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Invited Review

Mechanisms of synaptic dysfunction in Alzheimer's disease

E. Masliah

Departments of Neurosciences and Pathology, University of California, San Diego, La Jolla, California, USA

Summary. Alzheimer's disease (AD) is characterized by a progressive cognitive decline in which memory, initiation, learning and conceptualization are severely affected. The main histopathological alterations are the presence of amyloid ß/A4-containing plaques, tangles and amyloid angiopathy. It is believed that these brain alterations are associated with abnormal expression and/or processing of amyloid precursor protein (APP) and with abnormal assembly of cytoskeletal proteins. Recent quantitative studies with the electron microscope and with immunochemical/immunocytochemical assays, using molecular markers for synaptic proteins, have shown that synaptic loss in the cortex is the major correlate of the patterns of cognitive decline in AD. The synaptic loss in AD is accompanied by neuronal loss and aberrant sprouting, and studies in incipient AD cases have shown that this alteration occurs very early in the progression of the disease preceding tangle formation and neuronal loss. These results suggest that damage to the synaptic terminal plays a central role in the pathogenesis of AD. The mechanisms of synaptic pathology in AD are not yet clear, however, studies in transgenic animal models support the possibility that APP participates in synaptic stabilization and that abnormal metabolism of this molecule could lead to synaptic dysfunction which, in turn, results in neurodegeneration and dementia.

Key words: Synapses, Neurodegeneration, Alzheimer disease, Amyloid precursor protein

Introduction

Alzheimer's disease (AD) is a prevalent disorder among the elderly population and represents a major epidemiological challenge for the future in view of the projected growth of the population older than 65 years for the year 2000 (Khachaturian, 1985). Clinically AD is characterized by a progressive cognitive decline in which memory, initiation, learning and conceptualization are severely affected (Katzman et al., 1988; Salmon et al., 1989). The main histopathological alterations are the neurodegeneration of the association and limbic system accompanied by the formation of plaques, tangles and amyloid angiopathy (Terry et al., 1994). Plaques contain ß amyloid protein (ßAP) which is derived from the amyloid precursor protein (APP) (Selkoe, 1989). Embedded in the amyloid plaque core are dystrophic neurites, astroglial cells and microglial reaction (Terry and Wisniewski, 1970; Masliah et al., 1993b; Terry et al., 1994). The tangles are composed of polymerized phosphorylated microtubule associated protein - tau, neurofilaments and ubiquitin (Trojanowski et al., 1993). Although the density and distribution of the lesions is very important for the diagnosis of the disease, as well as for the understanding of physiopathological mechanisms of neurodegeneration (Mirra et al., 1993), the main substrate for the cognitive alterations is the loss of synapses in the association cortex and limbic system (DeKosky et al., 1990; Terry et al., 1991; Masliah and Terry, 1994). The objective of the present manuscript is to review the mechanisms involved in neurodegeneration and synapse loss in AD with special emphasis on their possible relationship with the genetic alterations associated with AD.

Offprint requests to: Dr. Eliezer Masliah, University of California, San Diego, School of Medicine, Department of Neurosciences, La Jolla, California 92093-0624, USA

The role of synaptic alterations in mechanisms of dementia in AD

Recent studies have shown that in addition to the traditionally described lesions (plaques and tangles) found in the AD brain (Alzheimer, 1907; Terry et al., 1964; Terry and Wisniwski, 1970; Dickson et al., 1988; Yamaguchi et al., 1988; Braak and Braak, 1991), this neurodegenerative disease is characterized by neuronal loss (Terry et al., 1981; Hof et al., 1990), disruption of the neuritic cytoskeleton with altered cortico-cortical connectivity (Morrison et al., 1987; Hof et al., 1990; Masliah et al., 1993a), and extensive synapse loss (Davies et al., 1987; Hamos et al., 1989; Masliah et al., 1989, 1991b,d; DeKosky et al., 1990; Honer et al., 1992;

