

Review

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Alzheimer's disease: synaptic dysfunction and A β

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Abstract

Synapse loss is an early and invariant feature of Alzheimer's disease (AD) and there is a strong correlation between the extent of synapse loss and the severity of dementia. Accordingly, it has been proposed that synapse loss underlies the memory impairment evident in the early phase of AD and that since plasticity is important for neuronal viability, persistent disruption of plasticity may account for the frank cell loss typical of later phases of the disease. Extensive multi-disciplinary research has implicated the amyloid β -protein (A β) in the aetiology of AD and here we review the evidence that non-fibrillar soluble forms of A β are mediators of synaptic compromise. We also discuss the possible mechanisms of A β synaptotoxicity and potential targets for therapeutic intervention.

Introduction

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys memory and cognitive skills. It is the most common human dementia and as such confers a huge emotional and economic burden on patients, caregivers and society [1]. Age is the most significant risk factor, with the chance for developing AD doubling every five years after 65 [2,3]. However, the disease can also strike in mid-life and so-called early-onset AD (EOAD) is designated as dementia developing before 65 years old. Fortunately EOAD is rare with an estimated incidence of 4.2 per 100,000 persons in the 45-64 year age group [4]. The cognitive and pathological changes evident in EOAD and late onset AD (LOAD) are highly similar with the former apparently an accelerated form of the more common LOAD. Because many EOAD cases have a strong Mendelian inheritance pattern they have proved instructive in identifying key gene products involved in the disease process. Specifically, autosomal dominant

mutations identified in familial AD all appear to converge on altering the processing of the amyloid precursor protein (APP) [5].

The precise onset of clinical AD is difficult to discern but is often manifested as subtle and intermittent deficits in episodic memory. After many months of gradually progressive impairment of first declarative and then also non-declarative memory, other cognitive symptoms appear and slowly advance. Over a further period of years a profound dementia develops that affects multiple cognitive and behavioral spheres [6]. Pathologically, the Alzheimer brain is characterized by atrophy and microscopically there are decreases in the numbers of neuronal cell bodies in the limbic and association cortices and in certain sub-cortical nuclei [7-9] and numerous amyloid plaques and neurofibrillary tangles litter the cerebrum [10-12]. Many studies have examined the relationship between cognitive impairment and plaque and tangle counts and while, in

general, the number of neurofibrillary tangles correlates better with severity of dementia than the number of amyloid plaques, the most robust correlation in the staging of dementia and early AD is the magnitude of synapse loss [13-15].

Synaptic impairment is likely to be the basis of memory loss in AD

The notion that dementia could result from some sort of synaptic degeneration has been with us for over 100 years and was eloquently expressed by Santiago Ramon y Cajal when he suggested that "dementia could result when synapses between neurons are weakened as a result of a more or less pathological condition, that is, when processes atrophy and no longer form contacts, when cortical mnemonic or association areas suffer partial disorganization" [16]. Quantification using electron microscopy or immunohistochemical staining for synaptic markers has documented significant decreases in synaptic density in the association cortices and hippocampus of AD brain [13,14,17-21] and biochemical and immunohistochemical analysis has revealed a similar loss of both pre-synaptic and post-synaptic components [22]. Moreover, the initial decrease in synapse number and density seems disproportionate to the loss of neuronal cell bodies [13,18,23], suggesting that pruning of synaptic endings may precede frank neuronal loss. Indeed, synaptic degeneration appears to be an early event in pathogenesis with synapse loss evident in patients with early AD and mild cognitive impairment (MCI) [14,15,24].

An important feature of the memory impairment associated with AD and MCI is the selective vulnerability of the ability to consolidate new memories; whereas, the capacity to recall information from the distant past is preserved. This selective impairment of recent memory together with the rapid decay of newly acquired information is reminiscent of the deficits seen in the temporal lobe amnesias and in patients with bilateral hippocampal damage [25,26]. Analogously, extensive experimentation has demonstrated that the memory deficits observed in aged rodents are highly similar to those in animals with bilateral lesioning of the hippocampus and that age-dependent memory impairment is associated with decreased hippocampal synaptic plasticity. Specifically, estimation of the number of axospinous synapses per neuron in the molecular layer of the dentate gyrus revealed that aged rats with impaired spatial memory had fewer simple and perforated synapses than did memory-intact aged or young rats [27-30]. Moreover, old animals with good spatial memory exhibited similar levels of synaptic enhancement as young animals, whereas memory-impaired old rats had impaired enhancement [31]. The prominent pathology evident in the perforant pathway in the early stages of AD [32] and the documented importance of the hippocampus in

encoding the memory of recent events [33-35] argue strongly that disconnection of the hippocampal formation is likely to underlie the progressive memory disturbances in AD.

