Intracellular and Extracellular Aβ, a Tale of Two Neuropathologies

A. Claudio Cuello

Departments of Pharmacology & Therapeutics, Anatomy and Cell Biology and Neurology and Neurosurgery, McGill University, Montreal, Canada.

Corresponding author:
A. Claudio Cuello, Dept of Pharmacology & Therapeutics, McGill University, 3655 Promenade Sir-William-Osler, Montreal, QC, Canada, H3G 1Y6
(E-mail: claudio.cuello@mcgill.ca)

The central pathological cause of Alzheimer disease (AD) is hypothesized to be an excess of β-amyloid (Aβ) which accumulates into toxic fibrillar deposits within extracellular areas of the brain. These deposits disrupt neural and synaptic function and ultimately lead to neuronal degeneration and dementia. In addition to the pathological roles attributed to Aβ, evidence from our laboratory would suggest that Aβ serves a physiological role in the modulation of CRE-directed gene expression. This commentary also highlights some of the pathological consequences of the accumulation of intracellular Aβ. Finally it discusses the impact of cortical Aβ burden on transmitter-specific synaptic numbers as well as the generation of dystrophic neurites. The fundamental thesis of my proposal is that the Aβ pathology seen in AD is a continuous process from an initial abnormal Aβ intracellular accumulation to the well-established extracellular Aβ aggregation, culminating in the formation of amyloid plaques and dystrophic neurites.

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INTRODUCTION

There is little doubt that overproduction of the Aβ peptide in the CNS plays a central causative role in unleashing the elaborate Alzheimer's neuropathology. The most compelling evidence stems from findings that the overproduction of its precursor protein (AβPP, the Aβ amyloid precursor protein) in Down syndrome leads to an Alzheimer-like pathology (for review, see 47). Similarly, mutations in AβPP and the presenilins result in the accelerated production of high levels of Aβ peptide in the familial forms of Alzheimer disease (AD) (21) with a neuropathology similar to the later onset and more protracted development of the sporadic forms of the disease. It needs to be acknowledged at this point that AD pathology is not only restricted to AβPP dysmetabolism and Aβ overproduction, but that it is also accompanied by neurofibrillar (25, 37) and synaptic pathologies (50), oxidative stress (22) and inflammation. Aβ production and deposition could be affected by a number of factors, such as high cholesterol levels (45) and brain trauma (53). As Aβ is found in diverse forms, amounts and localization in the CNS, the precise contribution of each in provoking Alzheimer syndrome remains an issue of great controversy and interest in the field.

In this commentary, I would like to suggest that AβPP fragments, including Aβ itself, serve a physiological role under normal circumstances, but that this changes progressively from an intracellular to an extracellular neuropathology in the AD scenario. These ideas are based on our own observations in animal models and from the observations of others as cited in this opinion paper.

A FUNCTIONAL ROLE FOR Aβ AND AβPP FRAGMENTS?

Aβ fragments appear to be produced under physiological conditions by the concerted action of 2 convertases referred to as β- and γ-secretases (for review, see 10, 62). The identities of these convertases have been elucidated. The main β-secretase is BACE 1 (beta amyloid convertase enzyme) (55). On the other hand, the γ-secretase is a membrane protein complex of which presenilin provides the catalytic function (for review, see 29). Activation of the γ-secretase generates intracellular C-terminal fragments from 2 membrane-resident proteins: Notch and AβPP. These resultant fragments are called NICD (Notch Intracellular C-terminal Domain) and AICD (AβPP Intracellular C-terminal Domain), respectively. There is evidence that these fragments migrate to the nucleus and can directly or indirectly modulate gene expression (for review, see 31, 32). The Notch pathway is implicated in the development of the CNS while the functions of the AICD fragment are less clear. Yet, the existence of such pathways suggests that therapies aimed towards the suppression of γ-secretase activity may eliminate vital neuronal regulatory functions. Moreover, cleavage of AβPP by α-secretases within the Aβ domain prevents the formation of Aβ amyloidogenic fragments and releases into the extracellular domain the AβPP N-terminal fragment referred to as soluble AβPP alpha (sAβPPα). Presently, the methaloprotease, ADAM 10, is thought to be the best candidate for this role (1). This α-secretase activity is apparently regulated by the CNS activation of muscarinic receptors (41), leading to the production of sAβPPβ fragments which apparently display neurotrophic effects in vitro (38). It is therefore plausible that in the early AD stages an overproduction of AβPP may generate sAβPPα, thus favoring the transient de novo formation of cortical-hippocampal synapses. If this were the case, it could explain the initial up-regulation in the number of cholinergic synapses in pre-plaque stages (63) as well as the transient up-regulation of cortical glutamatergic and GABAergic synapses in early stages of plaque formation observed in cortical areas of AD transgenic models (3). In line with this, De Kosky and collaborators (9, 24) have found an upregulation of choline acetyl transferase activity in the cortex and hippocampus of patients with mild cognitive impairments (9) in which a trophic response to sAβPPβ should not be rule out.