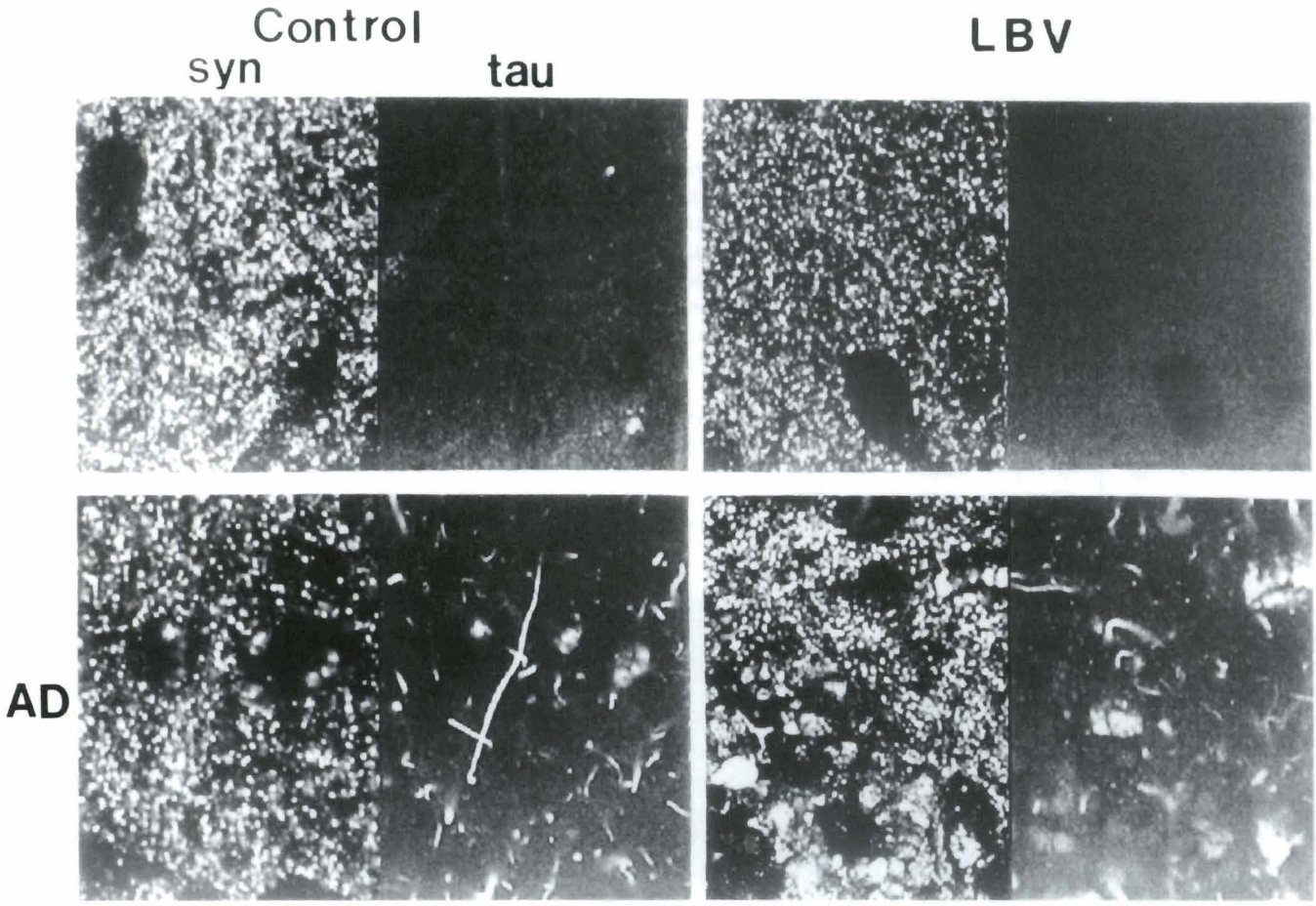
Lassman et al., 1992). It has been hypothesized that the dementia in AD could be caused by either the presence of these specific lesions alone or by the synergistic effect of some or all of these lesions (DeKosky and Scheff, 1990; Terry et al., 1991; Samuel et al., 1994). The original studies by Blessed et al. (1968) suggested that amyloid deposition and plaque formation might be the major correlate with cognitive alteration in AD, but more detailed studies where control cases were not included in the linear regression analysis did not support this view (Terry et al., 1991). Other groups have shown that neuronal loss in specific areas of the neocortex and subcortical regions correlated with clinical alterations seen in AD (Neary et al., 1986). However, these correlations are rather weak and do not completely explain all the clinical alterations observed in AD. Recently, several studies have shown that neuropil threads and neurofibrillary pathology could be contributing to the dementia in AD (Deleare et al., 1989; Arrigada et al., 1992; Masliah et al., 1992d; Samuel et al., 1994). However, it is important to remember that a subgroup of AD cases shows very little or no fibrillary pathology and yet display very significant clinical alterations (Terry et al., 1987b). An alternative hypothesis is that dementia in AD is directly associated with the disruption of neuritic substructure and loss of synaptic contact in specific neocortical and subcortical areas (Masliah et al., 1991d; McKee et al., 1991). In AD as well as in the Lewy body variant of AD (LBV) there is and approximate 30 to 50% loss of synapses in the frontal, parietal and temporal cortex (Davies et al., 1987; Masliah et al., 1989, 1991b,d, 1993c; Sheff et al., 1990; Scheff and Price, 1993; Lassmann et al., 1992) (Fig. 1). Studies of the progression of the lesions in AD have shown that synapse loss appears first in the molecular layer of the hippocampus dentate gyrus and is correlated with abnormal expression of APP in the entorhinal cortex (Masliah et al., 1994c,d). The damage to this circuit in AD correlates with the early symptoms of memory loss characteristic of this disorder (Hyman et al., 1986). Measurements by electron microscopy and immunocytochemistry have both shown very strong correlations between synaptic numbers in the frontal cortex and tests of global cognition in AD (DeKosky and Scheff, 1990; Terry et al., 1991). More recently, correlative studies between tests of cognition and immunochemical quantification of various synaptic proteins have confirmed this view (Lassmann et al., 1992; Zhan et al., 1993). Further supporting a central role of synaptic damage in the pathogenesis of AD, recent studies have shown that the dystrophic neurites of the plaques contains synaptic vesicles, synaptic proteins and neurotransmitters (Armstrong et al., 1989; Masliah et al., 1991b, 1994a; Lassmann et al., 1992; Masliah and Terry, 1993). Moreover ultrastructural studies have shown that in AD the synapses are swollen and contain abnormal accumulation of cytoskeletal proteins, vesicles and lysosomes (Gonatas et al., 1967, 1970; Masliah et al., 1991b, 1993b) (Fig. 2).

