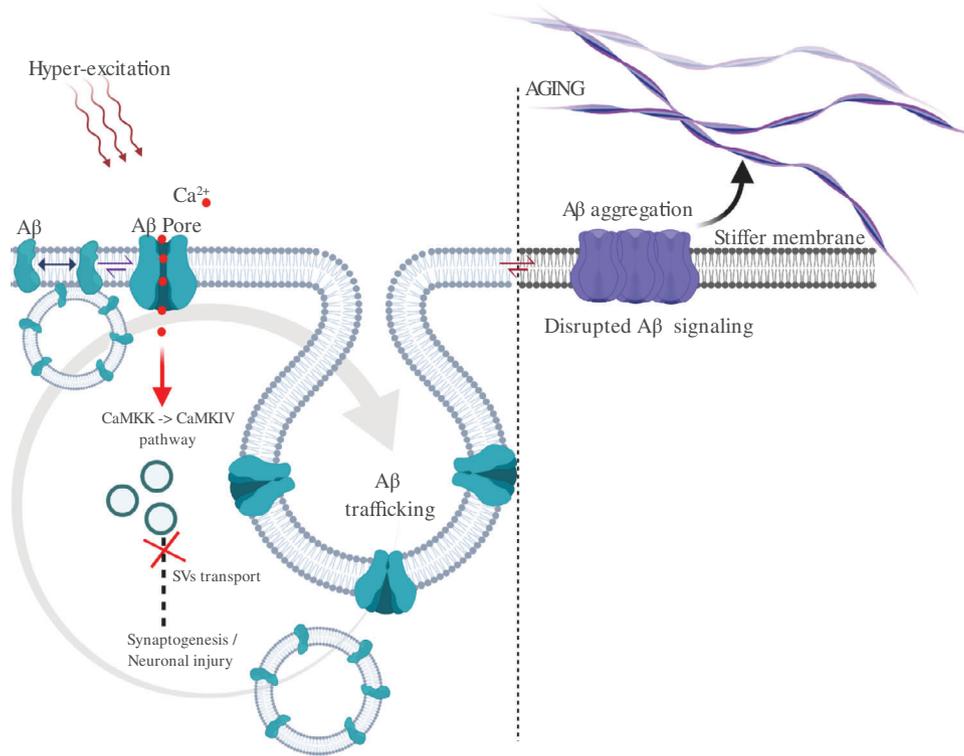
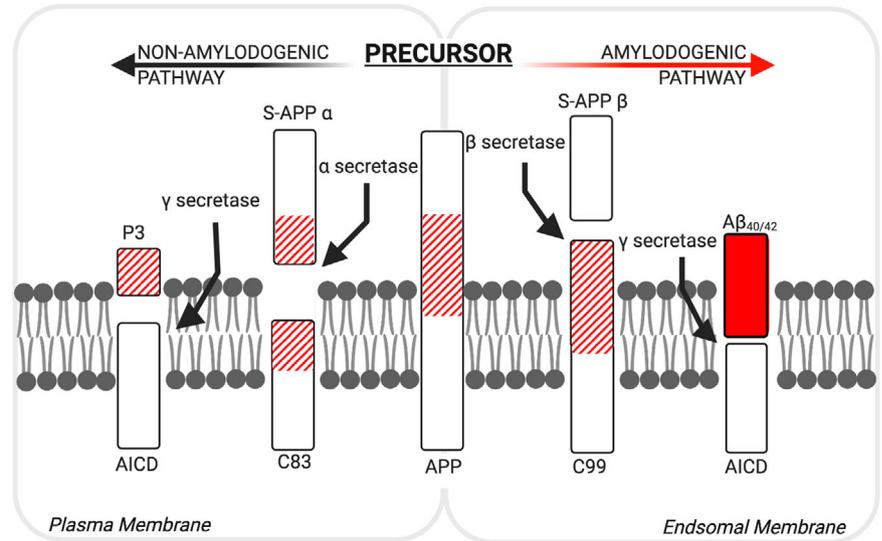
## 3 | A $\beta$ TRAFFICKING

In a healthy brain, A $\beta$ <sub>42</sub> is located in the outer membrane of multivesicular bodies (MVBs) within neurons. The manner in which hydrophobic peptides travel from endosomes to the PM and the extracellular space remains an open and crucial question, given that this partitioning may be critical in A $\beta$ -induced synaptic dysfunction.