Irrespective of educational or social status few humans will escape the age-dependent memory impairment that is a frequent companion of old age, yet the majority of elderly will not suffer the ignominy of AD. So, what distinguishes MCI and the early periods of AD from non-pathogenic age-dependent memory loss? Clearly the spectrum from healthy ageing to AD is a very broad one, though not necessarily a continuum, with age-dependent deficits often having a strongly frontal involvement [36,37]. However, it has been suggested that AD is an extreme and accelerated version of age-related memory loss [38], but that once this accelerated process is initiated it develops a pathogenic profile not seen in normal ageing. Here we propose that the amyloid β -protein ($A\beta$) is the molecular accelerant that hastens age-related memory decline and triggers AD pathogenesis.

Measures of synaptic plasticity provide a useful read-out to assess AD-related pathophysiology

Hebbs postulate describes a basic mechanism for synaptic plasticity wherein an increase in synaptic efficacy arises from the pre-synaptic cell's repeated and persistent stimulation of the post-synaptic cell [39]. Accordingly, long term potentiation (LTP) and long term depression (LTD) are widely used as models of learning and memory and such processes are believed to play important roles in neural circuits of the brain, with effects lasting for hours, days, or longer [34,35,40,41]. Similar to LTP, the induction of hippocampal LTD requires activation of NMDA receptors and/or metabotropic glutamate receptors (mGluR), depending on the experimental conditions [42,43]. Mechanistically, the balance between synapse potentiation versus depression is thought to depend on alterations in cytosolic Ca^{2+} concentration and the differential activation of certain kinases and phosphatases, such as calcium/calmodulin dependent kinase II (CamKII), calcineurin, and cyclic AMP response element binding protein (CREB) [42,43]. Ultimately, this balance in intraneuronal signalling appears to regulate the stability of the post-synaptic density [44] and the post-synaptic content of AMPA receptors [45,46]. Together, these structural changes dynamically modulate the magnitude of neurotransmission at the synaptic level.

Anatomical studies in normal rodents suggest that the induction of LTP is associated with spine formation and increased spine volume, whereas the induction of LTD results in decreased spine volume and spine elimination [47-49]. Similarly, processing of information for long-term storage requires specific patterns of activity that lead

to modification of synapse structure and eventually to change in neural connectivity [50]. Increases in synapse density and stabilization of dendritic spines in the mouse barrel cortex have been described following whisker stimulation, providing insights into structural changes in synapses associated with learning and memory [51]. It is also well-established that synapses throughout the CNS integrate synaptic activity to maintain and regulate receptors and channels at synapses [40]. Without regulation of synapse activity, maintenance of synaptic proteins is lost, leading to excitotoxicity and degradation of stored memory [40]. Consequently, we believe that studies of synaptic plasticity employing both electrophysiological and morphological measures are essential for developing an understanding of how memory mechanisms are disrupted in disease.

A exists in many different assembly forms

The molecular pathways leading to synapse loss and dysfunction in AD are not well understood, but substantial data indicate that A β may be responsible for these effects [52-54]. Over the past 17 years researchers have built up a detailed picture of the natural economy of brain A β . The steady state level of A β is controlled by its production, degradation and clearance [55,56] and it is proposed that in disease a defect leading to over-production or decreased clearance causes an accumulation of A β . This in turn triggers a pathogenic cascade culminating in the cognitive deficits that characterize AD [57]. Like several other disease-associated proteins, A β has the ability to self-associate [58], and can form an array of different assembly forms ranging from dimers all the way to aggregates of fibrils [59]. Initially, it was assumed that toxicity was mediated by fibrillar A β similar to that present in amyloid plaques. However, the quantity and temporal progression of amyloid plaques do not correlate well with the clinical progression of the disease [20,60,61], thus raising the simple question: if A β causes AD, then why doesn't the amount of A β in the form of amyloid plaques relate to the severity of dementia?

