

## Review Article

# A Window into the Heterogeneity of Human Cerebrospinal Fluid A $\beta$ Peptides

**Roberta Ghidoni,<sup>1</sup> Anna Paterlini,<sup>1</sup> Valentina Albertini,<sup>1</sup> Elena Stoppani,<sup>1</sup>  
Giuliano Binetti,<sup>2</sup> Kjell Fuxe,<sup>3</sup> Luisa Benussi,<sup>2</sup> and Luigi F. Agnati<sup>4</sup>**

<sup>1</sup>Proteomics Unit, IRCCS “Centro S. Giovanni di Dio-FBF”, 25125 Brescia, Italy

<sup>2</sup>NeuroBioGen Lab-Memory Clinic, IRCCS “Centro S. Giovanni di Dio-FBF”, 25125 Brescia, Italy

<sup>3</sup>Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden

<sup>4</sup>IRCCS San Camillo, Lido VE, 30126 Venice, Italy

Correspondence should be addressed to Roberta Ghidoni, rghidoni@fatebenefratelli.it

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The initiating event in Alzheimer’s disease (AD) is an imbalance in the production and clearance of amyloid beta (A $\beta$ ) peptides leading to the formation of neurotoxic brain A $\beta$  assemblies. Cerebrospinal Fluid (CSF), which is a continuum of the brain, is an obvious source of markers reflecting central neuropathologic features of brain diseases. In this review, we provide an overview and update on our current understanding of the pathobiology of human CSF A $\beta$  peptides. Specifically, we focused our attention on the heterogeneity of the CSF A $\beta$  world discussing (1) basic research studies and what has been translated to clinical practice, (2) monomers and other soluble circulating A $\beta$  assemblies, and (3) communication modes for A $\beta$  peptides and their microenvironment targets. Finally, we suggest that A $\beta$  peptides as well as other key signals in the central nervous system (CNS), mainly involved in learning and hence plasticity, may have a double-edged sword action on neuron survival and function.

## 1. Introduction

The “amyloid cascade hypothesis” suggests that the initiating event in Alzheimer’s disease (AD) is an imbalance in the production and clearance of amyloid beta (A $\beta$ ) peptides leading to the formation of neurotoxic soluble and insoluble brain A $\beta$  assemblies [1, 2]. Thus, A $\beta$  has become a major therapeutic target, with various anti-A $\beta$  strategies being pursued [3]. Biologically, monomeric A $\beta$  is formed through the enzymatic cleavage of the transmembrane amyloid precursor protein (APP). The discovery of the *APP* gene was followed by the identification of missense mutations associated with familial, early-onset AD. These mutations are found in and around the A $\beta$  region of APP (<http://www.molgen.ua.ac.be/ADmutations/>) and affect the production or aggregation properties of A $\beta$ . The physiopathological processing of APP involves various proteolytic activities leading to a complex set of A $\beta$  fragments. Full-length A $\beta$ 1-40 and A $\beta$ 1-42 peptides are generated by sequential proteolytic processing involving

$\beta$  and  $\gamma$ -secretases on APP [4]. These peptides (i.e., A $\beta$ 1-40, A $\beta$ 1-42) have been the dominant focus of research, but it is well established that N- and C-terminally truncated or modified forms of A $\beta$  peptides also exist in AD brains [5–9]. The detection of N-terminal truncated A $\beta$  peptides (especially A $\beta$ x-42) in young Down’s syndrome and in pre-clinical AD brains suggests that the amino-truncated species are implicated in the very first step of amyloidosis [10–12]. These forms are generated mainly by cleavage of APP between residues 16 and 17 of the A $\beta$  domain via the  $\alpha$ -secretase and by the alternative  $\beta'$  cleavage of APP triggered by the  $\beta$ -secretase  $\beta$ -site APP-cleaving enzyme (BACE)1 [13–15]. Heterogeneity at the C-terminus of A $\beta$  also contributes to the molecular variety of A $\beta$  peptides; according to some reports, due to its imprecise cleavage specificity,  $\gamma$ -secretase generates A $\beta$  peptides of variable length at the C-terminus [16]. Recently,  $\gamma$ -secretase has also been shown to cleave near the cytoplasmic membrane boundary of APP, called  $\epsilon$ -site cleavage [17]. In addition, it has been recently demonstrated