A pressing and somewhat neglected question is: Is there a physiological role for Aβ in the CNS? We know that a certain amount of Aβ appears to be generated intracellularly and is transported axonally un-

der normal conditions, however, its functions at the synaptic level is not yet clear (27, 30, 46, 61). The prevailing notion is that pathological amounts of Aβ accumulate in extracellular spaces of the brain and participate in AD's neuropathology as well as the demise of synapses. However, under basal physiological conditions, it is possible that Aβ plays a quite different role in reinforcing synaptic plasticity. For example, our laboratory has observed that undifferentiated rat pheochromocytome PC12 cells display an activity-dependent (high molarity K+ and forskolin-induced) up-regulation of phospho-ERK2 along with an elevation in CRE-regulated gene expression. These responses were markedly greater in PC12 cells transiently transfected with mutated forms of human AβPP and expressing modest amounts of endogenous Aβ (7, 13) (also Echeverria et al, unpublished data). In these transfected cells the stimulus-dependent increase in CRE-directed gene expression was blocked by γ-secretase inhibitors, suggesting that this response was mediated by either Aβ or the AICD peptide. Conversely, the over-expression of the AICD fragment did not replicate the increase in CRE-directed gene expression, suggesting that Aβ was the responsible agent. Our observations, thus, would indicate that endogenously produced Aβ peptides, acting on either intracellular or extracellular receptors, can facilitate CRE-directed gene expression. These observations are of functional relevance as CRE-regulated gene expression plays a key role in the development of late LTP formation requiring de novo protein synthesis (for review, see 28, 58). We speculate that, at this stage, the Aβ peptides are produced transiently and in relatively low amounts, and that they facilitate rather than inhibit the CRE-directed gene expression. This regulatory mechanism apparently requires the phosphorylation of the MAP kinase ERK 2, and the application of suitable inhibitors has shown that this signalling mechanism involves the Rap1-MEK-ERK pathway (7, 13)(also Echeverria et al, unpublished data). It is therefore feasible that in normal circumstances, low levels (pM-nM concentrations) of Aβ would stimulate CRE-directed gene expression and neural plasticity in the CNS. On the other hand, at higher, non-physiological levels, Aβ would inhibit CRE-directed gene expression. It is still unclear whether

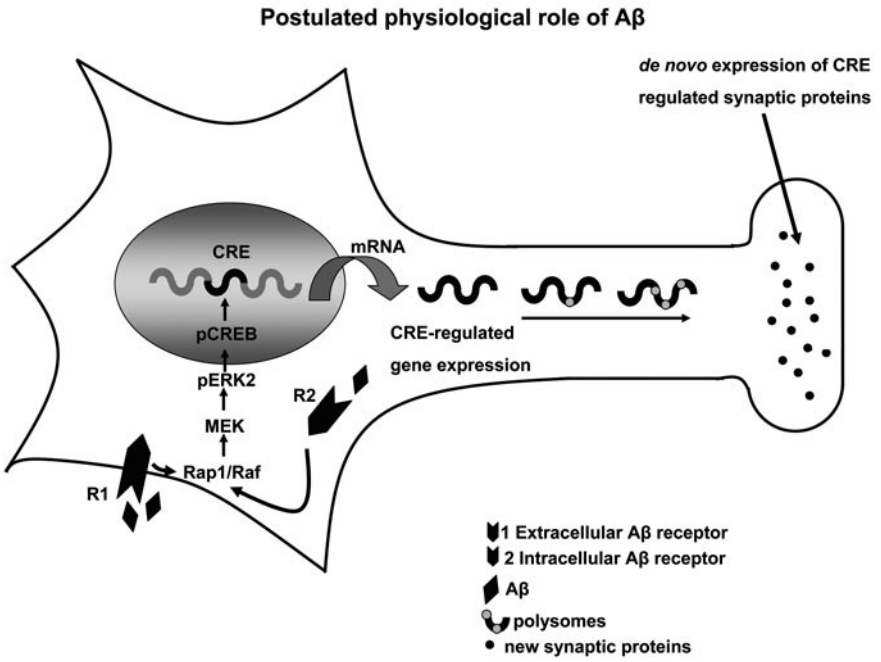

Figure 1. Schematic representation of Aβ-induced up-regulation of the activity-dependent expression of CRE-regulated genes and production of synaptic related proteins.