Recent studies suggest soluble non-fibrillar A β assemblies, which go by names such as ADDLs, oligomers, paranuclei, and protofibrils (Table 1) [62-66] may provide the missing link, but as yet the specific form(s) of A β which causes injury to neurons *in vivo* has not been identified. Perhaps the most compelling argument in support of a role for non-fibrillar soluble A β comes from a common sense inspection of available data. Extensive multi-disciplinary research provides incontrovertible evidence that A β plays an important role in AD [57]. This together with the findings that monomer is innocuous and that amyloid plaques alone cannot account for disease has led many to conclude, if it isn't fibrillar A β (akin to that found in amyloid plaques) and it isn't A β monomer then it must be

some other form of A β . Similarly, it seems reasonable that the synaptic and neuronal compromise seen at sites distant from plaques is mediated by an A β species that can readily diffuse and access the space in and surrounding the synaptic cleft.

In recent years biochemical analysis of AD brain have revealed a robust correlation between soluble A β levels and the extent of synaptic loss and severity of cognitive impairment [67-70]. But what exactly constitutes soluble A β is as yet not well-understood. The term soluble A β is an operational definition, embracing all forms of A β that remain in aqueous solution following high speed centrifugation of brain extracts [68-71]. Moreover, the origin of soluble A β is ambiguous. Extraction of A β from brain invariably involves homogenization and consequent cell fracture thus the extracted pool will include truly soluble extracellular A β , extracellular A β loosely associated or in equilibrium with plaques and a portion of intracellular A β . To date, most studies of soluble cerebral A β have employed ELISA methods that cannot disclose the aggregation state of the species detected and for the most part appear to preferentially detect A β monomer [72-76]. Although the detection methods used provide little information about the assembly state of A β the fact that they are not sedimented by ultracentrifugation indicates that they are not mature amyloid fibrils. While a huge amount of data has been gathered concerning the primary sequence of cerebral A β only limited attempts have been made to assess the assembly forms of A β present in human brain. Using aqueous buffer free of detergents or chaotropes, Kuo and colleagues [71] isolated a range of non-fibrillar forms of A β from both AD and control brain. Both sample populations contained a continuous distribution of A β species from monomer up to oligomers in excess of 100 kDa, with the major contribution coming from low-n oligomers ranging from dimers to octamers. In agreement with these results we have found that aqueous extracts of AD brain contain a distribution of A β species which migrate on non-denaturing size exclusion chromatography (SEC) [77] and glycerol velocity gradient centrifugation (GVGC) over a broad molecular weight range (J. McDonald and DMW unpublished). However, given that A β can also bind to other proteins, the molecular weight distribution determined by ultrafiltration, SEC and GVGC cannot be definitively ascribed to homo-oligomers of A β .

In a complementary study, McLean and colleagues extracted samples of frontal cortex and putamen in PBS and centrifuged these at 175,000 g for 30 min [68]. Western blot analysis of the supernates from AD brain revealed the presence of variable proportions of monomeric, dimeric and trimeric A β species [68]. Such SDS-stable low-n oligomers have also been detected in human CSF

Table 1: Water-soluble non-fibrillar A β assemblies

Name	Physical Characteristics	Biological activity
Protofibrils*	β -sheet-rich, curvilinear structures of 6-8 nm in diameter and 5-200 nm in length [63,118]. Under certain circumstances can also form annular structures [160].	Alter neuronal activity [108], block <i>in vitro</i> LTP [116] cause memory impairment and phosphorylation of tau [117].
ADDLs	A mixture of monomer and heterogenous high molecular weight oligomers [120] some of which appear similar to small protofibrils [121].	Block <i>in vitro</i> LTP [64] and cause loss of synapses and [111] at higher concentrations neuronal death [123].
Globulomers	Formed in the presence of SDS or fatty acids they are β -sheet-rich and by AFM appear as spheres of 1-5 nm [161] and elute from SEC with a molecular weight consistent for a globular protein of ~100 kDa.	Bind to hippocampal neurons, inhibit LTP <i>in vitro</i> [162] and block P/Q Ca ²⁺ currents [163].
Spheroids	Spherical structures of 3-20 nm diameter that co-migrate on glycerol gradients with thyroglobulin (669 kDa) [65]. Similar structures have been immunisolated from human brain [164].	Induce activation of GSK-3 β and cell death in cultured neurons [65].
Disulphide cross-linked A β	Isolated cysteine linked dimers of A β that lack significant secondary structure (O'Nuallain and DMW, unpublished) and behave as true dimers on SEC and analytical ultracentrifugation [77].	Block LTP <i>in vitro</i> [77] and <i>in vivo</i> [165] and impair the memory of learned behaviour (Cleary and DMW, unpublished).
Cell-derived SDS-stable low-n oligomers	Present in medium from CHO cells expressing human APP and which migrate on SDS-PAGE and SEC with molecular weights consistent for A β dimers and trimers [95,105].	Block LTP <i>in vitro</i> [104] and <i>in vivo</i> [102] and impair the memory of learned behaviour [107].
A β 56*	An A β -immunoreactive species isolated from brains of APP transgenic mice in the presence of 0.01% NP-40 and 0.1% SDS and which migrates on SDS-PAGE and SEC with a molecular weight comparable to a globular protein of 56 kDa [86].	Causes impairment of spatial memory [86].
Brain-derived SDS-stable A β dimer	Extracted from human brain using aqueous buffer and remain in solution following high speed centrifugation. These species migrate on SDS-PAGE as dimers, but elute from SEC as true dimers and high molecular weight species [77] (McDonald <i>et al.</i> Water- and triton-X100 extracted A β is specific for Alzheimer-type dementia, submitted).	Block LTP <i>in vitro</i> and <i>in vivo</i> and impair the memory of learned behaviour [77].

