Histol Histopathol (1995) 10: 509-519

Histology and Histopathology

*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;

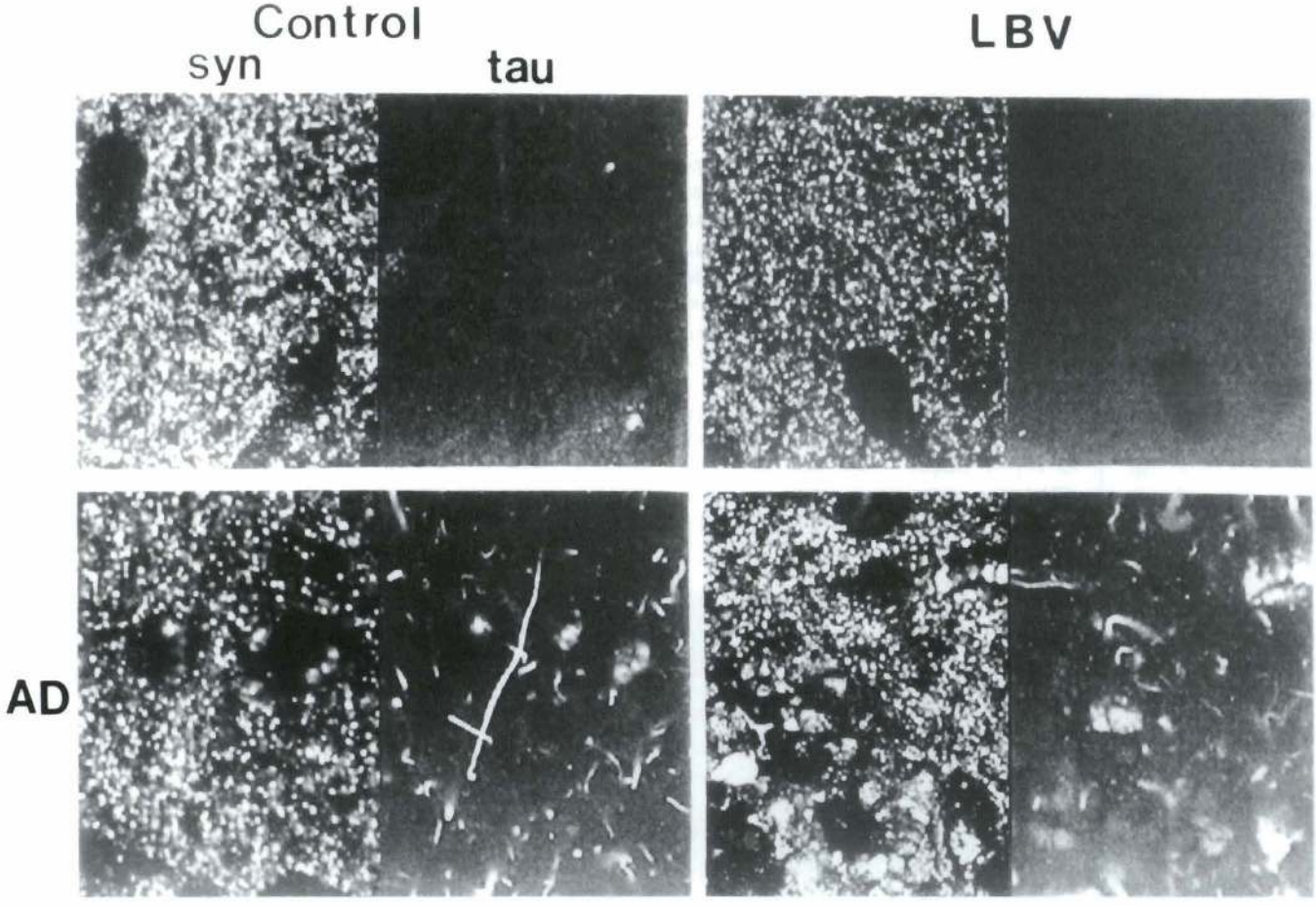
Lassman et al., 1992). It has been hypothesized that the dementia in AD could be caused by either the presence of these specific lesions alone or by the synergistic effect of some or all of these lesions (DeKosky and Scheff, 1990; Terry et al., 1991; Samuel et al., 1994). The original studies by Blessed 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).