a similar mechanism would occur in vivo. In this regard it is interesting to note that some transgenic animal models expressing the $A\beta PP_{Swe,Ind}$, unexpectedly display larger than normal LTP responses while in most models a down regulation of LTP formation is observed (for discussion, see 26). To explain these discrepancies, I would like to suggest that moderate levels of Aβ would, at least initially, facilitate synaptic plasticity through its effects on CRE-directed gene expression (Figure 1). As the levels of Aβ increase and accumulate extracellularly, the level of Aβ oligomers become detrimental to synaptic plasticity and to CRE-directed gene expression. Such changes are known to exert a negative effect on the formation of LTP in vitro (57) and can diminish the rate of the activation of CREB in isolated cortical neurons (51). Likewise, fibrillar Aβ inhibits the activity-dependent CRE-gene expression in PC12 cells (Echeverria et al, unpublished data).

INTRACELLULAR Aβ ACCUMULATION AND AD-RELATED NEUROPATHOLOGY

There is evidence that Aβ is generated and sequestered intracellularly early in AD's pathology (for review, see 11, 60, 61). In a review article (11), we referred to the accumulation of intracellular Aβ as "a sign of worse things to come." This assertion is based on our own observations in a rat transgenic model in addition to abundant findings in the literature. We and others (notably the laboratories of Gunnar Gouras, Frank La Ferla, Andrea Leblanc, Thomas Bayer and Mike D'Andrea) maintain the general hypothesis that the accumulation of intracellular Aβ within neurons plays a role in the early pathogenesis of AD. For example, Gouras and collaborators (49) proposed that while a certain amount of Aβ in intracellular compartments may always be present in the rodent and human CNS, the amounts of intracellular Aβ elevate markedly in Down syndrome (39) and in AD (2, 18). In addition, by injecting Aβ peptides in dissociated cortical human embryonic neurons, Leblanc and collaborators (65) have demonstrated that relatively low concentrations of intracellular Aβ peptides lead to apoptotic cell death with the involvement of a caspase cascade. Tabira and collaborators have also linked the accumulation of Aβ-immunoreactive material within cortical neurons with apoptotic signals in the AD human brain and in transgenic mice over-expression AD-related PS1 mutations (5). The possibility that Aββ plaques originate from the remnants of dead neurons that contain elevated amounts of Aββhas also been suggested from microscopical observations in AD brains (8, 17). It is therefore likely that the accumulation of intracellular Aβ leads