that the combined activity of  $\alpha$ - and  $\beta$ -secretases may generate the shortest forms (i.e., A $\beta$  1-15, A $\beta$  1-16) of C-terminally truncated A $\beta$  peptides [18]. Body fluids, such as cerebrospinal fluid (CSF), plasma, serum, or urine represent a cellular protein-rich information reservoir that contains traces of what has been secreted into these fluids. In particular, CSF, which is a continuum of the brain, is an obvious source of markers reflecting central neuropathologic features of the brain diseases.

This review provides an overview and update on our current understanding of the pathobiology of human CSF A $\beta$  peptides.

## 2. CSF A $\beta$ Peptides in Translational Research

Has knowledge on pathobiology of A $\beta$  been somehow translated to clinical practice? The criteria for the clinical diagnosis of AD were established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) workgroup in 1984 [19]. However, in the intervening 27 years, important advances in our understanding of AD, in our ability to detect the pathophysiological process of AD, and changes in conceptualization regarding the clinical spectrum of the disease have occurred [20, 21].

The revised diagnostic criteria proposed in 2011 by the National Institute of Aging and the Alzheimer's Association workgroup include the incorporation of biomarkers of the underlying disease state and formalization of different stages of disease—"preclinical AD," "mild cognitive impairment (MCI) due to AD," and "AD dementia"—in the diagnostic criteria [22–24]. Biomarkers are parameters (physiological, biochemical, anatomic) that can be measured *in vivo* and that reflect specific features of disease-related pathophysiological processes. In recent years, a number of reports have utilised specific protein/peptide quantitation techniques such as ELISA to study the levels of selective moieties in CSF as biomarkers of this neurodegenerative disorder. The three major alterations in AD brain are extracellular amyloid plaques, axonal degeneration, and intraneuronal tangles, which can be monitored with the CSF biomarkers A $\beta$ 1-42, total tau, and phosphorylated tau, respectively. The onset and progression of AD biomarkers likely follows an ordered temporal pattern. Biomarkers of A $\beta$  amyloid are indicative of initiating or upstream events which seem to be most dynamic (i.e., deviate most significantly from normal) before clinical symptoms. Biomarkers of neuronal injury and neuronal dysfunction are indicative of downstream pathophysiological processes which become dynamic later. There is evidence suggesting that combined assessment of CSF tau and A $\beta$ 1-42 have high diagnostic accuracy for established AD [25]. They may also be used to identify AD before onset of dementia at the stage of MCI, as shown in both mono-center and large-scale heterogeneous multicenter studies [26–30]. Since CSF levels of the shorter A $\beta$ 1-40 isoform are unchanged or increased in AD, it has been proposed that measurement of the A $\beta$ 1-42/A $\beta$ 1-40 ratio might be superior to A $\beta$ 1-42 alone [31–34]. Of note, A $\beta$ 1-42 is associated with impairment of

cognitive function from a potentially early to a later disease phase [35–37]. Decreased CSF A $\beta$ 1-42 is also seen in other neurodegenerative disorders [38]. Recent studies have shown associations between shorter forms of A $\beta$  peptides and specific dementias: decreased A $\beta$ 1-38 levels correlated with frontotemporal dementia [39] and A $\beta$ 1-37 with Lewy Body dementia [40]. Thus, the detection of the whole spectrum of A $\beta$  peptides in the CSF could be useful in order to improve early differential diagnosis.

