PERSPECTIVE

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

Martina Pannuzzo

Laboratory of Nanotechnology for Precision Medicine, Fondazione Istituto Italiano di Tecnologia, Genoa, Italy

Correspondence
Martina Pannuzzo, Laboratory of Nanotechnology for Precision Medicine, Fondazione Istituto Italiano di Tecnologia, Genoa, 16163, Italy.
Email: martina.pannuzzo@iit.it

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 β-secretase and γ-secretase in endosomal compartments (Figure 1).[1] 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.[2] 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.[3] Malinow's and Holtzman's groups demonstrated that increased synaptic activity enhances $A\beta$ secretion, while reduced activity inhibits it.[4,5] In physiological conditions, picomolar concentrations of $A\beta$ increase hippocampal long-term potentiation (LTP), whereas nanomolar concentrations inhibit it.[6] 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.[7] 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.

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

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.[11]

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.[12] Upon neuronal insults, an increase in intracellular calcium (Ca^{2+}) mediated by $A\beta_{42}$ activates the CaMKK-to-CaMKIV pathway to promote neuronal survival.[13] Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) mediated phosphorylation of synapsin S9 dissociates the SV-synapsin-actin ternary complex, inhibiting, in turn, the transport of SVs.[14] Hence, by indirectly suppressing the inter-synaptic vesicle trafficking needed for rapid functional synapse formation and transmission, $A\beta_{42}$ affords protection against neuronal injury.[15]

In early AD, synaptic plasticity is impaired, and synaptic density is reduced.[16,17]

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)[18,19] and are proposed to allow dysregulated Ca^{2+} entry in the cytoplasm of brain cells.[20] Intracellular Ca^{2+} levels are highly regulated to precisely modulate neuronal functions, including membrane excitability, neurotransmitter release, and synaptogenesis. Ca^{2+}-binding proteins (CaBP) contribute to the maintenance of Ca^{2+} homeostasis within neurons. CaBP variants with different kinetics and buffering capacities for Ca^{2+} are differentially expressed across the central nervous system. The selective susceptibility of cholinergic neurons to neurodegenerative insults correlates with a loss of the Ca^{2+}-binding protein calbindin with age and reduced Ca^{2+} buffering capacities.[21] Protracted Ca^{2+} dysregulation leads to an accumulation of reactive oxygen species and, ultimately, apoptosis and neuronal death.[22]

Consistent with this view, Ca^{2+} channel blockers have been evaluated to reverse $A\beta$-induced deficits. However, at least three clinical studies emphasized that older individuals administering Ca^{2+} 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^{2+} 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^{2+} 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[23,24,25] These outcomes suggest that blocking Ca^{2+} entry to restore its physiological intracellular concentration may aggravate the pathological scenario.

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

Considering the critical role of Ca^{2+} 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.[20] Such an argument can be extended to other amyloidosis, where amyloid proteins (including prion, Islet amyloid polypeptide, and α-synuclein) forming Ca^{2+}-permeable pores are considered to be implicated in the onset of spongiform encephalopathies, Type 2 diabetes mellitus, and Parkinson's disease, respectively.[26,27]

Experimental in vivo studies may help unravel the temporal relationship between synaptic plasticity, $A\beta$ accumulation, and Ca^{2+} homeostasis.[17,28]

3 | $A\beta$ TRAFFICKING

In a healthy brain, $A\beta_{42}$ 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.[29] 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 $A\beta_{42}$ can be released in association with exosomes upon the fusion of MVBs with the PM.[30] Furthermore, effective $A\beta_{42}$ uptake by the cell requires both the