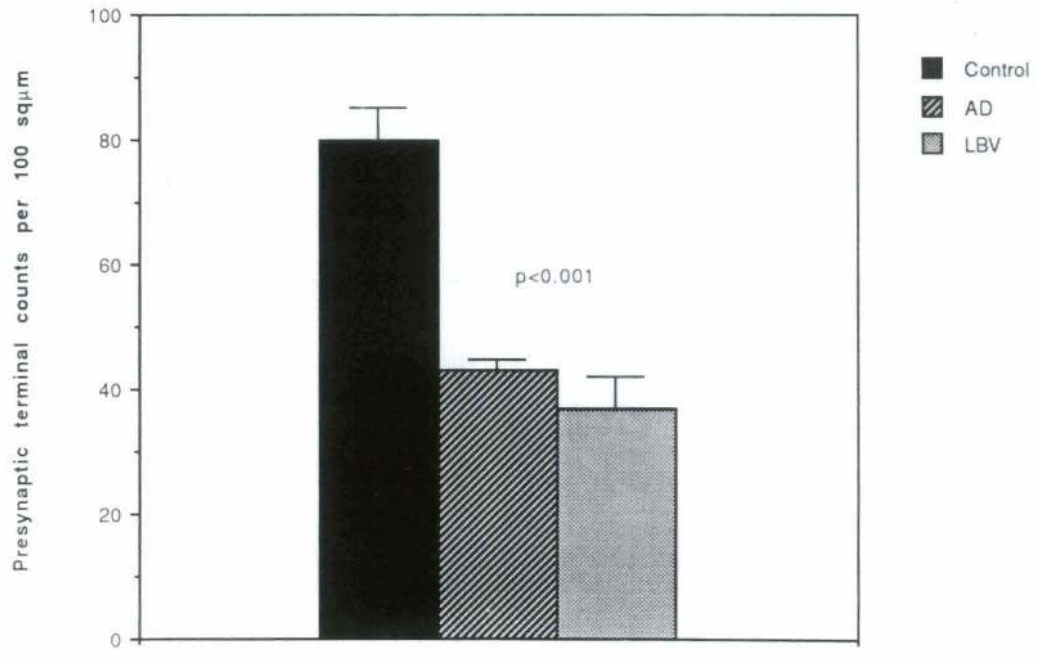
### Cellular mechanisms of synaptic damage in AD

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
syn
tau
LBV
AD


Presynaptic terminal counts per 100 sqμm
100
80
60
40
20
0
p<0.001
Control
AD
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