## 3. The Large Family of CSF A $\beta$ Peptides: The Mass Spectrometry-Based Detection

The predominant protein component of amyloid plaques are strongly aggregating peptides with an approximate molecular mass of 4 kDa. The main plaques component is the 42 amino acid isoform of A $\beta$ ; this isoform is highly hydrophobic and forms oligomers and fibrils that accumulate in extracellular plaques [41]. The deposition of the peptide in plaques is considered the underlying basis for the decrease in CSF A $\beta$ 1-42 levels seen in AD and incorporated in the new diagnostic criteria. In addition, other isoforms of A $\beta$ , for example, pyro A $\beta$ 3-42, A $\beta$ 4-42, pyro A $\beta$ 11-42, A $\beta$ 17-42, A $\beta$ 1-40, and A $\beta$ 11-40 have been detected in the brains of sporadic AD and familial AD cases [5–12, 42–46]. A $\beta$  peptides heterogeneity is observed also in the human CSF (see Table 1) [47–58]. The proteolytically processed A $\beta$  peptides, however, are difficult to detect in the CSF using standard methods, possibly because they comprise a heterogeneous set of both N- and C-terminally truncated peptides, some of which are present only at low levels. Many investigators used mass spectrometry (MS) for studying human CSF A $\beta$  peptides. MS allows for the detection of a variety of modified and truncated A $\beta$  peptides, thus enabling a more detailed and unbiased analysis of fragments that may play a role in neurodegeneration. The two main approaches are (1) the use of preactivated chip arrays that allow coupling with specific antibodies combined with surface-enhanced laser desorption and ionization time-of-flight (SELDI-TOF) MS (2) immunoprecipitation combined with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. An immunoproteomic approach—which combines specificity of 6E10 (against A $\beta$  epitope 1-16) mAb capture with precision of spectral analysis (i.e., SELDI-TOF MS)—has recently been successfully used to analyze A $\beta$  peptides in human CSF; Maddalena et al. [50] detected 9 C-terminally and 1 N-terminally truncated A $\beta$  peptides in CSF of AD patients and healthy controls subjects. While, with an analogous protocol, 10 A $\beta$  fragments were found by Lewczuk et al. [55, 58]. Immunoprecipitation experiments employing 4G8 mAb and MALDI-MS analyses of A $\beta$  peptides from 1 mL CSF revealed the presence of two previously unidentified N-terminally truncated A $\beta$  peptides (i.e., A $\beta$ 11-30, A $\beta$ 11-40), along with a number of C-terminally truncated forms [47, 48]. Since 6E10 and 4G8 mAbs bind different portions of A $\beta$  sequence, we tested whether the combined use of these two mAbs could improve the capture of N and C-terminally truncated A $\beta$  peptides; of note, applying this optimized immunoproteomic assay—that employs very low sample volume (5  $\mu$ L of CSF for each

TABLE 1: Summary of A $\beta$  peptides in human CSF.

A $\beta$ Peptides	Theoretical mass* (Da)	Literature
A $\beta$ 1-12	1424.61	[47]
A $\beta$ 1-13	1561.67	[47–49]
A $\beta$ 2-14	1583.70	[50]
A $\beta$ 1-14	1698.73	[47–49]
A $\beta$ 1-15	1826.78	[47–49]
A $\beta$ 3-17	1881.90	[48]
A $\beta$ 2-17	1952.94	[48]
A $\beta$ 1-16	1954.88	[47–49]
A $\beta$ 1-17	2067.96	[47–52]
A $\beta$ 1-18	2167.03	[47–51]
A $\beta$ 11-30	2212.11	[47]
A $\beta$ 1-19	2314.10	[47–49, 51, 52]
A $\beta$ 1-20	2461.17	[47–49]
A $\beta$ 6-27	2521.16	[53]
A $\beta$ 11-34	2608.39	[53]
A $\beta$ 1-27	3133.44	[53]
A $\beta$ 11-40	3150.68	[47, 51, 54]
A $\beta$ 6-34	3167.60	[53]
A $\beta$ 1-28	3261.53	[47, 48, 53, 55]
A $\beta$ 6-35	3298.63	[53]
A $\beta$ 12-43	3306.80	[53]
A $\beta$ 10-40	3313.74	[51, 54]
A $\beta$ 1-29	3318.56	[55]
A $\beta$ 11-42	3334.80	[51, 54, 56]
A $\beta$ 1-30	3389.59	[47–49, 53]
A $\beta$ 11-43	3435.85	[53]
A $\beta$ 3-34	3599.80	[53]
A $\beta$ 1-33	3672.78	[47, 49–51, 54, 55, 57]
A $\beta$ 1-34	3785.87	[47, 49–51, 53–55, 57]
A $\beta$ 1-35	3916.91	[51, 53, 57]
A $\beta$ 1-36	4015.98	[51]
A $\beta$ 1-37	4073.00	[47, 49–51, 54, 55, 57, 58]
A $\beta$ 1-38	4130.02	[47, 49–51, 53–55, 57, 58]
A $\beta$ 1-39	4229.09	[47, 49–51, 54, 55, 57, 58]
A $\beta$ 1-40	4328.16	[47, 49–51, 53–55, 57, 58]
A $\beta$ 1-42	4512.28	[47, 49–51, 54–58]
A $\beta$ 3-44	4526.33	[58]
A $\beta$ 1-45 or A $\beta$ 2-46	4825.48 or 4809.52	[55]
A $\beta$ 3-47	4851.56	[58]