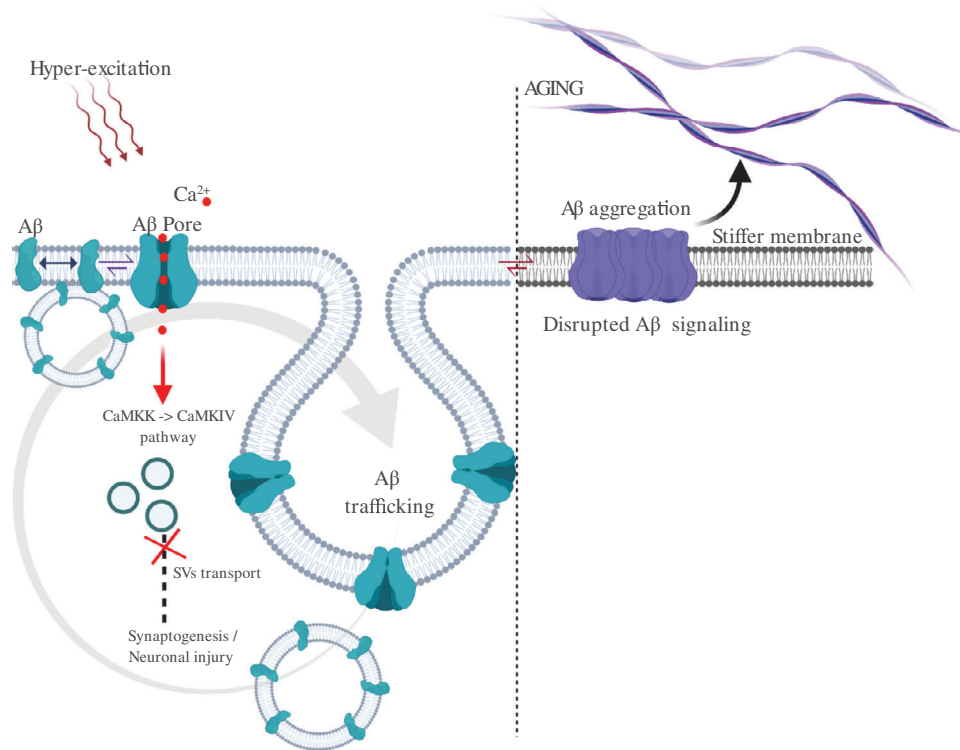**FIGURE 1** Schematic diagram of secretases cleavage of the precursor and derived products
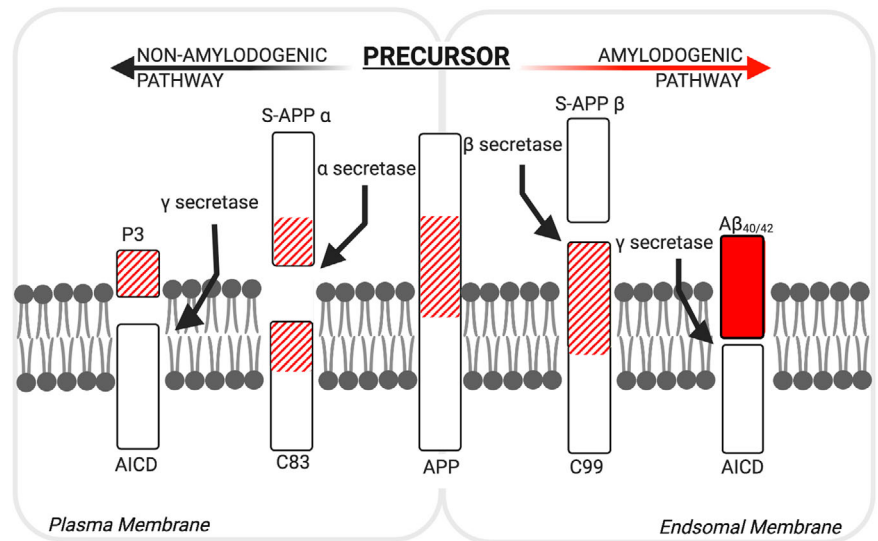

FIGURE 2 Proposed mechanisms for the physiological Aβ pore-mediated regulation of synaptic activity (left panel), and the affected Aβ signaling associated with aging (right panel). Figure created with BioRender.com

formation of an ordered aggregate on the PM and a critical concentration of membrane-bound Aβ_{42}.[31] 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.[32] However, endocytosis at lipid rafts, wherein Aβ is mainly distributed,[33,34] 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