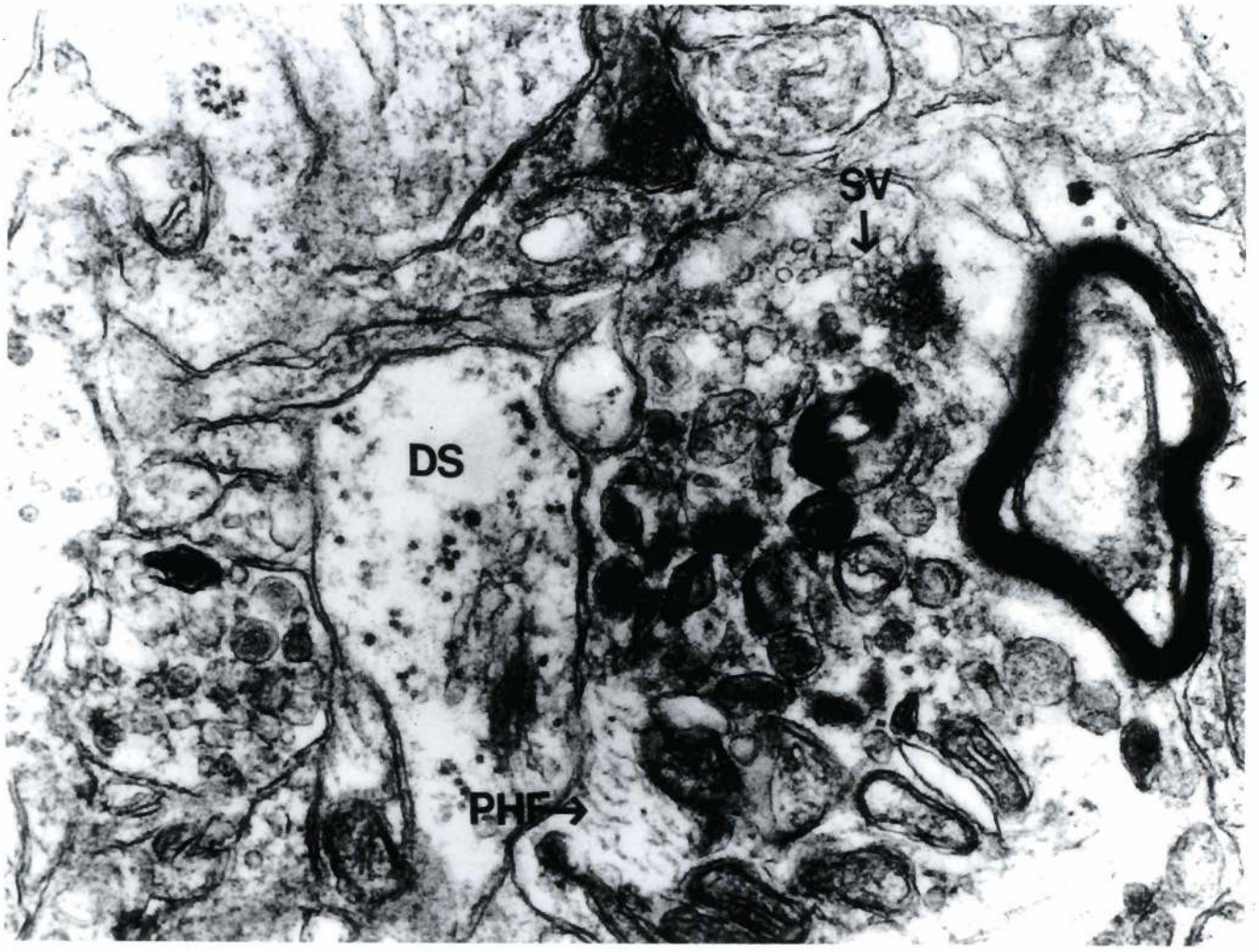



**Fig. 2.** Ultrastructural characteristics of the synaptic alteration in AD. The synaptic terminals and axons are enlarged and contain groups of synaptic vesicles (SV), paired helical filaments (PHF), and laminated bodies. This neurite is adjacent to a dendritic spine (DS). x 13,000