Cellular mechanisms of synaptic damage in AD

Synaptic pathology in AD could be either the direct (or primary) result of an underlying molecular defect affecting the synapses, or an indirect (or secondary) result of neuronal loss, plaque and tangle formation (Masliah and Terry, 1993, 1994). Recent studies in AD have shown that while there is a 20-30% loss of pyramidal neurons (Terry et al., 1987a), synaptic loss could be as high as 50% (Masliah et al., 1992d; Alford et al., 1994; Masliah and Terry, 1994). Furthermore, stepwise regression analyses of the different neuropathological, neuroanatomical and neurochemical markers in the AD neocortex have shown that loss of pyramidal neurons in the inferior parietal cortex contributes 45% ($r= 0.67$, $p<0.005$, $n= 16$) of the correlative strength to the synaptic loss in the mid-frontal cortex (Terry et al., 1990; Masliah and Terry, 1994), suggesting that neurodegeneration in AD might initiate with synaptic damage. Moreover, aging and plaque/tangle formation also contribute to synaptic loss in AD (Terry et al., 1990, 1991; Masliah et al., 1993e).

In addition, unsuccessful compensatory mechanisms are taking place in response to the ongoing synaptic pathology (Masliah et al., 1991c,e, 1992b; Cotman et al., 1991). Recent studies have shown that in AD approximately 30% of neuritic plaques express growth-associated protein 43 (GAP43) (Masliah et al., 1991e, 1993c) which is a molecule associated with plasticity and regeneration under normal conditions and its accumulation in abnormal neurites in AD could indicate aberrant sprouting (Masliah et al., 1991a). Moreover, GAP43-containing sprouting neurites in the plaque also display strong immunoreactivity with antibodies which detect both secreted APP (sAPP) and APP processed through the beta-secretase pathway (Masliah et al., 1992c, 1994a). These data suggest that accumulation of aberrantly processed APP products not only could mediate synaptic damage, but also trigger aberrant sprouting (Cotman et al., 1991; Masliah et al., 1992b,c). Supporting this view, previous studies have shown that, depending on concentration, APP is involved in neuronal survival, neuritic outgrowth, synaptogenesis and development of the nervous system (Whitson et al., 1989; Yankner et al., 1990; Milward et al., 1992; Roch et al., 1992; Masliah et al., 1992a, 1993f). Therefore, the

Fig. 1. Laser scanning confocal microscopy of the frontal cortex. Sections were double-immunolabeled with a monoclonal antibody against the synaptic-associated protein synaptophysin (left side of each panel) and polyclonal antibody against phosphorylated tau (right side of each panel), which identifies, cytoskeletal alterations in the neurites. Synaptophysin immunoreactivity appears as a punctate pattern, each dot represents an immunolabeled presynaptic terminals. In AD and LBV there is a significant decrease in the number of immunolabeled presynaptic terminals. In AD, the synaptic alterations are accompanied by formation of neuropil threads. No threads are observed in control and LBV cases. x 790