Besides the forms of A β described above a significant number of other A β preparations have been studied, but since most of these have not been characterized beyond the use of denaturing electrophoresis or reactivity with certain antibodies they are not dealt with here.

* Protofibrils appear prior to detection of mature amyloid fibrils and thus are considered as pre-fibrillar assemblies.

by LC-MS [78] and appear to represent highly stable non-covalently associated dimers of A β ₁₋₄₀ and trimers of either A β ₆₋₄₂ or A β ₁₋₃₅. Higher molecular weight SDS-stable assemblies have not been reported in human CSF or soluble extracts of human brain, but whether the dimers and trimers detected represent true low-n oligomers or are breakdown products of larger SDS-unstable assemblies is unclear. Moreover, the presence of SDS-stable dimers and trimers in the soluble fraction of human brain and in extracts of amyloid plaques [68,74,75,79] suggest that in addition to being the earliest mediators of neuronal dys-

function SDS-stable low-n oligomers of A β may be the fundamental building blocks of insoluble amyloid deposits.

Transgenic mouse models over-expressing human APP develop many facets of amyloid pathology [80,81] and have been studied in an effort to identify toxic A β assemblies [82-87]. Particularly noteworthy evidence supporting soluble forms of A β as the principal mediators of neuronal compromise comes from a report using PDAPP mice in which A β -mediated deficits of memory were

reversed by a single intraperitoneal injection of an anti-A β antibody [88]. In these acute (< 24 hr) experiments, brain amyloid burden was not decreased, suggesting that the antibody was acting on soluble, diffusible species of A β and that neutralization or clearing these small intermediates allowed rapid improvement in object recognition performance.

Using another well-characterized APP transgenic mouse model, Tg2576, Lesne and colleagues reported that in brain extracts from Tg2576 mice ~42 kDa (nonamer) and ~56 kDa (dodecamer) A β species were detected at an age that coincided with the first observed changes in spatial memory [86]. A β monomer, trimer and hexamer were seen at earlier time points and hence were not considered to be associated with a deleterious effect on cognition. Indeed, comparison of spatial memory and the levels of A β monomer, trimer, hexamer, nonamer and dodecamer revealed that only nonamer and dodecamer levels correlated with impairment of spatial memory. Importantly, ventricular injection of purified dodecamer into normal pre-trained wild-type rats dramatically perturbed the memory of learned behaviour [86,89], thus demonstrating that a soluble, brain-derived form of A β can directly mediate brain dysfunction. However, it is unlikely that nonamer and dodecamer alone are the only A β assemblies capable of altering brain function. For instance, Kawarabayashi and colleagues reported that the appearance of SDS-stable dimers present in lipid rafts also coincided with impairment of spatial memory [85]. In addition, the same Tg2576 mice demonstrate poor performance in a hippocampus-dependent contextual fear conditioning assay, decreased spine density in the dentate gyrus, and impairment of long term potentiation (LTP) at ages long before the first detection of A β dodecamer [86,90,91]. Thus, while the appearance of dodecamers correlate with the impairment of spatial memory in Tg2576 mice, it does not correlate with changes in other forms of memory, nor do dodecamer levels correlate with changes in synaptic form and function. Indeed, very recent data support the presence of multiple bioactive A β species in APP transgenic mouse brain [92,93]. In J20 APP transgenic mice, over-expression of neprilysin dramatically reduced total A β levels, but did not alter dodecamer and SDS-stable A β trimer levels nor did it recover spatial reference learning and memory impairments evident in J20 mice. In addition, changes in hippocampal Fos levels and hyperactivity were attributed to a third unidentified species [93]. Similarly, we found the presence of several different A β assemblies in the cerebrum of J20 mice, before, coincident with, and after the onset of detectable synapto-dendritic compromise [94] thus it seems likely that more than one A β species mediates the various synaptic deficits evident in APP transgenic mice.