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

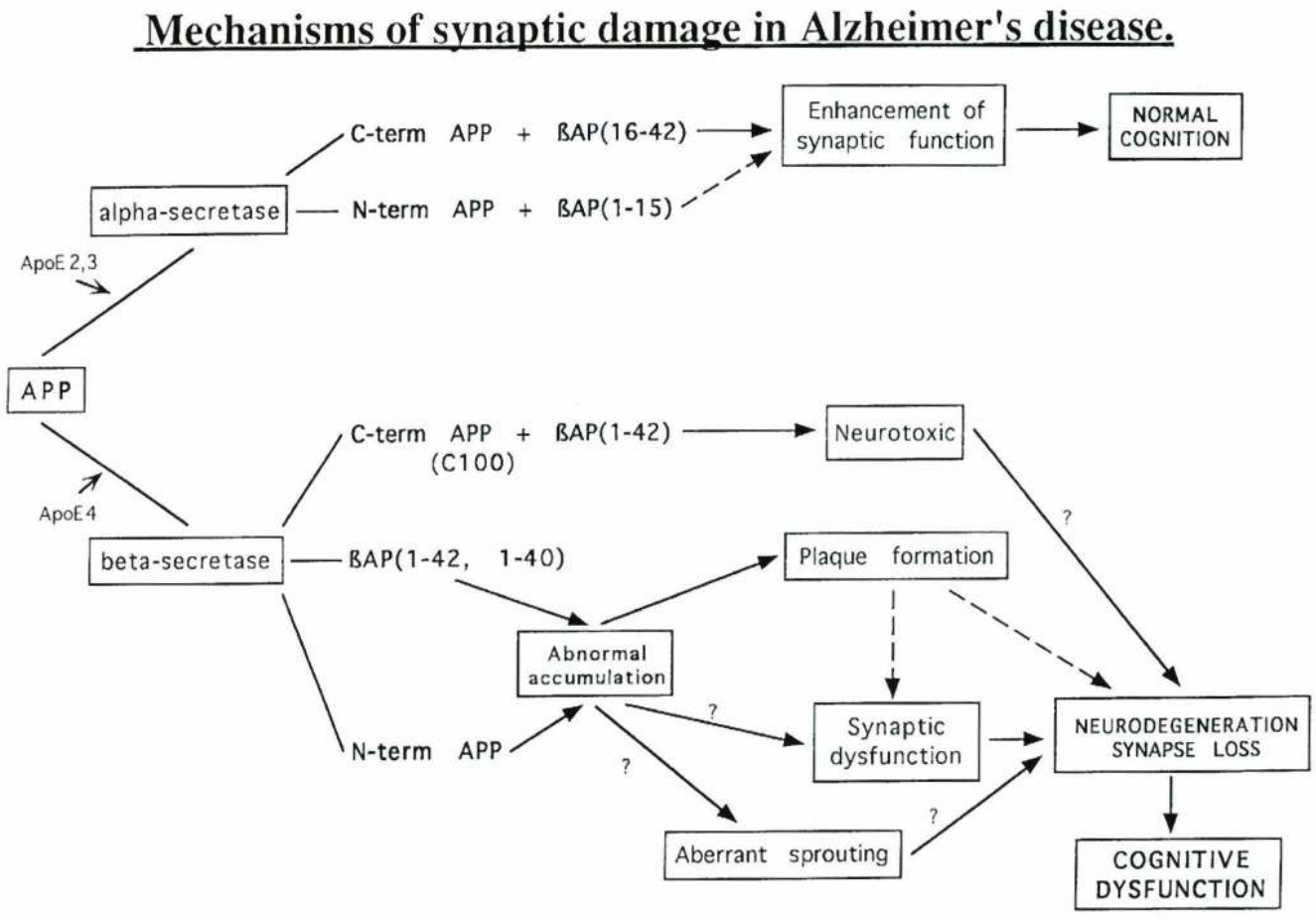



**Fig. 3.** Schematic representation of the enzymatic pathways involved in APP processing. Abnormal processing of APP through the ß-secretase pathway might not only result in the production of ßAP, but also in the generation of dysfunctional N-terminal APP fragments that might lead to synaptic dysfunction.

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).