Control
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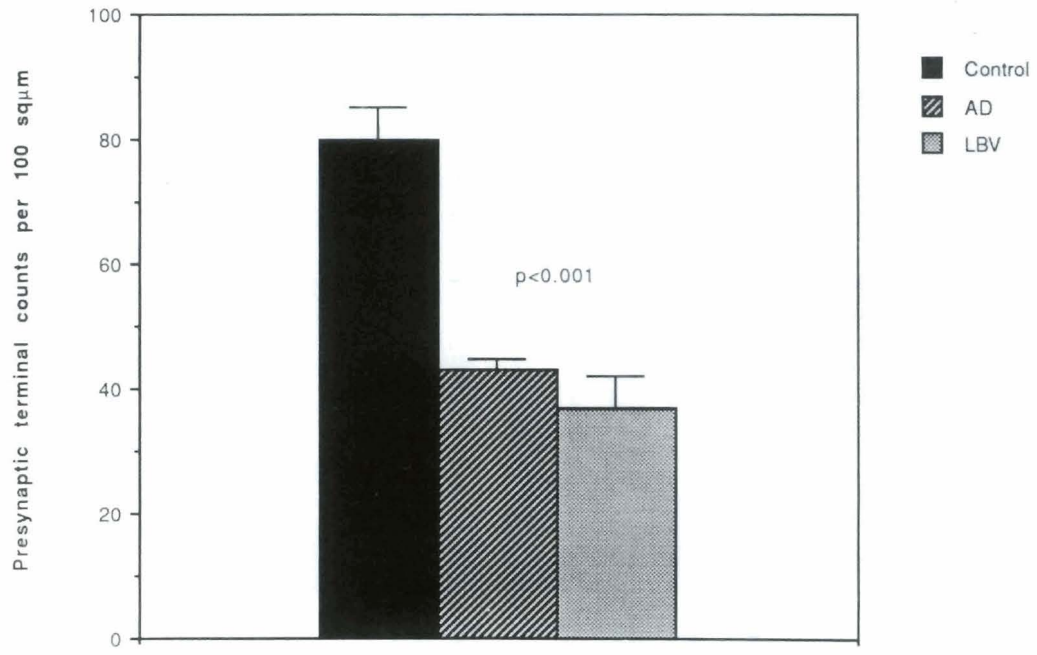
Presynaptic terminal counts per 100 sqμm
100
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Control
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LBV

neuritic plaque could represent a focal area of abnormal synaptic remodelling and since probably no successful synaptic circuitries are formed these neuritic process eventually degenerate (Dahl et al., 1989; Masliah et al., 1992b). Other lines of evidence supporting this possibility are: 1) the finding of other growth factors in neuritic plaques (Birecree et al., 1988; Gómez-Pinilla et al., 1990; Masliah et al., 1992c, 1993f), 2) decrease in growth-inhibitory factors in AD (Uchida et al., 1991), and 3) presence in AD of cells of neuroectodermal origin displaying aberrantly sprouting neuritic processes immunoreactive with antibodies against tau (Ihara, 1988), GAP43/neurofilaments (Masliah et al., 1993c), and brain spectrin (Masliah et al., 1991c).

To further understand how aberrant sprouting might contribute to neurodegeneration, we developed a rodent model where the growth-promoting agent phorbol 12-myristate 13-acetate (PMA) was administered into the neocortex of adult rats (Masliah et al., 1993d). In the first two weeks post-injection, PMA induced aberrant sprouting, followed by neurodegeneration at four weeks. PMA activates and eventually down-regulates protein kinase C and induces in the rat the expression of several genes, including APP (Nishiguchi et al., 1988). In addition, PMA increases the production of sAPP and reduces ßAP (Bieger et al., 1993; da Cruz de Silva et al., 1993; Fukushima et al., 1993; Gabuzda et al., 1993; Loeffler and Huber, 1993; Slack et al., 1993a,b). Taken together, these human and rodent studies support the concept that aberrant sprouting rather than contributing to the regenerative process, only enhances the synapse loss and neurodegeneration.

Neuronal loss, plaque/tangle formation, aging and aberrant sprouting only partially account for the synaptic pathology in AD, suggesting that there is a basic pathogenesis process affecting the synapses. Possible mechanisms involved in the pathogenesis of synaptic damage in AD could be related to either abnormal