Reportedly, extracellular and intracellular pools of A $\beta$  are interconnected.<sup>29</sup> Indeed, secreted A $\beta$  produced at the PM is taken up by the cell to form intracellular pools. Conversely, the clearance of intraneuronal A $\beta$  follows the removal of extracellular plaques.

There is evidence that membrane-associated A $\beta$ <sub>42</sub> can be released in association with exosomes upon the fusion of MVBs with the PM.<sup>30</sup> Furthermore, effective A $\beta$ <sub>42</sub> uptake by the cell requires both the

**FIGURE 1** Schematic diagram of secretases cleavage of the precursor and derived products



**FIGURE 2** Proposed mechanisms for the physiological Aβ pore-mediated regulation of synaptic activity (left panel), and the affected Aβ signaling associated with aging (right panel). Figure created with BioRender.com

formation of an ordered aggregate on the PM and a critical concentration of membrane-bound Aβ<sub>42</sub>.<sup>31</sup> Both pieces of evidence are compatible with the formation of Aβ pores.

However, which endocytic pathway (EP) is specifically involved in Aβ internalization remains controversial. The most frequently reported endocytic process depends on clathrin and dynamin.<sup>32</sup> However, endocytosis at lipid rafts, wherein Aβ is mainly distributed,<sup>33,34</sup> proceeds in a clathrin-independent manner, while it remains cholesterol-sensitive.

Molecular dynamics (MD) simulations have demonstrated how the membrane buckles whenever the membrane-embedded (round-shaped) Aβ cluster size exceeds a critical size (compatible with the size of a pore), depending on the distribution of cholesterol in the bilayer. In the presence of asymmetrically distributed cholesterol (healthy condition), the membrane will bend and vesiculate to maintain a critical Aβ-lipids ratio (Figure 2), whereas if cholesterol is symmetrically distributed (aging condition), the stiffer membrane will rather extrude

A $\beta$  than undergo invagination.<sup>35,36</sup> In the first scenario, Ca<sup>2+</sup> ions will freely pass through A $\beta$  pores in the PM, whereas Ca<sup>2+</sup> influx is reduced owing to A $\beta$  pore clustering in the second scenario.<sup>36</sup>

Here, it is hypothesized that in response to synaptic stimulation, exosomes carry and release A $\beta$  peptides upon fusion with the PM. Membrane-associated A $\beta$  peptides self-assemble to form selective pores and promote Ca<sup>2+</sup> influx to supply the cell's physiological demands. Above a critical density threshold, A $\beta$  pores activate their endocytic internalization by promoting membrane invagination and vesiculation. A $\beta$  pore clearance at the PM restores cell basal activity (Figure 2, left panel).

#### 4 | DISRUPTED A $\beta$ SIGNALING

Membrane composition affects its stiffness and ability to vesiculate. Membrane bending can be disrupted with aging owing to the stiffening associated with cholesterol dyshomeostasis (an AD hallmark).<sup>37</sup> Neuronal EP activation is a specific and extremely early response to AD. Early endosomes, a major site of A $\beta$  peptide generation, are markedly enlarged within neurons in the Alzheimer's brain, suggesting altered EP activity.<sup>38</sup>

The membrane's impaired ability to vesiculate causes accumulation of A $\beta$  pores-like oligomers, altered Ca<sup>2+</sup> transport, and additional membrane stiffening.<sup>39</sup> Alterations in membrane properties can also be responsible for the observed malfunction of other transmembrane receptors involved in the glutamatergic synaptic transmission (e.g., N-methyl-D-aspartate receptor) in AD.<sup>40</sup>

The rigid membrane, with restricted bending ability, may compensate for the stress associated with A $\beta$  accumulation by expelling A $\beta$  inclusions (Figure 2, right panel). MD simulations support this notion.<sup>36</sup> Once removed from the membrane, A $\beta$  seeds may then grow unrestrained (nucleation-dependent polymerization mechanism).<sup>20</sup>

#### 5 | MISLEADING EXPERIMENTAL TRIALS

There are numerous difficulties in experimentally working with A $\beta$ , including the peptide's low endogenous concentration, the dynamic nature of its configurational states, its heterogeneous membrane interactions and co-occurrence of aggregation, membrane permeabilization, and concomitantly induced deformation. It is difficult to capture signals uniquely associated with any of these events.