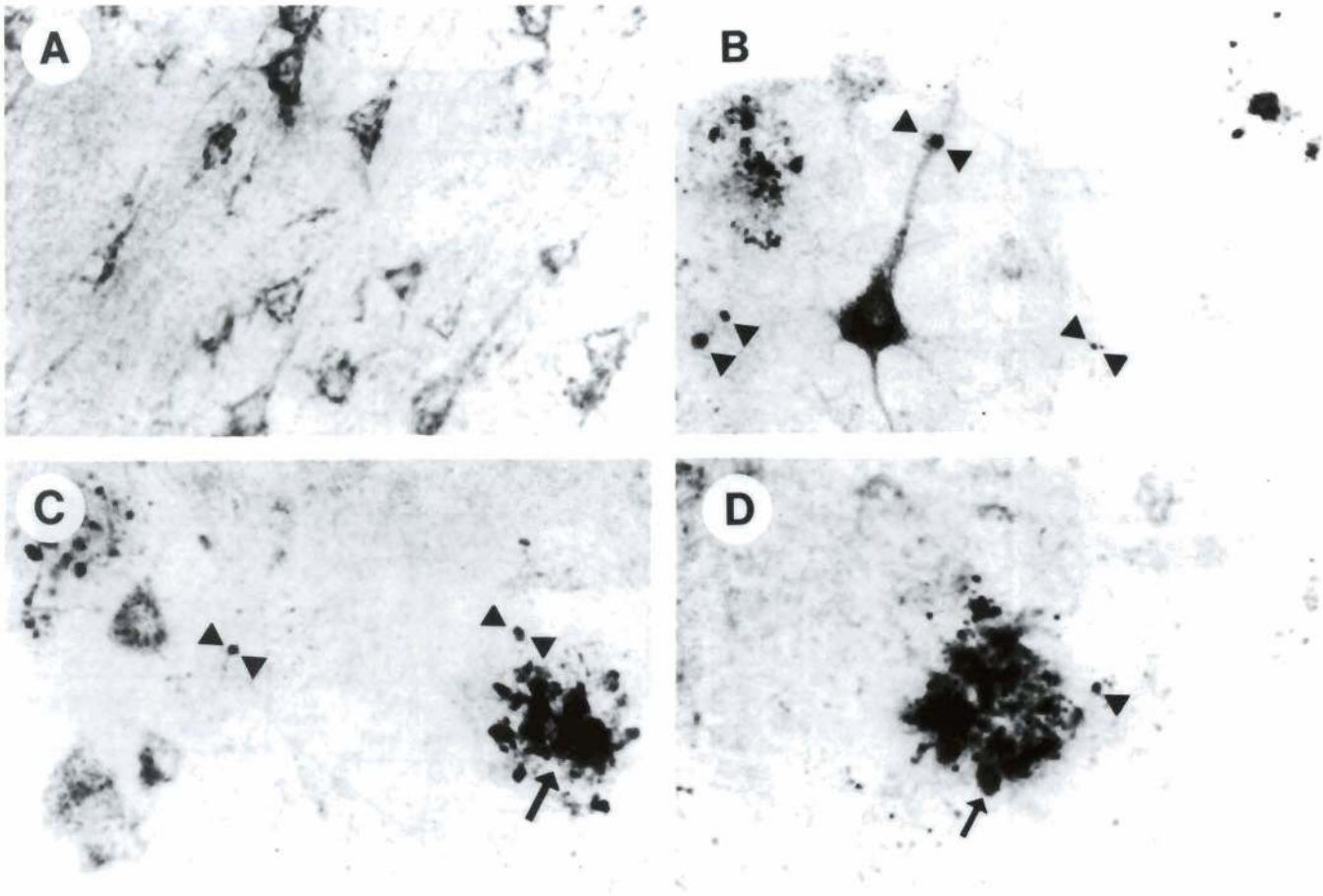



**Fig. 4.** Patterns of APP immunoreactivity in human frontal cortex. The monoclonal antibody specific for human APP (8E5, Athena Neurosciences) recognizes in the control cases (A) the neuronal cell bodies, as well as some synapses. In AD **(B, C, D)**, there is a significant increase in APP immunoreactivity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

*Acknowledgements.* The author wishes to thank Ms. Margaret Mallory for her expert technical assistance and Dr. P. Seubert at Athena Neurosciences for providing the antibody 8E5. This work was supported by NIH Grants AG05131, AG10689 and by the Department of Health Services, State of California (92-1539).

## References

Alford M.F., Masliah E., Hansen L.A. and Terry R.D. (1994). A simple dot-immunobinding assay for the quantification of synaptophysin-like immunoreactivity in human brain. J. Histochem. Cytochem. 42, 283-287.

Allsop D., Yamamoto T., Kametani F., Miyazaki N. and Ishii T. (1991). Alzheimer amyloid b/A4 peptide binding sites and a possible APP-secretase' activity associated with rat brain cortical membranes. Brain Res. 551, 1-9.

Alvarez J., Moreno R.D., Llanos O., Inestrosa N.C., Brandan E., Colby T. and Esch F.S. (1992). Axonal sprouting induced in the sciatic nerve by the amyloid precursor protein (APP) and other anti-proteases. Neurosci. Lett. 144, 130-134.

Alzheimer A. (1907). Uber eine eigenartige Erkrankung der Hinrninde. Algemeine Zeitschrift fur Psychiatric 64, 146-148.

Anderson J.P., Chen Y., Kim K.S. and Robakis N.K. (1992). An alternative secretase cleavage produces soluble Alzheimer amyloid precursor protein containing a potentially amyloidogenic sequence. J. Neurochem. 59, 2328-2331.

Armstrong D.M., Benzing W.C., Evans J., Terry R.D., Shields D. and Hansen L.A. (1989). Substance P and somatostatin coexist within neuritic plaques: implication for the pathogenesis of Alzheimer's disease. Neuroscience 31, 663-671.

Arrigada P.V., Growdon J.H., Hedley-Whyte E.T. and Hyman B.T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 42, 631-639.

Askanas V., Engel W.K. and Alvarez R.B. (1992). Strong immuno-reactivity of ß-amyloid precursor protein, including the ß-amyloid protein sequence, at human neuromuscular junctions. Neurosci. Lett. 143, 96-100.