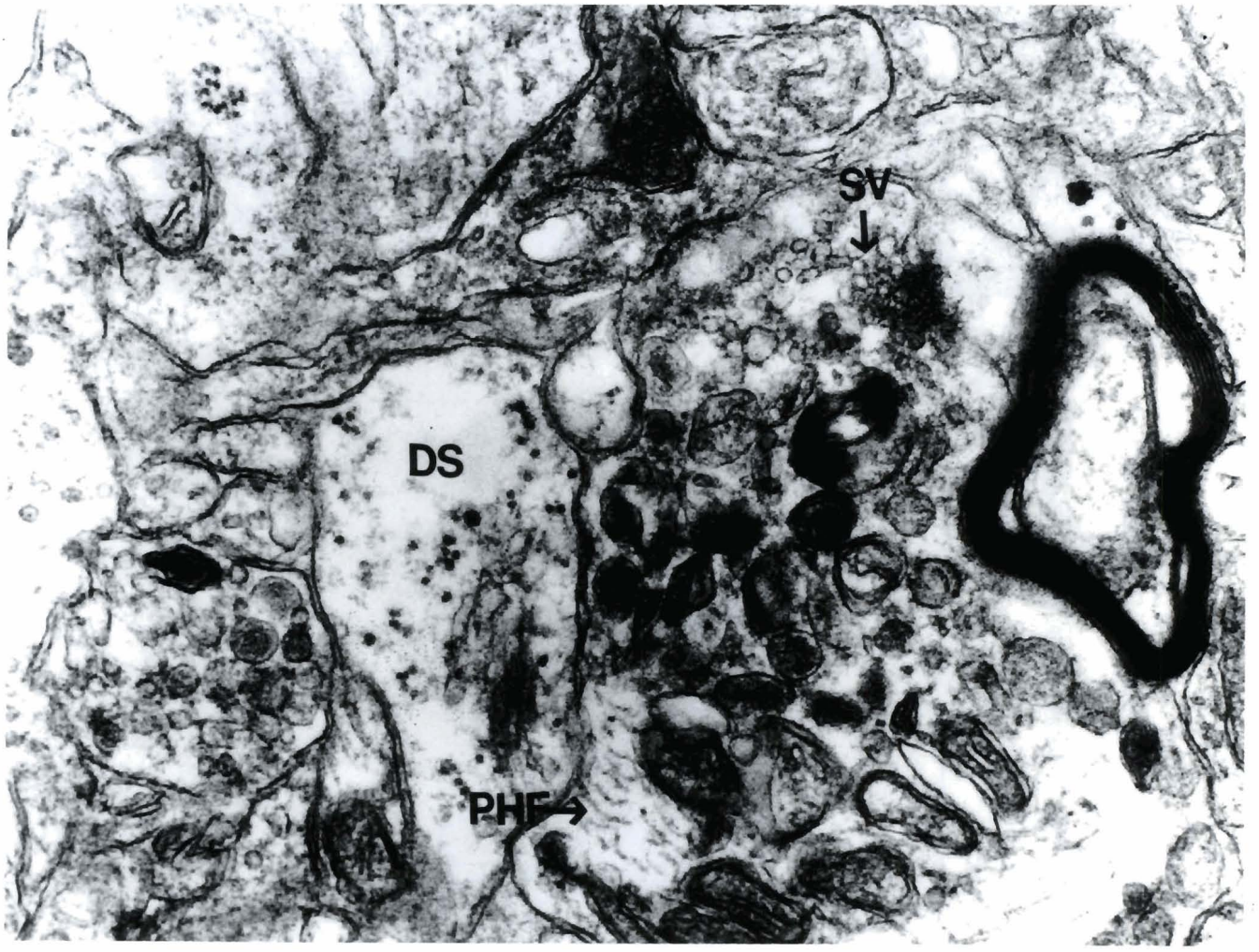

Fig. 2. Ultrastructural characteristics of the synaptic alteration in AD. The synaptic terminals and axons are enlarged and contain groups of synaptic vesicles (SV), paired helical filaments (PHF), and laminated bodies. This neurite is adjacent to a dendritic spine (DS). x 13,000

function of synaptic proteins or direct toxic effects at the presynaptic site (Masliah and Terry, 1993). In this regard, recent studies have shown that APP, which is believed to be centrally involved in AD (Selkoe, 1989), might play an important role as a synaptic regulator (Schubert et al., 1991; Alvarez et al., 1992; Askanas et al., 1992; Roch et al., 1994; Small et al., 1994). Moreover, APP metabolism appears to be abnormal in AD (Sisodia et al., 1990; Zhong et al., 1994). Taken together these findings suggest that altered APP processing may lead to synaptic dysfunction (Fig. 3).

APP processing and molecular mechanisms of synaptic damage in AD

APP might play an important role in regulating synaptic function since it is located in synapses, is axonally transported and may be released from nerve terminals (Schubert et al., 1991; Alvarez et al., 1992; Askanas et al., 1992; Roch et al., 1994; Small et al., 1994). Furthermore, APP is upregulated during CNS development, is present in the neuritic growth cones and promotes neuritic outgrowth and neuronal survival (Koo et al., 1990; Yankner et al., 1990; Fisher et al., 1991; Masliah et al., 1992a; Small et al., 1994). In addition, infusion of sAPP peptide into the rat brain and expression APP in transgenic mice promotes a synaptotrophic effect (Mucke et al., 1994; Roch et al., 1994). Recent studies (Allsop et al., 1991; Maruyama et al., 1991; Tagawa et al., 1991; Anderson et al., 1992; De Strooper et al., 1993; Mattson et al., 1993a,c) have suggested that APP is processed through two pathways (Fig. 3). In the alpha-secretase pathway, axonally transported APP is cleaved between amino acids ß16 and ß17 within the ßAP sequence, resulting in the release of sAPP [molecular weight (MW)>100 kDa] at the synaptic site. This pathway precludes the release of ßAP. In the beta-secretase pathway, APP is cleaved at the amino terminus of ßAP at Met596. This pathway results in the release of ßAP (1-40) and ßAP (1-42) (4kDa), as well as in the production of medium MW APP (68kDa) and the C100 fragment (14kDa). Recent studies suggest that the