\*The masses presented are the monoisotopic protonated molecules.

spot)—we detected a total of 15 A $\beta$  peptides (12 C-terminally and 3 N-terminally truncated forms) in human CSF [51].

In addition, we determined mass profiles of A $\beta$  peptides in the CSF of patients carrying familial AD-associated mutations (i.e., APP T719P, PS1 P117L, and PS2 T122R); these mutations were associated with an overall reduction of A $\beta$  species. Interestingly, the APP T719P mutation unbalanced the relative proportion of A $\beta$  peptides with a reduction of A $\beta$ 1-40 and A $\beta$ 1-42 paralleled by an increase of A $\beta$ 1-38 and

A $\beta$ 10-40 [54]. In accordance with these data, Portelius and coauthors [49] reported a reduction C-terminally truncated A $\beta$  peptides in CSF of affected and unaffected subjects carrying PS1 A431E mutation. An unbalance of A $\beta$  isoforms was also detected in CSF of sporadic AD and MCI patients [50, 52, 56, 57]. Interestingly, within a phase II clinical trial, it has been recently demonstrated that A $\beta$ 1-14, A $\beta$ 1-15, and A $\beta$ 1-16 are positive and very sensitive biomarkers for  $\gamma$ -secretase inhibition (even at doses that do not affect A $\beta$ 1-42 or A $\beta$ 1-40) [59]. Thus, A $\beta$  isoforms may be novel biomarkers to monitor the onset and progression of cognitive decline and the biochemical effect of disease-modifying drugs in AD clinical trials.