Bieger S., Klafki H.W. and Unsicker K. (1993). Synthesis and release of the beta-amyloid precursor protein by bovine chromaffin cells. Neurosci. Lett. 162, 173-175.

Birecree E., Whetsell W.O. Jr., Stocsckeck C., King L.E. Jr. and Nanney L.B. (1988). Immunoreactive epidermal growth factor receptors in neuritic plaques from patients with Alzheimer's disease. J. Neuropathol. Exp. Neurol. 47, 549-560.

Blessed G., Tomlinson B.E. and Roth M. (1968). The association between quantitative measures of dementia and senile change in the cerebral grey matter of elderly subjects. Br. J. Psych. 114, 797-811.

Braak H. and Braak E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 82, 239-259.

Cai X-D., Golde T.E. and Younkin S.G. (1993). Release of excess amyloid ß-protein from a mutant amyloid ß protein precursors. Science 259, 514-516.

Clinton J., Forsyth C., Royston M.C. and Roberts G.W. (1993). Synaptic degeneration is the primary neuropathological feature in prion disease: a preliminary study. NeuroReport 4, 65-68.

Corder E.H., Saunders A.M., Strittmatter W.J., Schemechel D.E., Gaskell P.C., Small G.W., Roses A.D., Haines J.L. and Pericak-Vance M.A. (1993). Gene dose of apoliprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921-923.

Cotman C.W., Cummings B.J. and Whitson J.S. (1991). The role of misdirect plasticity in plaque biogenesis and Alzheimer's disease pathology. In: Growth factors and Alzheimer's disease. Hefti F., Brachet P., Will B. and Christen Y. (eds). Springer-Verlag. New York. pp 222-233.

da Cruz e Silva O.A., Iverfeldt K., Oltersdorf T., Sinha S., Lieberburg I., Ramabhadran T.V., Suzuki T., Sisodia S.S., Gandy S. and Greengard P. (1993). Regulated cleavage of Alzheimer-beta-amyloid precursor protein in the absence of the cytoplasmic tail. J. Neurochem. 61, 2326-2329.

Dahl D., Labkovsky B. and Bignami A. (1989). Early and late appearance of neurofilament phosphorylation events in normal regeneration. Brain Res. 22, 225-232.

Davies C.A., Mann D.M.A., Sumpter P.Q. and Yates P.O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. J. Neurol. Sci. 78, 151-164.

De Strooper B., Umans L., Van Leuven F. and Van Den Berghe H. (1993). Study of the synthesis and secretion of normal and artificial mutants of murine amyloid precursor protein (APP): cleavage occurs in a late compartment of the default secretion pathway. J. Cell Biol. 121, 295-304.

DeKosky S.T. and Scheff S.W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann. Neurol. 27, 457-464.

Delaere P., Duyckaerts C., Brion J.P., Poulain V. and Hauw J.J. (1989). Tau, paired helical filaments and amyloid in the neocortex: a morphometric study of 15 cases with graded intellectual status in aging and senile dementia of Alzheimer type. Acta Neuropathol. 77, 645-653.

Dickson D.W., Farlo J., Davies P., Crystal H., Fuld P. and Yen S.C. (1988). Alzheimer disease. A double immunohistochemical study of senile plaques. Am. J. Pathol. 132, 86-101.

Fisher S., Gearhart J.D. and Oster-Granite M.L. (1991). Expression of the amyloid precursor protein gene in mouse oocytes and embryos. Proc. Natl. Acad. Sci. USA 88, 1779-1782.

Fukushima D., Konishi M., Maruyama K., Miyamoto T., Ishiura S. and Suzuki K. (1993). Activation of the secretory pathway leads to a decrease in the intracellular amyloidogenic fragments from the amyloid protein precursor. Biochem. Biophys. Res. Commun. 194, 202-207.

Gabuzda D., Busciglio J. and Yankner B.A. (1993). Inhibiton of beta-amyloid production by activation of protein kinase C. J. Neurochem. 61, 2326-2329.

Galasko D., Saitoh T., Xia Y., Thal L.J., Katzman R., Hill L.R. and Hansen L. (1994). The apolipoprotein E allele 4 is over-represented in patients with the Lewy body variant of Alzheimer's disease. Neurology 44, 1950-1951.