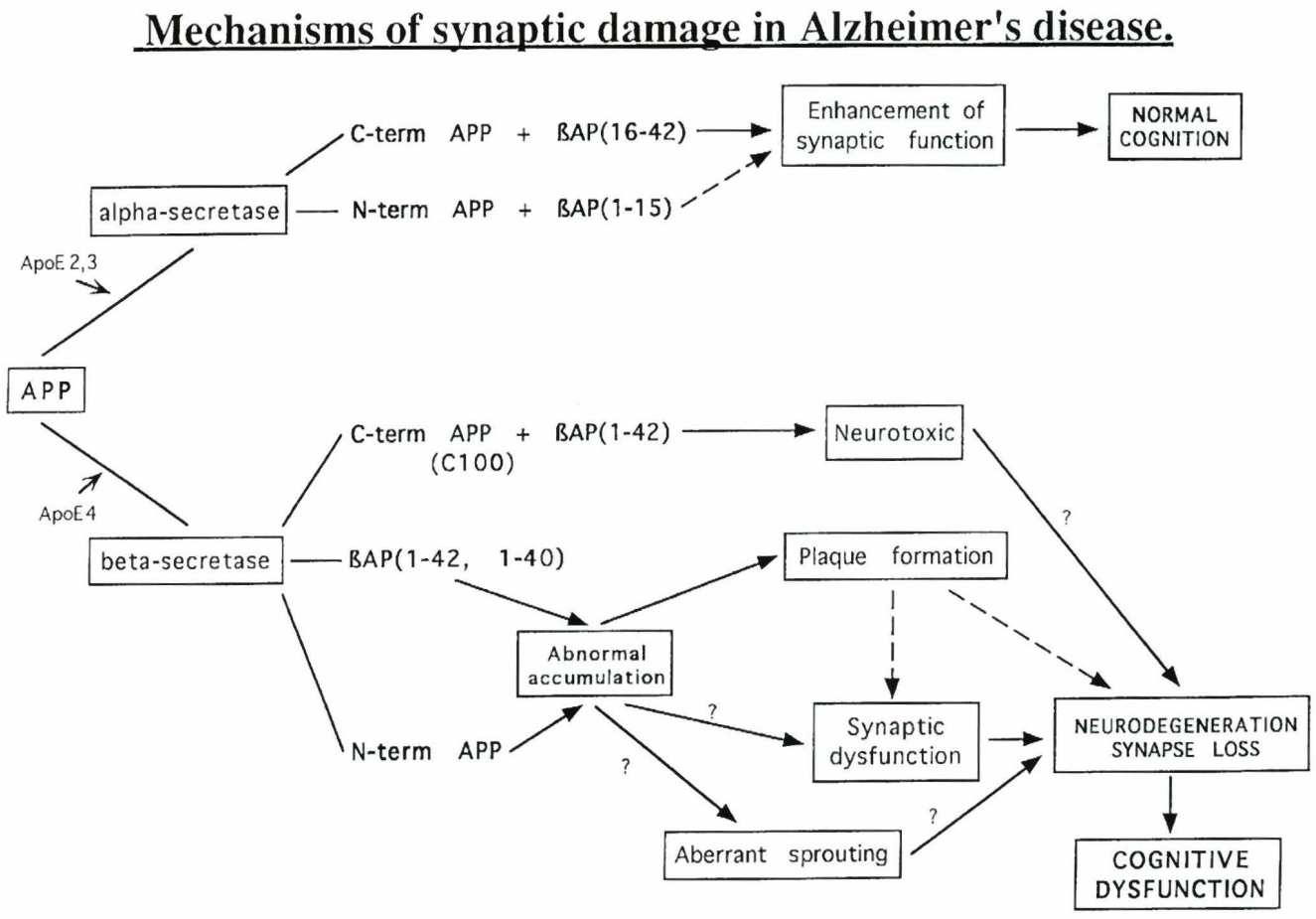

Fig. 3. Schematic representation of the enzymatic pathways involved in APP processing. Abnormal processing of APP through the ß-secretase pathway might not only result in the production of ßAP, but also in the generation of dysfunctional N-terminal APP fragments that might lead to synaptic dysfunction.