SDS-stable low-n oligomers bearing some similarities to those detected in human and mouse brain have been detected in the conditioned medium (CM) and/or lysates of a variety of cell lines [73,95-100]. Chinese hamster ovarian (CHO) cells that express mutant (V717F) human APP (referred to as 7PA2 cells) produce and secrete low nanomolar amounts of A β species that migrate in denaturing SDS-PAGE with molecular weights consistent for A β monomer, dimer and trimer [95,101]. Because of the easy maintenance and fast growth rate of 7PA2 cells, 7PA2 CM has become the media of choice to investigate the biological activities of cell-derived SDS-stable low-n A β oligomers. 7PA2 medium containing A β low-n oligomers has a variety of plasticity and memory impairing effects. It can block LTP *in vivo* [102,103] and *in vitro* [104,105], reduce spine density in cultured neurons [100,106] and decrease the density of synapses *in vivo* (Freir *et al.* Cell-derived A β oligomers inhibit synapse remodelling necessary for memory consolidation, submitted). When injected into the lateral ventricle 7PA2 CM or oligomer-containing fractions thereof impair performance on operant lever tasks such as the alternating lever cyclic ratio test [89,107], disrupt working memory in rats tested in a radial-arm maze [99] and show a time-dependent interference with consolidation of avoidance learning (Freir *et al.* Cell-derived A β oligomers inhibit synapse remodelling necessary for memory consolidation, submitted).

Importantly, biologically active non-fibrillar, non-monomeric assemblies of synthetic A β have also been identified and such species of A β have been shown to exert disease relevant toxicities [63,64,94,100,108-112]. Preparations containing toxicity mediating forms of A β are frequently referred to as oligomers, however, by and large, the assembly state of these have been poorly defined and it is very difficult to compare A β preparations used in different studies. Here we will review some of the most commonly encountered and better characterized A β preparations. Protofibrils (PFs) have been characterized using a combination of size exclusion chromatography, quasi-elastic light scattering, electron microscopy (EM) and atomic force microscopy (AFM) and derive their name due to the fact that these structures share some physical similarities with amyloid fibrils but appear prior to the detection of fibrils [62,63]. In solution PFs are highly polydisperse and by EM or AFM range in size from spheres ~5 nm in diameter to curvilinear structures up to 200 nm in length. Their formation is dependent on concentration, pH and ionic strength [113] and they appear to behave as true fibril intermediates in that they can both form fibrils and dissociate to low molecular weight species of A β [63,113]. Annular PFs with external and internal diameters of ~8 and ~2 nm have been detected and seem to be particularly well-populated in preparations of A β peptide bearing the Arctic mutation (E22G) [114]. Under conditions where

there appeared to be little conversion of PFs to fibrils, addition of PFs to rat cortical cultures caused a time-dependent decrease in neuronal viability as measured by LDH release or an increase in Hoechst staining [108,115]. Moreover, PFs caused a dose-dependent inhibition of MTT reduction by primary neuronal cultures that was detectable after only 2 hours of incubation with cells [63], an almost immediate enhancement of electrical activity of neurons [108] and a robust block of LTP [116]. It has also been demonstrated that certain naturally occurring lipids can cause disassembly of fibrils leading to the formation of protofibrils and that when these so-called "backward PFs" are injected into the ventricle can spread rapidly throughout the brain and alter learned behaviour [117].

A β -derived diffusible ligands (ADDLs) which are sometimes simply referred to as A β oligomers were first detected and studied by Mary Lambert, Bill Klein and colleagues [64]. Until relatively recently there was little physical definition of ADDLs. The original report simply documented the detection by AFM of imperfect spheres ~5-6 nm in diameter which appeared highly similar to the smallest PF species detected by AFM and EM [62,118,119]. In contrast solution state analysis of ADDLs using SEC, light scattering and analytical ultracentrifugation indicate that the classic "Klein ADDL preparation" contain a mixture of different sized species and include A β assemblies which are not well-detected by EM or AFM [120,121]. ADDL preparations contain a huge array of species ranging from monomer to large assemblies with molecular weights approaching a million Daltons [120], however, the relative abundance of the high molecular weight species and monomer appear to vary depending on the precise incubation conditions. For instance, Hepler and colleagues found monomer to be the major species in their ADDL preparation [120], whereas Lauren and colleagues found the high molecular weight species to predominant [121].