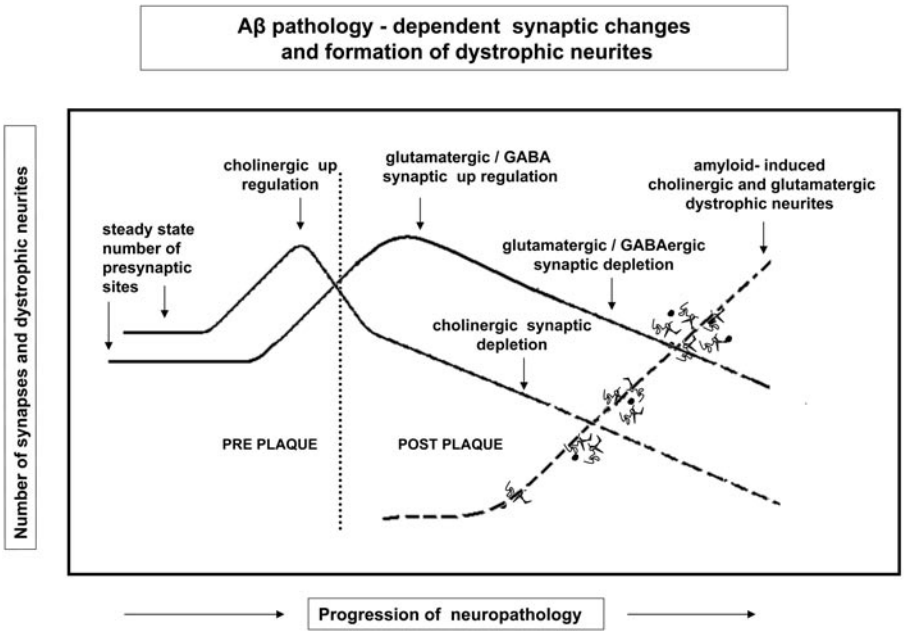

Figure 2. Schematic representation of the involvement of cortical transmitter systems as the Aβ burden. AD pathology evolves from a pre-plaque phase to the advanced formation of mature, neuritic plaques.

to neuronal dysfunction including changes in synaptic plasticity. We have observed that the overexpression of APP in neurally differentiated P19 cells can provoke considerable intracellular accumulation of Aβ peptides (19), and that this accumulation is accompanied by mitochondrial changes that include a shift to lower mitochondrial membrane potentials (20). This mitochondrial involvement is of interest in the context of the AD pathogenesis given that it has been shown that intracellular Aß can specifically bind and distort the active site of mitochondrial alcohol dehydrogenase, a change that favors the formation of free radicals and neuronal cell death (36).

Other organelles may also be negatively affected by the intracellular accumulation of Aβ. We have recently developed a number of transgenic rat models that over-express mutated forms of AβPP in which the most prominent phenotype has been the accumulation of intracellular Aβ in most neurons of the CA2 and CA3 regions of the hippocampus and within many large pyramidal neurons of the cerebral cortex (12). These Aβ-accumulating neurons displayed a significant increase in both the proportional area occupied by the Golgi apparatus elements as well as the size of individual saccules in the hippocampus of transgenic rats as compared to controls. We also found an elevated number of lysosomes and lipofuscin bodies in the hippocampi of transgenic rats, as well as an increase in the mean individual cross sectional area of lipofuscin bodies in the cortex of transgenic rats as compared to controls (35). These findings support the hypothesis that intracellular Aβ accumulation has a wide impact on subcellular compartments. Indeed, this rise in lipofuscin bodies signals the premature aging of Aβ-accumulating neurons. The up-regulation of lysosomes is also in line with observations for the involvement of this subcellular compartment in early stages of AD (42).

Besides alterations in organelles, the early intracellular accumulation of Aβ leads to changes in protein expression (56) (also Vercauteren et al, unpublished data) and, most importantly, in the activity of key proteins involved in cell signalling. In our transgenic rats with a phenotype of intracellular AββB accumulation, we observed a robust up-regulation of the MAP kinase ERK2 (12). This increase in ERK2 was accompanied by an augmented phosphorylation of tau, specifically in its MAP kinase phosphorylation sites (14). The dysregulation caused by persistent ERK2 activation, as opposed to activity dependent ERK2 activation (as discussed above), may have a negative impact on cell and synaptic function. Persistent ERK2 activation appears to diminish the degree of phosphorylation of pp90RSK, which would sequester the CREB binding protein (CBP) in cell systems (59). The sequestration of CBP leads to a down regulation of the CRE-directed gene expression of explicit genes that are involved in the protein-synthesis component of late LTP (for review, see 28). Therefore elevated intracellular Aβ should cause synaptic dysfunction and alterations in higher CNS functions. Evidence for the first was provided by La Ferla et al showing that a diminution of LTP responses occurs prior to the appearance of amyloid plaques, and corresponds to the accumulation intracellular Aβ in their triple transgenic mouse model (43). Evidence for the second effect of Aβ on higher CNS functions was provided with by our rat transgenic model which displays a phenotype of elevated intracellular Aβ in the absence of plaques. These animals displayed a mild but significant learning deficit along with marked hypophosphorylation of pp90RSK, which should compromise CRE-regulated gene expression (14). How these findings correspond to AD neuropathology is not yet fully established. However, Ferrer and collaborators (15) have found that activation of ERK2 is associated with early neurofibrillary pathology in AD. Likewise, an up-regulation of the phosphorylated form of ERK1/2, p-ERK1/2, has been reported in the same brain regions as AD patients with neurofibrillary neurodegeneration Braak stages I-II, but who are devoid of amyloid deposition (44). Furthermore, evidence for diminished CREB phosphorylation in the hippocampus of AD sufferers has been observed (64), which might indicate that in AD (as in the transgenic rat) sustained ERK2 activation might lead to dysregulation of protein phosphorylation patterns leading to the disarticulation of CRE-regulated gene expression and early tau phosphorylation, even in the absence of amyloid plaques.

