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Review Article

Synaptic vesicle cycle and amyloid β: Biting the hand that feeds

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Abstract The synaptic vesicle cycle (SVC) holds center stage in the biology of presynaptic terminals. Through recurrent exocytosis and endocytosis, it facilitates a sequence of events enabling chemical neurotransmission between functionally related neurons. As a fundamental process that links the interior of nerve cells with their environment, the SVC is also critical for signaling and provides an entry route for a range of pathogens and toxins, enabling detrimental effects. In Alzheimer's disease, the SVC is both the prime site of amyloid β production and toxicity. In this study, we discuss the emerging evidence for physiological and pathological effects of Aβ on various stages of the SVC, from postfusion membrane recovery to trafficking, docking, and priming of vesicles for fusion and transmitter release. Understanding of the mechanisms of Aβ interaction with the SVC within the unifying calcium hypothesis of aging and Alzheimer's disease should further elucidate the fundamental biology of the presynaptic terminal and reveal novel therapeutic targets for Alzheimer's disease and other age-related dementias.
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Keywords: Amyloid β; Presynaptic terminal; SNARE complex; Exocytosis; Transmitter release; Neuromodulation

1. Alzheimer's disease as a synaptic pathology

Alzheimer's disease (AD) is a chronic neurodegenerative brain disorder and the most common cause of dementia in the elderly. Progressive depositions of amyloid plaques and neurofibrillary tangles together with degeneration of neurons and synapses in selected brain areas are the most recognized histopathological features of the disease. From histochemical and functional studies, it emerges that the extent of synaptic loss in AD correlates closely with cognitive decline and memory deficit, with dysregulations of neuronal calcium and subtle impairments in synaptic function detectable from early preclinical stages, before the emergence of plaques and neurofibrillary tangles [4,5,60,133]. In the cerebral cortex, a 25%–35% decrease in synaptic connections has been reported within the first 2–3 years of clinical AD, while in the hippocampus these numbers exceed 50% [5,28]. Elucidating the mechanisms of synaptic impairments, thus, are of special interest for a better understanding of AD pathobiology and early therapeutic intervention, before slowing down the onset of irreparable damage with synaptic loss and cognitive decline [27,46].

According to the amyloid hypothesis of AD [45,47], synaptic impairments are triggered by a pathological increase in the amyloid β (Aβ) level in the brain, with soluble oligomers of Aβ42 known to be especially detrimental. Among the best-characterized negative effects of Aβ, the dysregulation of Ca^{2+} homeostasis and disruption of the fine balance between a wide range of kinases and phosphatases are of special relevance to the synaptic deficit and altered neuronal excitability [8,12,43]. Most reports of

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the synaptic effects of Aβ have been focusing on the postsynaptic side, with impairments of NMDA [126], metabotropic GluR5 [109], and M1 muscarinic cholinergic [37] receptors as well as deregulation of insulin and insulin growth factors [77], ephrin [24] and neurotrophin signaling [26,90]. The stimulation of Fyn kinase downstream of NMDAR and PrP activation appears to hold centre stage in the postsynaptic toxicity of Aβ, causing collapse of dendritic spines and synaptic degeneration [21,137]. Misplacement of microtubule-associated tau protein from axon to dendrites also contributes toward postsynaptic deficits with loss of dendritic spines, leading to degeneration of synaptic connections [50,149]. Reports also suggest a key role for GSK3β, CDK5, and other kinases in postsynaptic pathology of AD [25,86,114].

The presynaptic facets of AD, in the meantime, remain poorly elucidated, despite mounting evidence implying axon terminals as the prime site for Aβ production and the starting point of synaptic pathology [89,120]. Results of human and animal AD model studies demonstrate considerable changes in the expression and functions of presynaptic proteins, attributed in parts to direct effects of Aβ on the synaptic vesicle cycle (SVC) (Box 1). In this study, we present a detailed account of Aβ interference with different stages of SVC and transmitter release. Discussed herein, Aβ-related changes in presynaptic biology suggest a considerable overlap between the physiological and pathological effects of Aβ, unveiling numerous previously unrecognized challenges and therapeutic opportunities.

2. Modulation of presynaptic functions by Aβ

Discovery of the positive correlation between the cognitive decline and synaptic loss associated with AD [30,133] prompted penetrating research into the effects of Aβ on synaptic mechanisms [97,101]. Until recently, the general consensus was that at high dose, both, natural and synthetic Aβ oligomers suppress synaptic transmission and plasticity [39,71,121,122]. These effects mostly induced under experimental settings by application of exogenous Aβ have been ascribed in part to disruption of SVC and related changes in presynaptic release [57,59]. In extreme cases, over 50% reduction in the frequency of miniature excitatory postsynaptic currents has been observed in brain slices upon acute exposure to Aβ oligomers, implying a potent presynaptic site of action [121]. More recently, the focus has shifted on the effects of endogenous Aβ, with several reports demonstrating that both, the production and secretion of Aβ into the extracellular space is tightly controlled by neuronal activity (Box 1). Within the intact brain, strong association between Aβ secretion and synaptic functions has been observed during pathological events, such as epileptiform activity induced by electrical stimulation [23] and under certain type of physiological activity of brain circuits [53]. Such effects of Aβ were considered as part of a feedback loop that controls local and global neuronal excitability and circuit dynamics.