soluble forms of ßAP (1-40) are cleared and recycled, while ßAP (1-42) tends to accumulate in the neuropil eventually leading to plaque formation (Cai et al., 1993; Higgins et al., 1994; Murphy et al., 1994; Suzuki et al., 1994). It is conceivable that aberrant processing of APP might lead to the microdeposition of abnormal (and various MW) products at the synaptic site, which eventually results in damage to the synapto-dendritic apparatus (Fig. 3). Aberrant processing and/or clearance of APP products could lead to neuro-degeneration by: 1) direct toxic effect of elevated levels of aggregated ßAP, 2) since APP may turn out to be a important synaptic protein, its abnormal processing can result in synaptic dysfunction, 3) since APP might play an important role in neuronal survival, malfunctional APP could lead to lack of neuroprotection, and 4) any combination of the three. Abnormal deposits of aberrantly processed APP products at the synaptic site might cause damage by interfering with neurotransmission and/or by disturbing the calcium balance (Mattson et al., 1993b) at the synapses (Fig. 3). Supporting this possibility, recent studies have shown that in AD there is abnormal accumulation of APP, as well as several synaptic proteins, in neuritic plaques and synaptic terminals (Joachim et al., 1991; Masliah et al., 1992c, 1994a; Masliah and Terry, 1993) (Fig. 4).

Further evidence supporting the concept that abnormal accumulation of amyloidogenic proteins could alter synaptic function has been derived from studies of Creutzfeldt-Jakob disease (CJD), where prion protein $(Prp)^{CJD}$ accumulates in synapses (Kitamoto et al., 1992b). Moreover, in CJD and other prion protein diseases the patterns of synaptophysin and SNAP25 (another synaptic-associated molecule) immunostaining are abnormal, indicating a primary synaptic alteration in these conditions (Clinton et al., 1993). Recent studies have shown that in CJD, depending on the genetic alteration, PrP could accumulate either in a plaque-like fashion or in the synapses (Kitamoto et al., 1992b). Point mutation in codon 102 or 117/129 results in a plaque-type PrP accumulation (Kitamoto et al., 1992a,b), while a point mutation in codon 200 or no mutations in the PrP gene results in synaptic-type PrP accumulation (Kitamoto et al., 1992a,b).