Aβ than undergo invagination.[35,36] In the first scenario, Ca^{2+} ions will freely pass through Aβ pores in the PM, whereas Ca^{2+} influx is reduced owing to Aβ pore clustering in the second scenario.[36]

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

4 | DISRUPTED Aβ 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).[37] Neuronal EP activation is a specific and extremely early response to AD. Early endosomes, a major site of Aβ peptide generation, are markedly enlarged within neurons in the Alzheimer's brain, suggesting altered EP activity.[38]

The membrane's impaired ability to vesiculate causes accumulation of Aβ pores-like oligomers, altered Ca^{2+} transport, and additional membrane stiffening.[39] Alterations in membrane properties can also be responsible for the observed malfunction of other transmembrane receptors involved in the glutaminergic synaptic transmission (e.g., N-methyl-D-aspartate receptor) in AD.[40]

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

5 | MISLEADING EXPERIMENTAL TRIALS

There are numerous difficulties in experimentally working with Aβ, 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 Aβ with exposed synthetic membranes or live cells. In these studies, Aβ oligomers freely move in solution and interact with the membrane. However, upon APP cleavage, the Aβ peptide can be withheld in the membrane owing to a favorable interaction with cholesterol.[41] Alternatively, secreted Aβ monomers can bind glycolipid headgroups or anionic lipids on the surface and reinsert into the membrane.[42,43,44] Therefore, the measured Aβ in solution is only a fraction of the total concentration. Membrane-embedded Aβ is reasonably stable in a helical

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

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

6 | PERSPECTIVE

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

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

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

The proof of concepts can have implications for DS and ASD where the anomalous Aβ 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 $Aβ_{42}$ as a result of aging-dependent impaired trafficking, employing drugs for dissolving extracellular $Aβ_{42}$ plaques or blocking $Aβ_{42}$ 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 Aβ, the hypothesis of the physiological relevance of the amyloid pore in response to neuronal insults opens new perspectives for understanding AD.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

ORCID

Martina Pannuzzo https://orcid.org/0000-0001-8629-0173

REFERENCES

1. Tan JZA, Gleeson PA. The role of membrane trafficking in the processing of amyloid precursor protein and production of amyloid peptides in Alzheimer's disease. *Biochim Biophys Acta Biomembr.* 2019;1861(4):697-712..

2. Näslund J, Schierhorn A, Hellman U, et al. Relative abundance of Alzheimer A beta amyloid peptide variants in Alzheimer disease and normal aging. *Proc Natl Acad Sci U S A.* 1994;91(18):8378-8382.

3. Parihar MS, Brewer GJ. Amyloid-β as a modulator of synaptic plasticity. *J Alzheimers Dis.* 2010;22(3):741-763.

4. Kamenetz F, Tomita T, Hsieh H, et al. APP processing and synaptic function. *Neuron.* 2003;37(6):925-937.

5. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron.* 2005;48(6):913-922.

6. Puzzo D, Privitera L, Leznik E, et al. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci.* 2008;28(53):14537-14545.

7. Cramer N, Galdzicki Z. From abnormal hippocampal synaptic plasticity in down syndrome mouse models to cognitive disability in down syndrome. *Neural Plast.* 2012;2012:101542.

8. Wilson JF, Lodhia V, Courtney DP, Kirk IJ, Hamm JP. Evidence of hyper-plasticity in adults with Autism Spectrum Disorder. *Res Autism Spectr Disord.* 2017;43-44:40-52.

9. Markram H, Rinaldi T, Markram K. The intense world syndrome–an alternative hypothesis for autism. *Front Neurosci.* 2007;1(1):77-96.

10. Ray B, Long JM, Sokol DK, Lahiri DK. Increased secreted amyloid precursor protein-α (sAPPα) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. *PLoS One.* 2011;6(6):e20405.

11. Kimura T, Whitcomb DJ, Jo J, et al. Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos Trans R Soc Lond B Biol Sci.* 2014;369(1633):20130144.