Detailed analysis of the dose dependence of Aβ effects revealed that at low amounts, Aβ can also act as a positive regulator of presynaptic activity, enhancing the neurotransmitter release probability and increasing the neuronal excitability [2]. The facilitator effects of low Aβ dose on excitatory transmission does not involve postsynaptic NMDAR and AMPAR currents, but has shown dependence on activation of α7–nicotinic acetylcholine receptor, in agreement with the presynaptic action site [81,103,104]. From these studies, it emerges that the directionality of Aβ effects in addition to the dose also depends on the site of action. While in the first instance, the presynaptic modulator effects of exogenous Aβ42 on transmitter release were thought to be mediated only via stimulation of presynaptic α7–nicotinic acetylcholine receptor and downstream changes in the presynaptic calcium [32,139], other mechanisms underlying the presynaptic effects have been subsequently also considered. In terms of the action mode, it is important to note that both local autocrine and long-range paracrine action of Aβ on synaptic transmission have been documented, with potent effects on the strength of synaptic transmission and on the density of synaptic connections described [52,135,139] (Fig. 1A and B).

Soluble Aβ is present in the healthy brain, with its physiological levels in rodents estimated to be within the picomolar range [104,117]. In healthy humans, the concentrations of Aβ40 and Aβ42 in the cerebrospinal fluid are ∼1.5 and ∼2.0 nM, respectively [38]. It is noteworthy that while the level of Aβ in the cerebrospinal fluid of preclinical AD exceeds that of physiological, with the emergence of amyloid plaques and a cognitive deficit of clinical AD, the concentration of Aβ in the CFS declines [14,38,140]. The impact of such slow changes in endogenous Aβ levels on synaptic transmission in the human brain remains to be shown. Evidence from amyloid precursor protein (APP)-KO [118], PS1-KO [116], or BACE1-KO mice [68] lacking endogenous Aβ shows that both synaptic transmission and plasticity are notably reduced. Likewise, pharmacological inhibition of BACE1 caused a reduction in dendritic spine formation and synaptic plasticity in the cerebral cortex and hippocampus [36]. These findings agree with the positive effects of thiorphan (inhibitor of Aβ degradation) on the frequency of miniature excitatory postsynaptic current in mouse brain slices [2] (Fig. 1C and D). While in all these reports, the presynaptic effects of Aβ are viewed as a result of activation of surface receptors, the direct influence of intracellular Aβ42 oligomers injected into axon terminals, causing a blockade of synaptic transmission, has also been also documented [79] (Fig. 1E and F). Unchanged presynaptic Ca^{2+} currents and reduction in the size of the docked synaptic vesicle pool imply direct negative effects of intracellular Aβ with the SVC. As discussed in the following sections, behind these effects underlie Aβ action upon all major steps of the SVC, from postfusion membrane recovery to synaptic

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Box 1
Synaptic vesicle cycle and Aβ production

Based on the results of pioneering research into the mechanisms of neurotransmission at frog neuromuscular junctions, Katz and Fatt propose the quantal hypothesis [35,56]. According to this model, neurotransmitters at the presynaptic terminals of neurons are stored and released in small and relatively constant packages. Shortly after this landmark discovery, membrane-bound synaptic vesicles were visualized in the synaptic terminals of neurons using electron microscopy [96]. Follow-up biochemical and molecular biological studies revealed further mechanistic details of the storage and release of transmitters at axon terminals, summed up in the modern hypothesis of the synaptic vesicle cycle (SVC) [127], which present neurotransmitter secretion as a highly complex and multistep process. It starts with the loading of synaptic vesicles with transmitters followed by their trafficking and docking at specialized release sites known as active zones. Therein, synaptic vesicles become primed for rapid calcium-dependent fusion with the surface membrane to discharge their content into the synaptic cleft. The latter depends upon specific interactions of vesicular and target membrane-associated soluble N-ethylmaleimide sensitive factor attachment receptor proteins (v- and t-SNAREs) such as VAMP-1/2, SNAP-25, and Syntaxin-1/2 and an array of chaperone and regulator proteins [51,92]. This step is followed by rapid recovery of the synaptic vesicle membrane by clathrin-coated pits, which after shedding the propitious coat are recycled to the interior of the presynaptic terminal for refilling and preparation for the next cycle. The entire process of SVC is known to take approximately 60 seconds, with >90% of the time occupied by the recovery of the presynaptic membrane and transmitter reuptake [10].

The SVC is also the primary site for the amyloid β (Aβ) production [80,134]. Enriched at presynaptic terminals, amyloid precursor protein (APP) and fragments of APP are known to play major roles in cell-cell adhesion, synaptic signaling, and stability of dendritic spines. Reports investigating the mechanisms relating neuronal activity with Aβ production showed that SVC is essential for amyloidogenic processing of APP [44,108]. Indeed, while APP undergoes nonamyloidogenic cleavage on the cell surface, it is the internalization of APP within clathrin-coated vesicles that facilitates amyloidogenic processing of APP catalyzed by β- and γ-secretase (BACE1 and γ-secretase complex), resulting in Aβ production and release [76,138]. This process was shown to depend on regulated exocytosis and associated with it clathrin-dependent endocytosis [22,41,95] and involves membrane lipid rafts, which present the first point of contact between BACE1 and APP [1,34]. In the hippocampus of Tg2576 transgenic mice, SVC is responsible for over 70% of released Aβ in the interstitial fluid, with direct infusion of a dynamin dominant-negative inhibitory peptide (dynamin-DN), with pharmacological blockade of synaptic activity inhibiting both production and secretion of Aβ [22,23]. The remaining fraction of Aβ release appears to rely on housekeeping recycling of the surface membrane [44,67]. These observations agree with the results of sensory deprivation and functional brain imaging studies, which revealed strong association of neuronal activity and Aβ lodging in brain circuits [17,95,106,131].

vesicle trafficking, docking, priming, and regulated fusion of vesicles at the active zone.