#### 4. Beyond A $\beta$ Monomers: CSF Circulating A $\beta$ Oligomers

In the human brain it is likely that multiple A $\beta$  assemblies, that are in dynamic equilibrium almost simultaneously, alter brain cell function and that different toxic effects may occur virtually concurrently in various regions of the cerebrum. Several lines of evidence have converged to demonstrate that soluble oligomers of A $\beta$  may be responsible for synaptic dysfunction in AD animal models and in the brains of AD patients [46, 60, 61]. Small diffusible A $\beta$  oligomers have been shown to exert neurotoxic effects in cultured neurons [62–64]. It has been hypothesized that such prefibrillar assemblies might also be neurotoxic *in vivo* since synaptic, electrophysiological, and behavioral changes have been well documented in young APP transgenic mice before plaque formation [65, 66]. Accordingly, soluble A $\beta$  oligomers have been found to block, *in vivo*, hippocampal long-term potentiation (LTP), a synaptic correlate of memory and learning [67–71]. Importantly, A $\beta$  immunotherapy can protect against the neuropathology and cognitive deficits observed in APP transgenic mice and also prevent the LTP inhibition induced by A $\beta$  oligomers [68]. Soluble oligomeric A $\beta$  has been shown to be present in human CSF [72–74]. Human derived soluble A $\beta$  seems to have a pathophysiological role in the brain; the CSF-derived A $\beta$  dimers—and not the monomers—potently disrupt synaptic plasticity *in vivo* [75]. Of note, it has been reported that CSF circulating oligomers are increased in AD and MCI patients, and their levels are negatively correlated with Mini-Mental State Examination scores [76, 77]. Thus, an emerging strategy within the AD field is to use oligomeric A $\beta$  as a possible biomarker/therapeutic target for the disease. The actual identity of the oligomer participating in AD pathogenesis remains elusive although several lines of evidence suggest that AD-associated oligomers are primarily composed of A $\beta$ 42. Nevertheless Gao and coworkers, using a novel misfolded protein assay, found an enrichment of A $\beta$ 40-containing oligomers in AD CSF [78] and suggested these assemblies as biomarker for early diagnosis of AD. Although A $\beta$  oligomers are attractive AD biomarker candidates, several issues relating to these molecules persist. The levels of these A $\beta$  species in CSF seem to be very low in comparison with A $\beta$  monomers and the precise molecular identity of these soluble toxins remains unsettled; thus more precise mass spectrometry analyses are needed in order to better

characterize the molecular weight and composition of the most neurotoxic species. Furthermore, assays suitable for large clinical studies are still to be developed for these molecules. The development of conformation-sensitive antibody domains targeting the  $A\beta$  oligomers [79–83] is of great interest for research in this field. Targeting the pathological assemblies of  $A\beta$  with specific probes, for mechanistic studies, for intracellular imaging, or for therapeutic purposes, is therefore very important.

## 5. $A\beta$ Peptides Are Double-Edged Sword Signals Transmitted Both via Volume and Wiring Transmission

As discussed above,  $A\beta$  peptides have been regarded as the principal toxic factor in the neurodegeneration of AD. Intense research effort has, therefore, been directed at determining their sources, activities, and fates, primarily with a view of preventing their formation or toxic actions, or promoting their degradation.

These are important studies and very promising ones for a better understanding of the pathogenesis of AD. However, in our opinion, a crucial aspect is the discovery of the physiological role of these peptides.

Thus, the following points will be briefly discussed as far as the  $A\beta$  peptides are concerned:

- (a) communication modes for these peptides, hence (volume transmission (VT)) versus (wiring transmission (WT)) versus (VT and WT);
- (b) micro-environment where the targets for  $A\beta$  peptides are located, hence plasma membrane versus intracellular environment;
- (c) possible physiological roles of  $A\beta$  peptides.

Finally, a previously published theoretical proposal [84] will be summarised since it can give a possible frame for interpreting otherwise contradictory data on  $A\beta$  peptides functions. The hypothesis is based on the concept that  $A\beta$  peptides as well as other key signals in the central nervous system (CNS) mainly involved in learning, and hence plasticity may have a double-edged sword action on neuron survival and function.

**5.1. Communication Modes for  $A\beta$  Peptides and Their Microenvironment Targets.** It has been proposed that two main modes for intercellular communication are in operation in the CNS, namely, the VT and the WT [85].

The characteristics of the channel connecting two nodes of the network, that is, the cell source of the signal with the cell-target of the signal allow distinguishing the VT from the WT.

(i) VT is characterized by a channel with a poorly defined physical substrate and signal transmission takes place via diffusion (or vector migration) in the medium interposed between nodes. Recently, it has been shown that several messages can be sent via microvesicles (acting as protective containers hence *like the bag of a roamer*), dispatched into

the extracellular space (ECS) and diffusing until the proper targets are reached [86–88].

Different types of microvesicles have been described, which are the result of specific cellular phenomena [86]. In particular, exosomes are microvesicles contained within a special class of membrane-bound organelles (endosomes), which can be released by fusion of the limiting membrane of the MVB with the plasma membrane.