Single-molecule imaging techniques are commonly employed to examine the interactions of labeled exogenous A $\beta$  with exposed synthetic membranes or live cells. In these studies, A $\beta$  oligomers freely move in solution and interact with the membrane. However, upon APP cleavage, the A $\beta$  peptide can be withheld in the membrane owing to a favorable interaction with cholesterol.<sup>41</sup> Alternatively, secreted A $\beta$  monomers can bind glycolipid headgroups or anionic lipids on the surface and reinsert into the membrane.<sup>42,43,44</sup> Therefore, the measured A $\beta$  in solution is only a fraction of the total concentration. Membrane-embedded A $\beta$  is reasonably stable in a helical

configuration,<sup>45</sup> whereas it is highly prone to form beta-sheets in water.<sup>46</sup> Membrane-embedded and soluble oligomers are probably two different entities and may follow completely different pathways. Thus far, the perceived toxicity associated with exogenous A $\beta$  may result from an experimental artifact rather than representing the real function of A $\beta$  in vivo during the disease process. Furthermore, Saito et al. demonstrated that 60% of Alzheimer's model mice overexpressing mutant APP, for assessing A $\beta$  in vivo, demonstrated artifactual phenotypes.<sup>47</sup> Herein, it is crucial to establish an experimental protocol that can overcome the need for overexpressing A $\beta$  or deal with exogenous A $\beta$ .

In this regard, PET combined with electrophysiological studies has shown promising potential for assessing the progression of synaptic loss in AD patients.<sup>17</sup>

#### 6 | PERSPECTIVE

The involvement of A $\beta$  pores in plastic regulation upon neuronal excitability needs to be verified. A potential strategy may involve examining the increased intracellular Ca<sup>2+</sup> concentration upon synaptic excitation in three different disorders where endogenous A $\beta$  is differently expressed (AD, DS, and ASD). To ascertain the intercession of A $\beta$  pores in Ca<sup>2+</sup>-mediated neuronal protection, the intracellular Ca<sup>2+</sup> concentration should be assessed in the presence or absence of A $\beta$  pore blockers,<sup>48</sup> with A $\beta$  physiologically expressed or silenced by knocking out, at rest, or upon synaptic excitation.

Two other hypotheses need validation: (1) whether A $\beta$  endocytic trafficking is A $\beta$ -mediated and more substantial upon neuronal insults than rest conditions, and (2) whether membrane aging affects A $\beta$ -mediated vesiculation.

The dimension and the number of endosomes with their A $\beta$  content in ASD, DS, and AD can suggest whether A $\beta$  endocytic trafficking/turnover is stimulated in response to synaptic excitation in an A $\beta$  concentration-dependent manner: an increased density of endosomes would be expected in young DS individuals where A $\beta$  is highly expressed, and decreased in the case of ASD where A $\beta$  is poorly expressed, with respect to healthy controls. Assuming that above a critical density threshold, A $\beta$  pores promote membrane vesiculation to maintain a critical A $\beta$ -lipids ratio, endosomes generated in different numbers are, however, expected to be similar in size and A $\beta$  content in DS, ASD, and healthy controls. Instead, larger endosomes should be observed in AD and elderly DS individuals as compared with healthy controls, if A $\beta$ -mediated vesiculation is impaired with aging.

The proof of concepts can have implications for DS and ASD where the anomalous A $\beta$  concentration levels might be therapeutically targeted and adjusted to restore memory and learning activities.

Second, if AD insurgence is provoked by the endosomal accumulation of A $\beta_{42}$  as a result of aging-dependent impaired trafficking, employing drugs for dissolving extracellular A $\beta_{42}$  plaques or blocking A $\beta_{42}$  pores at the PM would fail to resolve the problem. EP may be exploited to deliver such therapeutics in situ.

After decades of unsuccessful therapies targeting A $\beta$ , the hypothesis of the physiological relevance of the amyloid pore in response to neuronal insults opens new perspectives for understanding AD.

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## CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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