3. Reaching and wrecking synapses from within

Although most intracellular Aβ is contained within membranous compartments, substantial amounts have also been found in the cytoplasm of neurons [13,42]. The first evidence for intracellular Aβ came shortly after the discovery of Aβ as the main constituent of AD plaques. However, as early studies used anti-Aβ antibodies with cross-reactivity with APP, the validity of conclusions drawn remained a matter of controversy [66]. Interestingly, in autopsy samples tested from individuals between 38 and 83 years of age, Aβ deposition in neurons proved to be age-independent [66]. It is important to note that unlike the bulk of extracellular Aβ terminating at AA40, most intracellular Aβ terminates at AA42 [40,42,130]. There seems to be a close mechanistic link between extracellular and intracellular pools of Aβ, with deposition of extracellular Aβ in plaques causing a reduction of intracellular Aβ [64,85]. From the clinical standpoint, it is important to note that increases in intracellular Aβ can be detected from early, mild cognitive impairment stages of AD, with its levels particularly high in neurons of the hippocampus and entorhinal cortex, two brain regions affected most severely by AD [40,130]. Whether the buildup of intracellular Aβ in diseased brains results from reduced secretion or enhanced reuptake of extracellular Aβ remains to be determined. It is clear, however, that pathological loading of neurons with Aβ occurs primarily when the levels of extracellular soluble Aβ are abnormally high and depends on its specific binding to a range of receptor proteins and membrane biomolecules (e.g., lipids and proteoglycans).

The first and the best-characterized mechanism of receptor-dependent internalization of Aβ is mediated via the α7-nicotinic acetylcholine receptor mechanism [82]. Lipoprotein receptor protein represents the second best-studied receptor that facilitates the uptake of Aβ by neurons, involving additional molecules such as apolipoprotein E (APOE) [16]. In basal forebrain cholinergic Q9

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neurons, p75NTR can mediate the uptake of Aβ [90,93]. Finally, Aβ has been shown to bind multiple scavenger receptors and advanced glycation end products in neurons and glia [29,115] as well as formyl peptide receptor-like-1 expressed in the brain [49]. As both, membrane turnover and receptor internalization are tightly coupled with SVC at axon terminals, presynaptic compartments present the primary site for Aβ entry into neurons. These processes not only can enrich the intracellular membrane-bound organelles with Aβ but are thought also to favor the buildup of Aβ in the cytoplasm, with cytotoxic effects [148,150]. It is noteworthy that the extent of Aβ internalization can be influenced by specific mutations, with especially high intracellular Aβ deposits detected in the brains affected by APPswe (K595M and M596L) [18,111]. Analysis of the intracellular Aβ distribution in SH-SY5Y neuroblastoma cells revealed that monomers of both Aβ40 and Aβ42 can colocalize with markers for RAB-8 (trans-golgi network and golgi), RAB-9 (trans-golgi network and recycling endosomes), LAMP 1/2 (late endosomes and lysosomes), RAB-5 (early endosomes), RAB-3 (exocytosis vesicle marker), and VAMP-2 (synaptic vesicles). Importantly, however, a substantial fraction of Aβ42 granules do not colocalize with any of these, implying cytoplasmic Aβ aggregates [150]. Using high-power electron microscopy, it was shown recently that a large fraction of intracellular Aβ is present in the cytosol [150]. While cellular mechanisms leading to deposition of Aβ therein remain a matter of controversy, the leakage of Aβ from membrane-bound compartments and active export from the ER to the cytoplasm for degradation through the ER-associated protein degradation pathway have been closely considered [69]. It appears that under physiological conditions, only limited amounts of Aβ are transported into the cytoplasm and timely degraded therein. If dysregulated, this process leads to excessive depositions of cytoplasmic Aβ, which interferes with a range of proteins, including the proteasome complex [3,88] and SVC proteins at presynaptic terminals. Analysis of the functional consequences of intracellular Aβ for synaptic functions in the human brain is limited to correlational studies in postmortem tissue. Experimental data from animal models and neuronal cultures, however, show manifold effects of intracellular Aβ with a direct and indirect impact on the neurotransmitter release machinery. Within endosomes, Aβ leads to disruption of endosomal sorting via inhibition of the ubiquitin-proteome system [3]. This mechanism has been discussed particularly in the context of the buildup of tau protein and its abnormal distribution, critical for synaptic functions [87,136]. As noted, proteasome inhibition also accelerates the accumulation of intracellular Aβ, with detrimental effects on the molecular machinery of neurotransmitter release [79,145]. Intracellular Aβ interferes with presynaptic functions also via disruption of mitochondrial biology, depleting presynaptic and axonal mitochondria and changing their size and number [150]. Finally, presynaptic Aβ has been shown to interfere with molecular scaffolds governing the trafficking of synaptic vesicles and their priming for regulated exocytosis [112,144]. While the exact mechanisms underlying these abnormalities remain unclear, the clues gained from recent studies in this direction highlight a considerable variety of mechanisms and functional outcomes.