(ii) WT is characterized by the transmission of the signal along a channel with a well-defined physical substrate; thus, a “wire” links the source node with the target node. Classically, in the case of neural networks, the WT-channel is formed by an axon and a chemical synapse.

However, two more subclasses of WT play a role in the CNS. The first one is represented by the well-characterized gap junctions, while the second one, the clear-cut *in vivo* demonstration of which has not yet been provided, is represented by the tunnelling nanotubes (TNTs) that are transient structures forming a “private” direct channel connecting two cells. They have a diameter of 50–200 nm and a length up to several cell diameters. Several *in vitro* studies demonstrated that these structures make possible the exchange of proteins, mtDNA, RNA, and whole organelles between cells [89]. It is interesting to note that  $A\beta$  peptides can be transmitted according to both VT and WT. Actually, it has been shown that these signals can use several possible modes of intercellular communication:

- (i) the classical VT mode that is diffusion in the ECS [90–94],
- (ii) the Roamer Type of VT that is diffusion via exosomes [95–99],
- (iii) the TNT mode of WT [100].

The targets for the  $A\beta$  peptides are located both at the plasma membrane level [101, 102] and at intracellular level where they may exert an “intracrine function” [95, 103, 104].

**5.2. Possible Functional Roles of  $A\beta$  Peptides.** We completely agree with Pearson and Peers’ view that  $A\beta$  peptides should have important physiological roles and may even be crucial for neuronal cell survival and CNS function. Thus, the view of  $A\beta$  being a purely toxic peptide requires a reevaluation [105]. In support of such a proposal, there are several papers, two of these will be cited since while the first one shows a role of  $A\beta$  peptides on learning [106], the other one opens a new field by giving evidence for a possible role of these peptides as antimicrobial agents [107].

Thus, it has been shown that, in contrast with its pathological role when accumulated, endogenous  $A\beta$  in normal hippocampi mediates learning and memory formation probably via nicotinic acetylcholine receptors. Furthermore, hippocampal injection of picomolar concentrations of exogenous  $A\beta$ 1–42 enhances memory consolidation. Hence,  $A\beta$  peptides, including  $A\beta$ 1–42, play an important physiological role in hippocampal memory formation.

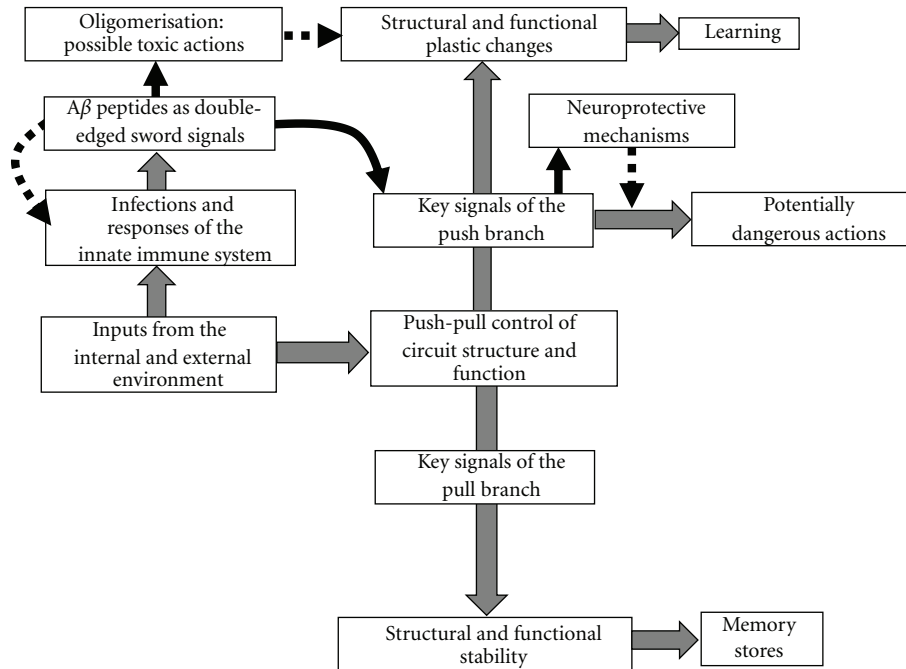


FIGURE 1: Schematic representation of the “Push and Pull Control” of structural and functional plasticity of neuronal circuits and how this control is related to learning processes (plastic changes of the circuits) and maintenance of the memory traces (stability of the circuits). The possible actions of  $A\beta$  peptides as double-edged sword signals are indicated. Broken arrows indicate reduction or inhibition.