12. Chi P, Greengard P, Ryan TA. Synapsin dispersion and reclustering during synaptic activity. *Nat Neurosci.* 2001;4(12):1187-1193.

13. McCullough LD, Tarabishy S, Liu L, et al. Inhibition of calcium/calmodulin-dependent protein kinase kinase β and calcium/calmodulin-dependent protein kinase IV is detrimental in cerebral ischemia. *Stroke.* 2013;44(9):2559-2566.

14. Park D, Na M, Kim JA, et al. Activation of CaMKIV by soluble amyloid-β1-42 impedes trafficking of axonal vesicles and impairs activity-dependent synaptogenesis. *Sci Signal.* 2017;10(487). eaam8661.

15. Staras K. Share and share alike: trading of presynaptic elements between central synapses. *Trends Neurosci.* 2007;30(6):292-298.

16. Shankar GM, Walsh DM. Alzheimer's disease: synaptic dysfunction and Abeta. *Mol Neurodegener.* 2009;4(1):48.

17. Mecca AP, Chen M-K, O'Dell RS, et al. In vivo measurement of widespread synaptic loss in Alzheimer's disease with SV2A PET. *Alzheimers Dement.* 2020;16(7):974-982.

18. Lin H, Bhatia R, Lal R. Amyloid beta protein forms ion channels: implications for Alzheimer's disease pathophysiology. *FASEB J.* 2001;15(13):2433-2444.

19. Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT Jr. Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature.* 2002;418(6895):291.

20. Sciacca MFM, Kotler SA, Brender JR, Chen J, Lee D-K, Ramamoorthy A. Two-step mechanism of membrane disruption by Aβ through membrane fragmentation and pore formation. *Biophys J.* 2012;103(4):702-710.

21. Riascos D, de Leon D, Baker-Nigh A, et al. Age-related loss of calcium buffering and selective neuronal vulnerability in Alzheimer's disease. *Acta Neuropathol.* 2011;122(5):565-576.

22. Supnet C, Bezprozvanny I. The dysregulation of intracellular calcium in Alzheimer disease. *Cell Calcium.* 2010;47(2):183-189.

23. Maxwell CJ, Hogan DB, Ebly EM. Calcium-channel blockers and cognitive function in elderly people: results from the Canadian Study of Health and Aging. *CMAJ.* 1999;161(5):501-506.

24. Heckbert SR, Longstreth WT Jr, Psaty BM, et al. The association of antihypertensive agents with MRI white matter findings and with Modified Mini-Mental State Examination in older adults. *J Am Geriatr Soc.* 1997;45(12):1423-1433.

25. Wagner G, Icks A, Abholz H-H, Schröder-Bernhardi D, Rathmann W, Kostev K. Antihypertensive treatment and risk of dementia: a retrospective database study. *Int J Clin Pharmacol Ther.* 2012;50(3):195-201.

26. Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem.* 2005;280(17):17294-17300.

27. Volles MJ, Lansbury PT. Vesicle permeabilization by protofibrillar α-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. *Biochemistry.* 2002;41(14):4595-4602.

28. Francesco DL, Koch G. Synaptic impairment: the new battlefield of Alzheimer's disease. *Alzheimers Dement.* 2021;17(2):314-315.

29. Oddo S, Caccamo A, Smith IF, Green KN, LaFerla FM. A dynamic relationship between intracellular and extracellular pools of Aβ. *Am J Pathol.* 2006;168(1):184-194.

30. Rajendran L, Honsho M, Zahn TR, et al. Alzheimer's disease β-amyloid peptides are released in association with exosomes. *Proc Natl Acad Sci U S A.* 2006;103(30):11172-11177.

31. Jin S, Kedia N, Illes-Toth E, et al. Amyloid-β(1-42) aggregation initiates its cellular uptake and cytotoxicity. *J Biol Chem.* 2016;291(37):19590-19606.