4. Obstructing SNARE "zippering"

Sharma et al. demonstrated for the first time that in the postmortem AD brains, the level of SNARE complex formation, which is necessary for driving synaptic vesicle fusion at the presynaptic active zone, is significantly reduced [123]. In the absence of changes in expression of individual SNARE proteins in their study, this finding has been interpreted as evidence that Aβ hinders the "zippering" of vesicle SNARE VAMP-2 with target SNAREs syntaxin-1 and SNAP-25 into a four-helical SNARE complex (Fig. 2A). Such effects of Aβ could contribute toward a wide range of synaptic impairments and network dysfunctions found in AD [5,97]. In the search for molecular correlates of this effect, Yang et al. recently analyzed the impact of Aβ42 monomers and oligomers on SNARE complex formation in APP-PS1 mice, using biochemical assays in vitro and a transgenic approach in vivo [144] (Fig. 2B and C). As APP-PS1 mice are engineered to overexpress Aβ but no other AD-related proteins (e.g. tau, presenilin) [48], they are very suitable as a model for exploring specific effects of pathologically enhanced levels of Aβ on SNARE interactions. In APP-PS1 mice, like in humans, the Western blot bands corresponding to the super-stable SNARE complex are significantly reduced, without a change in the expression of SNARE proteins. The outcome of these biochemical experiments is in agreement with functional data, which demonstrate inhibition of exocytosis by intracellular Aβ42 oligomers [79]. Detailed analysis of Aβ interactions with v-SNAREs and t-SNAREs showed that the hampering effects of Aβ42 oligomers on SNARE complex formation were due to its high-affinity interactions with t-SNARE syntaxin 1a, and specifically with the SynH3 motif, known to play a critical role in the formation of four-helical SNARE bundles [100,129] (Fig. 2D and E). Indeed, it is the disruption of the association of syntaxin-1 with VAMP-2 and SNAP-25 that limits the formation of the trans-SNARE complex, essential for setting vesicle fusion into motion.

In similar experiments with Aβ42 monomers, while Aβ42 displayed the ability to interact with syntaxin-1, it failed to prevent the formation of the SDS-resistant SNARE complex or membrane fusion reaction, implying the unique capability of Aβ42 oligomers to interfere with the assembly of SNAREs and exocytosis [144]. Intriguingly, no evidence was found for impairments of synaptic vesicle docking by either Aβ monomers or oligomers, an observation that

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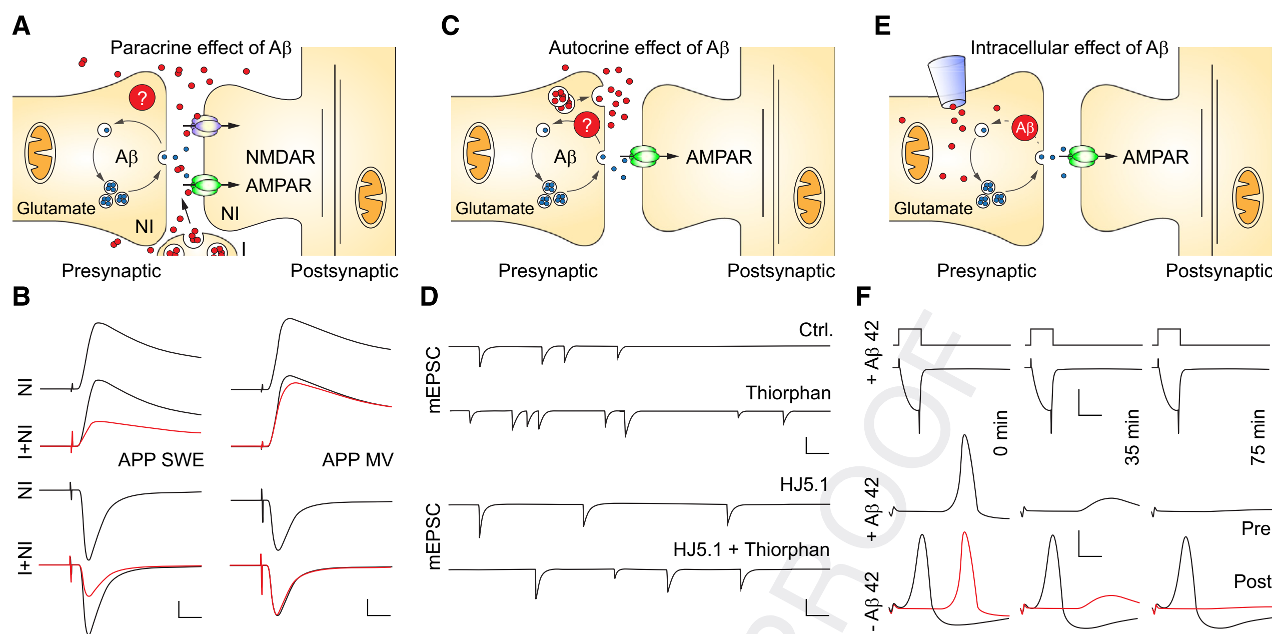