ADDLs have been shown to cause neuronal death, block LTP [64,109] and inhibit reduction of MTT by neural cell lines [109,122,123]. When incubated with organotypic mouse brain slice cultures for a 24 h period low concentrations of ADDLs (5 nM) caused ~20% loss in cell number whereas at higher concentrations (500 nM) and brief incubation periods (45-60 minutes) cell loss was not evident but a near complete abrogation of LTP was observed [64,109]. Consistent with their synaptotoxic activity, ADDLs have been shown to avidly bind and decorate dendritic arbors of certain cultured neurons [121,124,125] and to mediate dendritic spine loss [111]. The *in vivo* relevance of ADDLs is suggested by the finding that antibodies (M93 and M94) to synthetic ADDLs prevented A β -induced toxicity and that both synthetic A β ₁₋₄₂

and some forms of brain-derived A β bound specifically to the surface of cultured hippocampal neurons [125,126]. These antibodies only weakly reacted with monomer and preferentially recognized assembled forms of A β [126], and dot blot analysis of soluble extracts from human AD brain revealed a dramatic increase in M93 immunoreactivity compared to control brain [125]. Similarly, soluble brain extracts from old Tg2576 mice displayed significant anti-ADDL immunoreactivity [127]. However, given the highly heterogeneous nature of the synthetic ADDL immunogen used to generate M93 it is unclear exactly what species of A β this antibody recognizes.

Besides the use of ADDLs and PFs a number of studies have employed a variety of other non-fibrillar preparations and found these also to be toxic to cultured neurons [65,128-132]. For example, when Deshpande and colleagues examined the effect of 3 distinct assembly forms of synthetic A β , they found that all preparations tested were toxic to primary human cortical neurons, but that the extent and mechanism of toxicity differed [133]. The forms of A β investigated were, high molecular weight oligomers (formed as described by [134]) ADDLs and fibrillar A β . Low micromolar concentrations (5 μ M) of high molecular weight synthetic oligomers caused widespread death within 24 h, whereas similar concentrations of ADDLs took 5-times longer to cause cell loss, and 4-fold higher concentrations of fibrillar A β took 10 days to induce only modest cell death. Both high molecular weight oligomers and ADDLs bound rapidly and avidly to synaptic contacts. High molecular weight oligomers caused activation of the mitochondrial death pathway, but activation of this pathway also occurred when *sub-lethal* levels of the same oligomers were used, suggesting that such changes may underlie defective synaptic activity in neurons that are still viable. In contrast, Wogulis and colleagues found that overt neuronal loss required the presence of both unaggregated soluble A β and fibrillar A β [135]. The apparent conflict between this report and those ascribing toxicity to a specific A β species likely results because of the use of relatively long incubation conditions. During prolonged incubation with neurons intermediates have the potential to further associate and transition to higher ordered aggregates and fibrils have the ability to dissociate. Thus such experimental formats render it difficult to unambiguously ascribe cytopathological activity to a discrete species. Other studies have reported that the application of sub-lethal concentrations of various non-fibrillar A β assemblies can alter neuronal architecture, cause perturbations in axonal transport and reduced cell surface levels of NMDA receptors [130,131,136-139].

The role of low-n oligomers of A β in the range of dimer to tetramer is supported by results from studies using pep-

tides bearing disease-associated and design mutations. A mutation associated with EOAD which results in the deletion of glutamic acid 22 produces A β which *in vitro* exhibits accelerated oligomerization without fibrillation and which potently blocks LTP [140]. In contrast, design mutations substituting glycine for leucine within the GxxxG repeat motif of A β show a greater propensity for aggregation and decrease the abundance of mass spectrometry detected dimers and trimers relative to wild type A β ₁₋₄₂. Accordingly, such mutants exhibit reduced toxicity when incubated with cultured neurons [141]. Using a similar approach Harmeier and colleagues found that A β ₁₋₄₂ peptides containing G33A or G29/33A substitutions formed reduced amounts of low-n oligomers, and that the low-n oligomers formed did not block LTP. This latter finding indicates that aggregation size alone is not the sole determinant of synaptotoxicity, but that the solution structure of assemblies is also critical. Interestingly, in comparison to wild type A β , over-expression of these peptides in *Drosophila* resulted in lower cell loss and reduced eye degeneration [142].

In summary, studies using synthetic A β peptides, A β -containing cell culture medium, APP transgenic mouse and human brain demonstrate that A β toxicity is a complex and multifaceted phenomenon that may be induced by multiple assembly forms of A β and which can result in a variety of effects ranging from reversible changes in synaptic form and function all the way to frank neuronal loss.