ELEVATED EXTRACELLULAR Aβ AND AD-RELATED NEUROPATHOLOGY

A complete discussion of the impact of extracellular Aβ is beyond the scope of this commentary, and can be found in many well noted and authoritative reviews and also within this volume. In this section, I will highlight key features regarding the relationship between toxic species of ex-

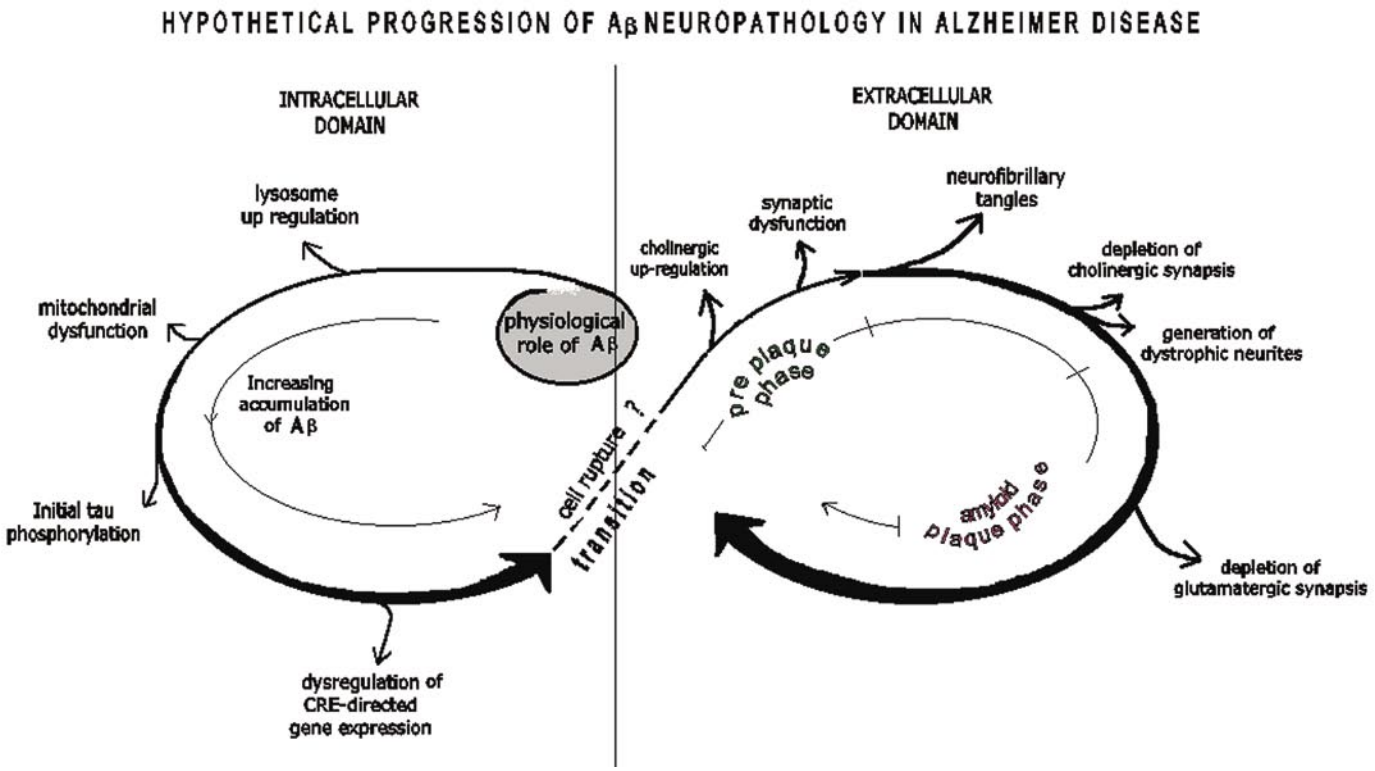

Figure 3. Schematic representation of the evolution of the Aβ-dependent AD neuropathology from an early intracellular phase to advanced stages of extracellular Aβ aggregation in the form of amyloid plaques. The broken lines of transition between intracellular to extracellular Aβ pathology highlight the lack of direct knowledge as to how one process relates to the other.

tracellular Aβ and synaptic structure and function.