Fig. 1. Three distinct modes of amyloid β (Aβ) αχτιον on neurotransmission. (A and B) Inhibition of the synaptic transmission in hippocampal slices by endogenous Aβ mediated via a long-range paracrine mechanism. (A and B) Overexpression of amyloid precursor protein (APP) harboring Swedish mutation (APP_{SWE}) in selected neurons leads to excessive Aβ release (red granules, A) with suppression of evoked excitatory postsynaptic currents (EPSCs) mediated via NMDA and AMPA receptors in noninfected neighboring neurons (infected, I vs. noninfected, NI) (B). No significant changes in neurotransmission could be detected in slices infected with a BACE-1–resistant mutated variant of APP (APP MV) (B). In the same study, authors report decreased frequency of miniature EPSCs in APP_{SWE} infected slices, implying reduced density of synaptic connections due to Aβ effects (not shown). Adapted with permission from [52]. (C and D) Presynaptic inhibition of neurotransmission by endogenous Aβ mediated via an autocrine mechanism (red granules). Pharmacological blockade of Aβ degradation by thiorphan lowers the frequency of mEPSCs (i.e., presynaptic effects) and can be countered by a monoclonal HJ5.1 antibody against Aβ supplemented to the recording medium (D). Adapted with permission from [2]. (E and F) Inhibition of glutamate release and neurotransmission by Aβ dialyzed in the presynaptic terminal of a giant squid synapse through the patch pipette (E). Complete blockade of synaptic transmission (75 min after Aβ injection), with no changes of presynaptic calcium currents (F, top traces) and action potentials implies selective effects on synaptic vesicle cycle. Adapted with permission from [79].

suggests the differential sensitivity of synaptic vesicle docking and fusion to Aβ [144]. Given the pivotal role of t-SNARE syntaxin-1 in synaptic vesicle docking, the differential effects of Aβ on docking versus fusion have been interpreted as a result of steric hindrance of Aβ oligomers (but not monomers) to "zippering" of SNAREs into a four-helix complex (*cis*-SNARE), while sparing their partial assembly required for docking (*trans*-SNARE) (Fig. 3D and E). In this context, it is worth stressing that presynaptic terminals represent the principal site of Aβ reuptake, which may subsequently leak from early and recycling endosomes into the cytoplasm [66,112]. As mentioned above, in hippocampal neurons, internalized Aβ42 interferes with specific interactions between synaptophysin and VAMP-2, which is essential in priming synaptic vesicles for regulated exocytosis, another major step in SVC [112]. These findings agree with functional measurements detailed below, consolidating the disruptive effects of Aβ on synaptic vesicles trafficking and recovery after fusion.

5. Synaptic vesicle recovery and trafficking

Endocytosis is a critical step in the SVC, which affords recovery of the synaptic membrane after exocytosis. Four main types of endocytosis have been defined, with clathrin-dependent endocytosis enabling the retrieval of synaptic vesicles. In neurons, this process is controlled by a set of regulatory and adaptor proteins (AP-2, AP-180, dynamin, epsin, and others), which promotes the fission, pinching off, and uncoating of the surface membrane followed by formation of synaptic vesicles [113,143]. Considerable evidence suggests that in AD, clathrin-dependent recovery of synaptic vesicles may be severely compromised [141,145], with both genomic and proteomic studies showing also reduced expression of regulator proteins in AD autopsies [145–147]. Indeed, analysis of the expression of dynamin 1, AP180, and synaptophysin across various brain regions showed a notable decrease in their levels in the hippocampal CA1 region and the entorhinal cortex. The expression of AP180 and synaptophysin was also lower in the hippocampal dentate gyrus and CA4 region, as well as in the wider temporal cortex.

Similar studies in APPswe AD transgenic mice revealed reduced dynamin 1, AP180, and synaptophysin expression in the hippocampus, particularly prominent in the CA1 and CA4 subfields [19]. These findings imply that Aβ can act not only as a modulator of exocytosis but is a potent regulator of synaptic vesicle recovery after fusion. It is

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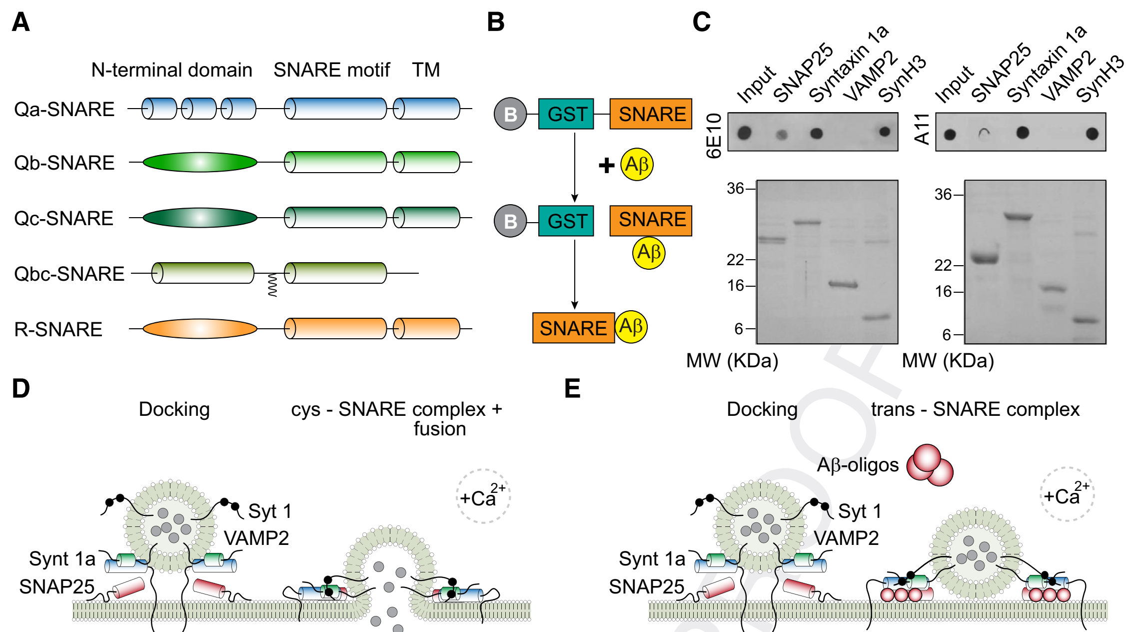