Mechanisms of A β -mediated synaptic dysfunction

How A β mediates its effects on synaptic plasticity may take many years to fully understand, but already we know that it is likely to involve three different levels. The first and most important mechanism impugns a toxic gain of function for A β which results due to self-association and attainment of new structures capable of novel interactions that lead to impaired plasticity. The other two scenarios predicate that A β has a normal physiological role. On the one hand insufficient A β could lead to a loss of normal function, whereas excess A β may precipitate dysfunction.

A growing body of literature supports a physiological role for A β in normal synapse function. For instance, in organotypic hippocampal slices, β -secretase activity is increased by synaptic activity and the resulting A β peptides depress excitatory transmission through AMPA and NMDA receptors, suggesting a role for A β in homeostatic plasticity [143]. Indeed in APP transgenic mouse brain there is a strong positive correlation between synaptic activity and the concentration of A β in the interstitial fluid [56] and in humans cerebral A β concentration increases as neuronal function and mental status recover in patients with traumatic brain injury [144]. A β may also have a role in regulating well-described forms of synaptic plasticity.

Specifically, exogenous application of A β partially occludes mGluR-dependent LTD, suggesting shared pathways that elicit and modulate, this form of endogenous synaptic plasticity [145]. It has also been suggested that A β is important for neuronal survival [146] and it has been shown that picomolar concentrations of human A β ₁₋₄₂ increase the magnitude of LTP generated in hippocampus by recruiting an α 7-acetylcholine receptors pathway [147]. While a physiologic role for A β can be surmised from such studies, the A β assembly form responsible for these effects is not known. However, it seems likely that monomeric A β would mediate these effects, not least since monomer would be the predominant form of A β present in freshly reconstituted synthetic peptide or cortical A β . Consequently, pathologic effects on synapse physiology may not only arise from the appearance of higher order A β assemblies, which assume a toxic gain of function, but also rising level of monomeric A β . Thus increased concentrations of A β could lead to synaptic dysregulation mediated by abnormally high levels of monomer and the formation of toxic oligomers. For instance it seems plausible that the increase in non-convulsive seizures observed in APP transgenic mice result due to an A β -dependent imbalance of excitatory and inhibitory activity [148]. In this scenario A β promotes neuronal over-excitability, which results in GABAergic sprouting of inhibitory synapses as a compensatory mechanism. Similarly, using multiphoton imaging of intraneuronal calcium fluctuations high focal levels of A β were shown to increase heterogeneity in the excitability of neurons within 60 μ m of amyloid plaques [149]. Moreover, dendritic spine loss observed within 20 μ m of amyloid plaques in the Tg2576 APP transgenic mouse provides a structural correlate to the physiologic findings [150].

Interactions between A β and various receptors have been shown through biochemical and pharmacologic techniques. Given the profound loss of cholinergic transmission in AD nicotinic and muscarinic acetylcholine receptors have drawn considerable attention. Synthetic A β has been shown to bind the calcium permeable α 7 nicotinic acetylcholine receptors with high affinity [151]. Functionally, this interaction has been proposed to account for the internalization of NMDA receptors through a calcineurin dependent pathway [137,152]. Because these studies focus on post-synaptic cholinergic transmission, it is unclear whether interactions with acetylcholine receptor signalling directly account for the disruption of pre-synaptic cholinergic projections in AD, such as those extending from the nucleus basalis of Meynert.

Hebbian mechanisms of synapse modulation often implicate involvement of NMDA receptors, which transduce the level of synaptic activity into a calcium signal that can

initiate an array of signalling pathways. The current-voltage relationship that is generated by the magnesium block of this receptor is a key determinant in the amount of calcium that enters upon glutamate binding. Whether NMDA receptor activation will result in LTP or LTD appears to be dependent on the amplitude and kinetics of the calcium transient through the channel [153,154]. A number of studies have reported that the effects of A β on the viability, morphology and physiology of neurons are dependent on NMDA receptor activation [106,111,155]. Memantine, an activity dependent NMDA receptor antagonist is used for the treatment of AD. Initially it was proposed to mitigate glutamate excitotoxicity, but it may also block more subtle effects of NMDA receptor activation that lead to synaptic depression and loss.