32. Kumari S, Mg S, Mayor S. Endocytosis unplugged: multiple ways to enter the cell. *Cell Res.* 2010;20(3):256-275.

33. Kawarabayashi T, Shoji M, Younkin LH, et al.. Dimeric amyloid βprotein rapidly accumulates in lipid rafts followed by apolipoprotein E and phosphorylated tau accumulation in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci.* 2004;24(15):3801-3809.

34. Wesén E, Jeffries GDM, Matson Dzebo M, Esbjörner EK. Endocytic uptake of monomeric amyloid-β peptides is clathrin- and dynamin-independent and results in selective accumulation of Aβ(1-42) compared to Aβ(1-40). *Sci Rep.* 2017;7(1):2021.

35. Pannuzzo M, Raudino A, Böckmann RA. Peptide-induced membrane curvature in edge-stabilized open bilayers: a theoretical and molecular dynamics study. *J Chem Phys.* 2014;141(2):024901.

36. Pannuzzo M. On the physiological/pathological link between Aβ peptide, cholesterol, calcium ions and membrane deformation: a molecular dynamics study. *Biochim Biophys Acta.* 2016;1858(6):1380-1389.

37. Larbi A, Douziech N, Dupuis G, et al. Age-associated alterations in the recruitment of signaltransduction proteins to lipid rafts in human T lymphocytes. *J Leukoc Biol*. 2004;75(2):373-381.

38. Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA. Endocytic pathway abnormalities precede amyloid β deposition in sporadic Alzheimer's disease and down syndrome: differential effects of APOE genotype and presenilin mutations. *Am J Pathol*. 2000;157(1):277-286.

39. Lulevich V, Zimmer CC, Hong H-S, Jin L-W, Liu G-Y. Single-cell mechanics provides a sensitive and quantitative means for probing amyloid-beta peptide and neuronal cell interactions. *Proc Natl Acad Sci U S A*. 2010;107(31):13872-13877.

40. Li S, Jin M, Koeglsperger T, Shepardson NE, Shankar GM, Selkoe DJ. Soluble Aβ oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. *J Neurosci*. 2011;31(18):6627-6638.

41. Di Scala C, Yahi N, Lelièvre C, Garmy N, Chahinian H, Fantini J. Biochemical identification of a linear cholesterol-binding domain within Alzheimer's β amyloid peptide. *ACS Chem Neurosci*. 2013;4(3):509-517.

42. Bokvist M, Lindström F, Watts A, Gröbner G. Two types of Alzheimer's β-amyloid (1-40) peptide membrane interactions: aggregation preventing transmembrane anchoring versus accelerated surface fibril formation. *J Mol Biol*. 2004;335(4):1039-1049.

43. Rondelli V, Salmona M, Colombo L, et al. Aβ beyond the AD pathology: exploring the structural response of membranes exposed to nascent Aβ peptide. *Int J Mol Sci*. 2020;21(21):8295.

44. La Rosa C, Scalisi S, Lolicato F, Pannuzzo M, Raudino A. Lipid-assisted protein transport: a diffusion-reaction model supported by kinetic experiments and molecular dynamics simulations. *J Chem Phys*. 2016;144(18):184901.

45. Pannuzzo M, Raudino A, Milardi D, La Rosa C, Karttunen M. α-helical structures drive early stages of self-assembly of amyloidogenic amyloid polypeptide aggregate formation in membranes. *Sci Rep*. 2013;3(1):2781.

46. Cerf E, Sarroukh R, Tamamizu-Kato S, et al. Antiparallel beta-sheet: a signature structure of the oligomeric amyloid beta-peptide. *Biochem J*. 2009;421(3):415-423.

47. Saito T, Matsuba Y, Yamazaki N, Hashimoto S, Saido TC. Calpain activation in Alzheimer's model mice is an artifact of APP and presenilin overexpression. *J Neurosci*. 2016;36(38):9933-9936.

48. Martinez Hernandez A, Urbanke H, Gillman AL, et al. The diphenylpyrazole compound anle138b blocks Aβ channels and rescues disease phenotypes in a mouse model for amyloid pathology. *EMBO Mol Med*. 2018;10(1):32-47.