Fig. 2. Amyloid β (Aβ) blocks SNARE-dependent exocytosis. (A) Types of SNAREs with their gross structure schematized. The C-terminal of SNAREs corresponds to the transmembrane domain; the central portion contains the SNARE motif, and the N-terminal of Qa-SNAREs (syntaxin in neurons) possesses antiparallel three-helix bundles. In Qbc SNAREs (SNAP23 and SNAP-25 in neurons), duplicated SNARE motifs are connected by a linker that is frequently palmitoylated (zig-zag line) and anchors the protein to the membrane. During vesicular fusion at nerve terminals, Qbc SNAREs (SNARE23/25 variants) contribute two α-helices to the four-helical core complex, with the other two provided by Qa (syntaxin) and R-SNAREs (VAMP). Adapted with permission from [92]. (B and C) Schematics of the GST pull-down assay for Aβ binding to individual SNARE proteins (B, glutathione agarose bead) with the amounts of Aβ monomers bound to the SNARE proteins measured by dot blot using anti-β amyloid antibody (6E10, top) and a similar experiment conducted using anti-amyloid oligomer antibody (A11, top). SNARE proteins cleaved from GST were visualized by Coomassie blue staining after SDS-PAGE (bottom). Adapted with permission from [144]. (D and E) Schematized illustration of the SNARE-dependent synaptic vesicle fusion and impeding effects of Aβ oligomers on fusion of the synaptic vesicle with the surface membrane, which prevents the zippering of the SNAREs, leading to exocytosis block.

interesting to note that reduction of dynamin 1 and synaptophysin was also observed in rats injected with Aβ42 into the hippocampus, with RNA and protein levels of AP180, however, unchanged or even elevated. Also, changes in dynamin-1 and AP180 expression under chronic elevation of the Aβ levels were far more pronounced than those of

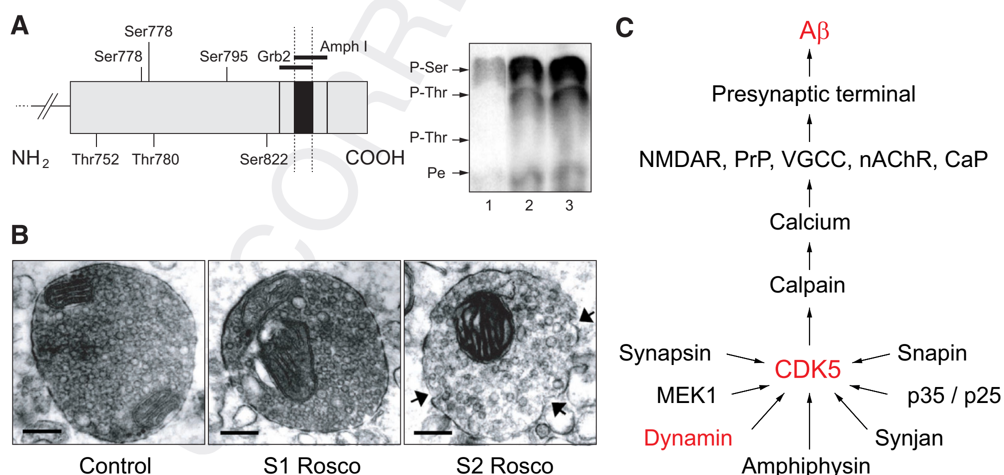

Fig. 3. Regulation of postfusion recovery of the presynaptic membrane by amyloid β (Aβ). (A) Potential phosphorylation sites for CDK5 plus the PKC phosphorylation site (Ser 795) in the proline-rich domain of dynamin 1 implicated in Aβ-induced block of endocytosis. Binding sites for the SH3 domains of Grb2 and amphiphysin-1 are also indicated. (B) High-power electron micrographs of synaptosomes incubated with or without 100 μM CDK5 antagonist roscovitine (Rosco). Typical morphology found in control untreated synaptosomes at rest (left), S1, and repolarization or S2. The terminals are filled with synaptic vesicles and contain a mitochondrial profile sealed in the plasma membrane. After depolarization in the presence of roscovitine (S2), the morphology is essentially unaltered but leads to a greatly depleted vesicle population and numerous plasma membrane invaginations (arrows) suggestive of an endocytic block. Scale bar represents 0.2 μm. Adapted with permission from [98,132]. (C) Putative mechanisms and key molecular players mediating CDK5-dependent effects of Aβ on synaptic membrane recovery at the presynapse. Adapted with modifications from [54].

synaptophysin, implying that these effects result from downregulation of AP180 and dynamin 1 by Aβ42 and are not a by-product of synaptic degeneration [59]. Assessment of the functional effects of Aβ42 on stimulation-induced endocytosis and recovery of the synaptic membrane in neuronal cultures with FM1-43 fluorescence dye showed that in the presence of Aβ42, the dye uptake coupled with synaptic activity is markedly reduced [19]. This effect was attributed to the inhibition of dynamin 1 activity, as in similar experiments in control neurons (i.e., in the absence of Aβ42) post-exocytosis membrane recovery was not altered.