As mentioned above, recently a new possible function for  $A\beta$  peptides has been demonstrated, namely, the antimicrobial action. Thus, it has been shown that many of the physiochemical and biological properties previously reported for  $A\beta$  are similar to those of a group of biomolecules collectively known as “antimicrobial peptides” (AMPs; also called “host defense peptides”) which function in the innate immune system. These peptides are potent, broad-spectrum antibiotics that target several infective agents. In particular, the pleiotropic LL-37 peptide is a widely expressed archetypal AMP present also in humans that exhibits striking similarities to  $A\beta$ , including a propensity to form cytotoxic soluble oligomers and insoluble fibrils with classical histochemical properties of tinctorial amyloid. Soscia et al. [107] findings reveal that  $A\beta$  exerts antimicrobial activity against eight common and clinically relevant microorganisms with a potency equivalent to, and in some cases greater than, LL-37. These findings obviously impose a great caution in developing future AD treatment strategies based on the drastic reduction of synthesis and levels of  $A\beta$  peptides.

**5.3. Double-Edged Sword Action of  $A\beta$  Peptides on Neuron Plasticity and Survival.** More than one century ago, Tanzi proposed that learning processes in the CNS are basically due to plastic changes of neuronal networks [108].

As pointed out by Taylor and Gaze, neuronal plasticity allowing continuous CNS adaptation to the challenges of the environment plays a fundamental role not only for learning processes. Actually, *plasticity in the nervous system means a patterned or ordered alteration in structure and function brought about by development, experience, or injury* [109].

Thus, this definition mentions age, learning, and lesions as factors triggering out plasticity.

In this paper the concept is introduced that physiological processes (such as learning and memory) as well as repairable processes (such as those occurring after lesions or during ageing), being all rooted in CNS rearrangements, are competing for the brain plasticity [110], which exists as a fixed amount (“total brain plasticity capability,” see [84]).

It has been demonstrated that some signals, such as excitatory amino acids,  $A\beta$  peptides, and  $\alpha$ -synuclein ( $\alpha$ -syn), are not only involved in information handling by the neuronal circuits, but also trigger out CNS plasticity [84]. It has also been shown that these signals are potentially dangerous possibly since, inter alia, they force the neuronal circuits to move from one stable state towards a new state. Several mechanisms are put in action to protect neurons and glial cells from these potentially harmful signals and hence favouring the emergence of only their physiological functions. However, ageing and neurodegenerative diseases, on one side, increase the need of plasticity for the CNS repair but, on the other side, cause a reduction in the secretion of several trophic factors (e.g., BDNF and NGF) leading to a less effective neuroprotection and deficits in neural plasticity [111, 112].

Against this background, it has been shown that in ageing and neurodegenerative diseases functionally ambivalent (i.e., double-edged sword) signals such as  $A\beta$  and  $\alpha$ -syn are secreted at a high rate possibly in the attempt of maximizing neuronal plasticity. It has been proposed that in the long run these peptides do not exert their possible physiological actions but on the contrary may favour neurodegenerative processes.

Soscia et al. [107] have demonstrated that an increased A $\beta$  generation/accumulation leading to AD pathology may be mediated by a response of the innate immune system to a perceived infection. This model is in agreement with data supporting a central role for neuroinflammation in AD neuropathology [113].

Thus, not only genetic factors may contribute to activation of the innate immune system by regulating A $\beta$  production and clearance but also a transient infection may lead to a self-perpetuating innate immune response.

These findings allow an update of the hypothesis made in the JNT 2009 [84] (see Figure 1).

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