Activation of metabotropic glutamate receptors (mGluR) recruits a number of signalling pathways (such as p38 MAP kinase or ERK), stimulates release of intracellular calcium stores through generation of inositol triphosphate, or modulates associated ionic channels. These effects may result in post-synaptic AMPA receptor endocytosis [156] and decreased pre-synaptic neurotransmitter release probability [157], both of which decrease synaptic strength. Understanding the contribution of these receptors to A β -mediated synaptic depression is difficult because of the high variability in mechanisms linked to mGluRs across brain regions and developmental time periods. However, various groups have reported that A β mediates synaptic depression and loss through activation of group I mGluRs with p38 MAP kinase and calcineurin as downstream effectors [77,94,104,145,152].

We have found that A β oligomers from a variety of sources can facilitate LTD through both mGluRs and NMDA receptors and that this appears to involve dysregulation of the neuronal glutamate transporter [94]. A role for aberrant glutamate transport is supported by *in vivo* microdialysis experiments in which micro-injection of oligomer-containing 7PA2 CM caused a rapid and massive increase in hippocampal interstitial fluid glutamate [158].

In addition to receptors well-known for their involvement in synaptic plasticity, A β also appears to engage with other synapse proteins. For example, ADDLs appear to perturb insulin signalling by inducing preferential somatic distribution of the receptor via an NMDAR-dependent pathway [159]. Similarly, non-infectious conformations of the cellular prion protein (PrP) have also been demonstrated to bind ADDLs and the role of PrP as a disease-relevant receptor for A β is supported by the finding that knock-out of PrP rescues the block of LTP mediated by ADDLs [121].

But how can the myriad interactions demonstrated between A β and several classes of receptors be explained?

One possibility is that these reports are all correct, but that A β does not directly target a single receptor *per se*, but rather it interacts with synaptic membranes and influences the expression and distribution of receptors thus mimicking the same effect as if A β had directly bound and antagonized the effected receptors. Another possibility lies in the diversity of the different assembly forms and conformations of A β used. It is quite possible that different A β assemblies or conformation have different targets thus depending on the form of A β used one group can report activation of the insulin receptor and another antagonism of $\alpha 7$ nicotinic acetylcholine receptors.

Therapeutic targeting of synaptotoxic A oligomers

Although our understanding of how and what forms of A β mediate synaptic dysfunction is incomplete, there is a growing consensus that soluble non-fibrillar A β interacts either directly or indirectly with one or more receptors initiating transduction mechanisms that result in decreased plasticity (see appendix 1). If correct, this information already provides for the rational design of disease-modifying therapeutics. Plausible approaches include neutralizing one or more forms of synaptotoxic, non-fibrillar A β , and preventing A β -mediated perturbations of receptors. As our understanding of the mechanisms contributing to synapse dysfunction continues to develop so too additional therapeutic targets are likely be revealed. In this regard the application of unbiased paradigms in which synaptic alterations are delineated following exposure to well defined soluble A β assembly forms should yield novel insights on the pathogenesis of synapse loss (see appendix 2). This information may not only assist in drug design to halt neurodegeneration in AD, but may also promote synapse regeneration. Thus the dream of curing AD may be realized not by just preventing neuronal death, but by actively promoting synaptic connectivity.

Competing interests

DMW is a shareholder and member of the scientific advisory board of Senexis, plc.

Authors' contributions

DMW designed the layout and content of the review and DMW and GMS co-wrote the text.

Appendix I

Key observations

1. Synaptic loss is an early and invariant feature of AD the extent of which correlates closely with severity of dementia.
2. MCI and early stage AD have a strong amnesic presentation implicating dysfunction of hippocampal and medial temporal lobe circuitry.

3. Soluble forms of A β present in human brain correlate well with synaptic loss and dementia status.
4. Synaptic plasticity as measured by changes in LTP and spine density are perturbed in transgenic animals models of AD and are associated with impaired spatial learning and memory.
5. Changes in plasticity and memory often occur at intervals before transgenic animals have detectable amyloid deposits and can be reversed by acute treatment with anti-A β antibodies.
6. Synthetic, cell culture- and brain-derived A β inhibits LTP, facilitates LTD, reduces spine and synaptic densities and impairs the memory of learned behaviour.

Appendix 2

Critical next steps

1. Employ sophisticated biophysical techniques to determine the size and structure of synaptotoxic human brain-derived A β .
2. Identify the cellular and molecular targets of A β oligomers.
3. Elucidate the mechanism by which A β mediates synaptic compromise: delineating whether these effects involve disruption of receptors secondary to membrane destabilization or are a result of direct A β receptor binding.
4. Determine if and how A β oligomers alter tau aggregation and/or phosphorylation and how this is linked to neuronal loss.

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