Reduced dynamin 1 and AP180 activity are likely to contribute toward the altered size of synaptic vesicle pools in AD mouse models and AD autopsies [19,99]. At a typical presynaptic terminal, three discrete but interconnected pools of synaptic vesicles can be distinguished: (1) releasable, (2) recycling, and (3) reserve, with all three of major relevance to synaptic physiology and plasticity [110]. Using vGlut-pHluorin fluorescence protein expression in cultured neurons, Park et al. observed a reduction of recycling and increase of the synaptic vesicle reserve pool by Aβ42 oligomers [98]. These effects occur without alterations in the total synaptic vesicle content of axon terminals. In a separate experiment, the effects of Aβ42 oligomers on the rate of endocytosis in individual synaptic boutons were analyzed, with endocytosis and reformation of synaptic vesicles found to be slowed down, with only 50% of released vesicles recycled back to the releasable pool [98]. Although the molecular mechanisms of delayed recovery of synaptic vesicles remain unknown, CDK5, known to regulate synaptic vesicle pool size [63], has been suggested to play a major role [98,132] (Fig. 3A–C). Aβ oligomers are known to activate CDK5 via calpain, with levels of CDK5 in AD brain autopsies reported being significantly enhanced [70,105]. Failure of Aβ42 oligomers to inhibit endocytosis and alter the size of recycling and resting vesicle pools in the presence of CDK5 inhibitors is consistent with this mechanism [98]. Other factors such as depletion of dynamin 1 by Aβ oligomers [58] with knock-on effects on synaptic vesicle recovery and trafficking as well as vesicle exchange between different pools could also potentially contribute.

6. Voltage-gated calcium influx and Aβ

Calcium is a ubiquitous regulator of neuronal functions, with intracellular Ca^{2+} dynamics tightly regulated by multiple presynaptic and postsynaptic mechanisms. The causative link between dysregulation of calcium homeostasis, brain aging, and AD was first proposed by Z. Khachaturian [60–62] in the calcium hypothesis of AD and brain aging [4,60]. According to this hypothesis, sustained disruptions of intracellular Ca^{2+} signaling are not only the key for triggering aging-related adverse changes in the functioning of neurons but are also crucial for the initiation of pathological processes underlying synaptic deficit and neurodegeneration of AD (Box 2). In turn, both Aβ monomers and oligomers have been shown to disrupt Ca^{2+} homeostasis, with oligomers capable of forming calcium-permeable pores, causing a pathological increase in the level of intracellular calcium, with cytotoxicity [6,7,65]. These reports received backing from biophysical studies, which demonstrated activation of transmembrane cation currents in cells exposed to Aβ oligomers [33,72], capable of disrupting the fine ionic balance and causing oxidative stress, which can lead to cell death. Increase in presynaptic Ca^{2+} induced by Aβ pores is expected also to interfere with neurotransmitters release [31,99,124].

Perforation of the surface membrane, however, is not the only mechanism of calcium dysregulation by Aβ at the presynaptic terminals. When in excess, both monomers and oligomers modulate biophysical properties of voltage-activated calcium channels as well as calcium release from the endoplasmic reticulum, with knock-on effects on transmitter release and synaptic plasticity [125,128]. Rapid and localized calcium influx mediated via voltage-gated N- or P/Q-type calcium channels at presynaptic terminals are the principal regulators of transmitter release, coupled also to postfusion membrane recovery. In all neuron types tested, Aβ modulates voltage-gated calcium currents [83]. In cortical synapses, for instance, low concentrations (10 nM) of Aβ42 oligomers enhance spontaneous release of glutamate and noradrenaline, which can be reversed by an N-type channel blocker ω-conotoxin GVIA but not by ω-agatoxin or diltiazem, inhibitors of P/Q- and L-type calcium channels [15]. Under prolonged Aβ42 treatment, however, significant inhibition of N-type calcium was also observed [55]. In cerebellar granule cells, at potentials positive to 0 mV, calcium currents are significantly enhanced by prolonged exposure to 1-μM Aβ [102]. The increase in calcium currents was accompanied by a 5-mV shift in channel activation in the positive direction and increased deactivation. Similarly, in cortical neurons, inhibition of L-type channels with nifedipine (2 μM) did not prevent the rise in calcium channel currents or affect current activation and deactivation. N-type calcium channel antagonist ω-conotoxin GVIA (1 μM), on the other hand, abolished the augmentation of Ca^{2+} current and deactivation rate changes but did not prevent the shift in the current activation curve. These data suggest that Aβ could exert presynaptic effects via disruption of calcium influx through N-type calcium channels. Subsequent reports showed that in cortical neurons, both monomeric and oligomeric Aβ40 facilitate P-type calcium currents [73], while their effects on N-type channels depend on the Aβ aggregation state, causing bilateral changes [107]. Interestingly, a more recent study in cultured hippocampal neurons demonstrated that at micromolar dose, Aβ42 oligomers inhibit P/Q-type calcium currents [84], while the same preparation of Aβ increased P/Q-type calcium channel currents expressed in *Xenopus laevis* [78]. This discrepancy could be due to the fact that in an expression system, enhanced effects of Aβ40 oligomers are due to direct interaction of

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Box 2
Calcium hypothesis of Alzheimer's disease