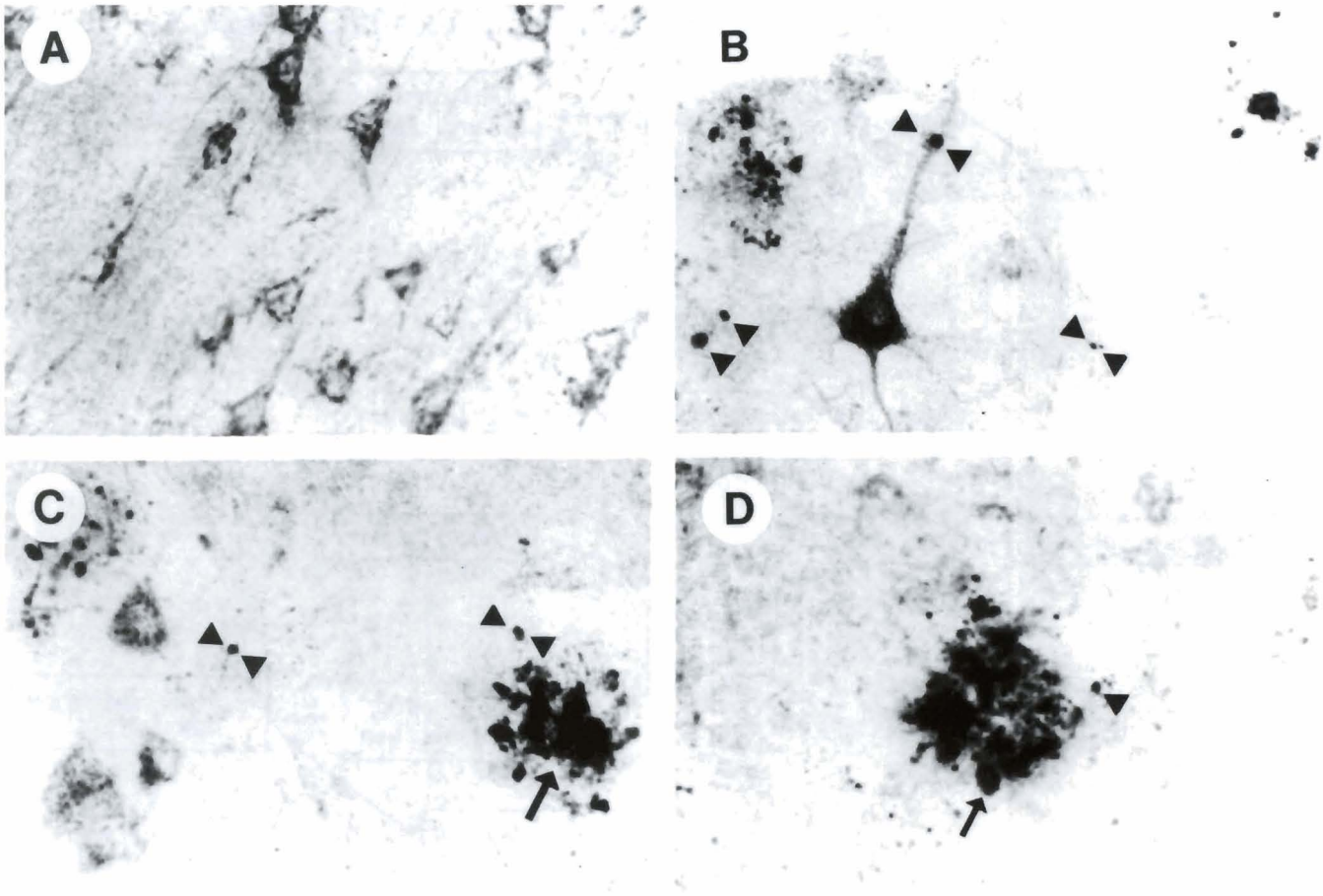

Fig. 4. Patterns of APP immunoreactivity in human frontal cortex. The monoclonal antibody specific for human APP (8E5, Athena Neurosciences) recognizes in the control cases (A) the neuronal cell bodies, as well as some synapses. In AD **(B, C, D)**, there is a significant increase in APP immunoreactivity in synaptic terminals (arrow heads) and dystrophic neurites in the plaque (arrows). x 350

Taken together, these findings suggest that abnormal accumulation of potentially amyloidogenic proteins at the synaptic site might be responsible for synaptic dysfunction and neurodegeneration observed in these disorders (Probst et al., 1991; Kitamoto et al., 1992a,b).

Concluding remarks: The role of genetic abnormalities in pathogenesis of synaptic alterations in AD

Currently it is unclear how the expression of abnormal genotypes (APP mutations) (Goate et al., 1991; Peacock et al., 1993), Chr14 mutations (Schellenberg et al., 1992), APOE ε4 (Corder et al., 1993; Saunders et al., 1993; Strittmatter et al., 1993a) might lead and/or confer susceptibility to the same clinical/pathological entity - AD. However, the prevalent hypothesis is that APP metabolism and processing is affected leading to neurodegeneration by either ßAP deposition (Strittmatter et al., 1993a) and/or disruption of synaptic function (Masliah and Terry, 1993). The levels of APP within the nervous system, especially at the synaptic site, may depend on the rate of production/transport, proteolytic metabolism and clearance of APP products. In consequence, genetic alterations that might disturb any or all of the steps involved in metabolism and transport of APP function could lead to alterations of the function of this molecule at the synaptic site. While recently described mutations within the APP molecule in familial AD appear to affect cleavage and processing of APP (Suzuki et al., 1994; Zhong et al., 1994), the polymorphism in APOE might affect the clearance of metabolically processed APP products (Schmechel et al., 1993; Strittmatter et al., 1993a,b; Wisniewski et al., 1993). In either case, the end result will be the abnormal accumulation of degraded products in the neuropil (Fig. 3).

Recent studies have shown that the presence of APOE ε4 allele is the major risk factor for AD, since more than 50% of patients with sporadic and familial AD (Corder et al., 1993; Saunders et al., 1993; Strittmatter et al., 1993a) and LBV (Galasko et al., 1994) display this allele. The mechanisms by which apoE is associated with AD are not known. However, it has been shown that apoE binds high affinity ß-amyloid and that AD patients with the APOE ε4 allele have more dense amyloid deposits within their brains (Schmechel et al., 1993; Strittmatter et al., 1993a,b; Wisniewski et al., 1993). Furthermore, apoE appears to be an important CNS molecule which is centrally involved in synaptic regeneration after injury and in neuritic outgrowth (Poirier et al., 1993; Nathan et al., 1994). In this regard, we have recently shown that in AD cases displaying the APOE ε4 allele synaptic loss is more severe than in cases with APOE ε3 allele (Miller et al., 1994). Furthermore, aged homozygous APOE-knockout mice show significant loss of dendrites and presynaptic terminals, accompanied by microgliosis and abnormal regeneration after lesion (Masliah et al., 1994b).

In conclusion genetic alterations that interferes with the processing of APP could result in the abnormal function of this protein. This not only leads to the deposition of amyloid and plaque formation, but it also interferes with the synaptotrophic and stabilizing functions (Saitoh et al., 1994) of this molecule promoting eventually synaptic damage, neurodegeneration and cognitive dysfunction.

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