Recently a link between extracellular and neurofibrillar pathology has been reported. Thus, the extracellular accumulation of endogenously expressed Aβ in double transgenic mice (34) or the application of exogenous Aβ (16) resulted in the generation of neurofibrillary tangles akin to those observed in AD, and with those observed in transgenic mice that carry tau transgenes with mutations observed in frontotemporal dementias. The cytoskeletal disruption arising from tangle formation should compromise axonal transport and, consequently, contribute to the severe cortical synaptic depletion observed in AD (50). It is likely, however, that the initial deleterious effects of extracellular Aβ on synapses are functional in nature. In some AD transgenic models there is evidence that cognitive deficits can be observed prior to plaque appearance (33, 54) (intracellular Aβ? Aβ oligopeptides?). These observations correlate with the demonstration that oligomeric Aβ molecules in the extracellular space are sufficient to inhibit LTP generation in the hippocampus (57), as discussed above. Although early functional deficits can be observed in AD transgenic models, there is well-supported evidence that the extent of the compromise of LTP formation correlates well with the amyloid plaque evolution (52).

Synaptic structural alterations in transgenic models might occur prior to the formation of amyloid plaques. Mucke and collaborators (40) have found a down-regulation of synaptophysin-immunoreactive sites while we have consistently observed an up-regulation (dysfunctional?) of cholinergic presynaptic boutons in pre-amyloid plaque phase in the cerebral cortex of murine transgenic models of AD neuropathology (23, 63). In our experience with transgenic models, these marked synaptic losses manifest themselves with the appearance and progression of amyloid plaque formation (4). We have been able to demonstrate that these structural synaptic losses follow a transmitter-specific pattern of vulnerability in which the cholinergic cortical presynaptic sites are the first to be affected followed by glutamatergic and GABAergic synapses (3, 4, 23, 63). Furthermore, the overt formation of transmitter-immunoreactive dystrophic neurites in AD transgenic models only appears with amyloid plaque formation (3, 48, 63). In our lab, Bell and collaborators (6) have used several transgenic mouse models to demonstrate that cholinergic fibres in the cerebral cortex are the first to be engulfed by the peri-plaque amyloid pathology and that a second wave of dystrophic neurites is composed of glutatergic elements, while GABAergic neurites appear fairly resistant to the plaque aggregation of fibrillar Aβ These views are schematically summarized in Figure 2.

These observations from transgenic models illustrate that the presence of extracellular Aβ in the CNS per se is sufficient to produce functional and structural synaptic changes, even in the absence of overt neurofibrillary tangles. While current transgenic models do not reproduce the full repertoire of AD neuropathologies, they have provided important insights into the diverse modalities by which Aβ, soluble or fibrillar, contributes to the synaptic pathology of AD.

CONCLUSIONS

The conventional hypothesis for the pathophysiology of AD—the so-called "amyloid hypothesis"—states that Aβ plaques are the main cause of neurodegeneration and dementia. Consequently, the majority

of studies have focused on the neurotoxic effects of aggregated extracellular Aβ fragments in AD and in AD animal models. However, in recent years the participation of intracellular Aβ accumulation has been seriously considered. In this commentary I have proposed that Aβ, in non-pathological concentrations and situations, plays a physiological role stimulating, post-synaptically, the expression of proteins related to synaptic plasticity via CRE-regulated gene expression. Second, I have proposed that the abnormal accumulation of Aβ within intraneuronal compartments is an initial step in the AD neuropathology and leads to profound alterations in protein expression, their phosphorylation patterns (and thus activity), and thereby affects the structure and function of subcellular organelles and ultimately synaptic function. These changes are most probably insufficient to provoke a dramatic change in higher CNS function however they might "prime" it for downstream pathologies. Finally, I have briefly discussed the possible consequences of Aβ fragment accumulation in the extracellular space, offering support for the notion that there is an incremental functional-to-structural compromise of synaptic elements and cortical neurites as the accumulation of Aβ proceeds from soluble oligomers to fibrillar aggregates and, finally, to toxic amyloid plaques. Indeed, I believe that this extracellular Aβ pathology affects CNS transmitter systems in a highly hierarchical and sequential manner. In short, I am proposing that the Aβ pathology seen in AD is a tale of 2 neuropathologies, which starts at the intracellular level and proceeds into the extracellular space, and which recruits in both compartments diverse cellular and molecular components as the pathology proceeds. These ideas are schematically represented in Figure 3.

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