The principal assertion of the calcium hypothesis of Alzheimer's disease (AD) is that sustained dysregulation of intracellular Ca^{2+} dynamics plays a critical role in the pathobiology of AD and other age-related dementias [60,74]. In addition to disruption of synaptic function and plasticity mechanisms, deregulation of Ca^{2+} with impairments of downstream signaling are viewed as the key to the neurodegenerative process in AD [11,43]. As a ubiquitous regulator of cellular functions, changes in Ca^{2+} handling are expected to affect virtually all major molecular processes in neurons, including amyloid precursor protein hydrolysis, phosphorylation of MAP tau, mitochondrial functions, synaptic plasticity and dendritic spine dynamics. There is growing consensus that abnormal Ca^{2+} handling could play a crucial role in the synaptic deficit during the mild cognitive impairment stage of AD, well before the onset of degenerative changes [9,20]. Initial alterations in synaptic functions are thought to be related to the reversible remodeling of intracellular Ca^{2+} signaling, which may switch brain circuits from memory storage to memory loss mode. Perhaps the most progressive tenet of the calcium hypothesis of AD is that it offers a heuristic framework unifying AD with physiological brain aging and related changes in neuronal mechanisms [4,62]. The recently updated calcium hypothesis of AD seeks to reinforce the association between age-related cognitive decline and dementia of AD [4]. It postulates that decline in synaptic and neuronal functions associated with normal aging could contribute toward the abnormal performance of neural circuits in the AD, thus presenting a unifying view on AD and aging, which could explain a spectrum of pathophysiological and histopathological changes leading to cognitive deficit and neuronal loss in the elderly. Similar to AD, aging displays compromised neuronal and synaptic energetics, heightened stress susceptibility, metabolic impairments, DNA damage, a deficit of lysosomal functions, as well as reduced Ca^{2+} buffering and sequestration by endoplasmic reticulum and mitochondria. From the therapeutic standpoint, age- and AD-related remodeling of Ca^{2+} signaling and synaptic plasticity mechanisms and particularly long-term depression are of special interest, given their relevance to encoding or erasing of traces of synaptic memory [75,119]. In effect, sustained alterations in Ca^{2+} signaling change the functional states of synapses, with disruptive effects on storage and processing of information by neural circuits and the brain as a whole. Due to the reversible nature of these changes, restoration of Ca^{2+} dynamics via dietary restrictions, physical exercise, and intellectual challenges, as well as via pharmacological means hold the potential of forestalling the progression of brain aging and AD.

Aβ with the Cav2.1 α-subunit of the P/Q-type channel, in the absence of axillary subunits [83]. Functional measurements of the effects of Aβ42 oligomers via Ca^{2+} channels showed inhibition of spontaneous postsynaptic currents, an outcome that could be partly attributed to the reduction of calcium-dependent transmitter release at presynaptic terminals [84]. In light of the key regulatory functions of N- and P/Q-type calcium currents in transmitter release, changes in presynaptic Ca^{2+} currents induced by Aβ are expected to have a major impact on synaptic transmission and plasticity mechanisms, with knock-on effects on neural circuit dynamics and information processing.

7. Concluding remark

The SVC plays a twofold role in the pathobiology of AD. On one side, it facilitates the amyloidogenic processing of APP by β-secretase BACE1 and γ-secretase complex, leading to Aβ production and release. On the other hand, it presents the prime target for Aβ toxicity, resulting in failure of synaptic function and leading to degeneration of synaptic connections. Throughout this review, we considered and discussed emerging data illustrating the effects of Aβ on major steps in the SVC, from postfusion membrane recovery to vesicle trafficking, docking, and fusion as well as on presynaptic voltage-gated calcium currents. Conceivably, the most important notion that threads across most studies reviewed here is that SVC (and neurotransmitter release) is subject to regulation by both, extracellular and intracellular Aβ. In fact, the early onset of functional changes associated with the rise in intracellular Aβ, in the absence of extracellular amyloid deposits, emerges to be of major relevance to cognitive impairments and changes in neural network dynamics in preclinical AD. At more advanced stages, these alterations become aggravated by the added effects of extracellular Aβ, disturbing all major synaptic functions and plasticity mechanisms, leading to the collapse of dendritic spines and loss of synaptic connections. From the above discussion, it follows that in addition to the most widely used therapeutic approaches targeting Aβ production and clearance mechanisms by developing specific APP protease inhibitors and anti-Aβ immunotherapies, modulators of Aβ uptake mechanisms and presynaptic Ca^{2+} channel functions as well as inhibitors of the translocation of Aβ into the neuronal cytosol could hold major therapeutic potential. Dampening the hyperactivity of cortical circuits and acceleration of the extracellular glutamate clearance are other areas of potential interest, given the tight coupling between the synaptic activity with Aβ release and plaque formation [91,95,142]. While still at the premature stage, research into the "Frankensteinian" drama unfolding at axon terminals, where the product of synaptic activity Aβ relentlessly

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degrades all core synaptic functions and mechanisms has already shown great promise for clarifying major facets of the pathobiology of AD, for better understanding and management of this highly complex brain disorder.

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RESEARCH IN CONTEXT

1. Systematic review: As a fundamental neurobiological process that links the interior of neurons with their external environment, the synaptic vesicle cycle provides an entry route for a range of pathogens and toxins, including amyloid β (Aβ), into neurons, to enable their signaling and toxic effects.
2. Interpretation: This study critically reviews the recent evidence for the physiological and pathological effects of the Aβ peptide on various stages of the synaptic vesicle cycle. Effects of several forms of Aβ on different stages of the synaptic vesicle cycle, from postfusion membrane recovery to trafficking, docking, and priming of vesicles for fusion and transmitter release are discussed.
3. Future direction: Future research and interpretation of the mechanisms of Aβ interaction with the synaptic vesicle cycle should elucidate the role played by Aβ in the biology of the presynaptic terminal and reveal novel therapeutic targets for Alzheimer's disease.

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