Goate A., Chartier-Harlin M.-C., Mullan M., Brown J., Crawford F., Fidani L., Guiffra L., Haynes A.,Irving N., James L., Mant R., Newton P., Rooke K., Roques P., Talbot C., Williamson R., Rossor M., Owen M. and Hardy J. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 394, 704.

Gomez-Pinilla F., Cummings B.J. and Cotman C.W. (1990). Induction of basic fibroblast growth factor in Alzheimer's disease pathology. NeuroReport 1, 211-214.

Gonatas N.K., Anderson W.W. and Evangelista I. (1967). The contribution of altered synapses in the senile plaque: an electron microscopic study in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 26, 25-39.

Gonatas N.K. and Gambetti P. (1970). The pathology of the synapse in Alzheimer's disease. Ciba Foundation Symposium on Alzheimer's disease and related conditions. J & A Churchill. New York.

Hamos J.E., DeGennaro L.J. and Drachman D.A. (1989). Synaptic loss in Alzheimer's disease and other dementias. Neurology 39, 355-361.

Higgins L.S., Holtzman D.M., Rabin J., Mobley W.C. and Cordell B. (1994). Transgenic mouse brain histopathology resembles early Alzheimer's disease. Ann. Neurol. 35, 598-607.

Hof P.R., Cox K. and Morrison J.H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: I. Superior frontal and inferior temporal cortex. J. Comp. Neurol. 301, 44-54.

Honer W.G., Dikson D.W., Gleeson J. and Davies P. (1992). Regional synaptic pathology in Alzheimer's disease. Neurobiol. Aging 13, 375-382.

Hyman B.T., Van Hoesen G.W., Kromer L.J. and Damasio A.R. (1986). Perforant pathway changes in the memory impairment of Alzheimer's disease. Ann. Neurol. 20, 472-481.

Ihara Y. (1988). Massive somatodendritic sprouting of cortical neurons in Alzheimer's disease. Brain Res. 459, 138-144.

Joachim C., Games D., Morris J., Ward P., Frenkel D. and Selkoe D. (1991). Antibodies to non-beta regions of the beta-amyloid precursor protein detect a subset of senile plaques. Am. J. Pathol. 138, 373-384.

Katzman R., Terry R., DeTeresa R., Brown T., Davies P., Fuld P., Renbing X. and Peck A. (1988). Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous recortical plaques. Ann. Neurol. 23, 138-144.

Khachaturian Z.S. (1985). Diagnosis of Alzheimer's disease. Arch. Neurol. 42, 1097-1105.

Kitamoto T., Doh-ura K., Muramoto T., Miyazono M. and Tateishi J. (1992a). The primary structure of the prion protein influences the distribution of abnormal prion protein in the central nervous system. Am. J. Pathol. 141, 271-277.

Kitamoto T., Shin R-W., Doh-ura K., Tomokane N., Miyazono M., Muramoto T. and Tateishi J. (1992b). Abnormal isoform of prion proteins accumulates in the synaptic structures of the central nervous system in patients with Creutzfeldt-Jakob disease. Am. J. Pathol. 140, 1285-1294.

Koo E., Sisodia S.S., Archer D.R., Martin L.J., Weidemann A., Beyreuther K., Fischer P., Masters C.L. and Price D.L. (1990). Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. Proc. Natl. Acad. Sci. USA 87, 1561-

1565.

Lassman H., Weiler R., Fischer P., Bancher C., Jellinger K., Floor E., Danielcky W., Seitelberger F. and Winkler H. (1992). Synaptic pathology in Alzheimer's disease: immunological data for markers of synaptic and large dense-core vesicles. Neuroscience 46, 1-8.

Loeffler J. and Huber G. (1993). Modulation of beta-amyloid precursor protein secretion in differentiated and nondifferentiated cells. Biochem. Biophys. Res. Commun. 195, 97-103.

Maruyama K., Kametani F., Usami M., Yamao-Harigaya W. and Tanaka K. (1991). «Secretase», Alzheimer amyloid protein precursor secreting enzyme is not sequence-specific. Biochem. Biophys. Res. Commun. 179, 1670-1676.

Masliah E. and Terry R. (1993). The role of synaptic proteins in the pathogenesis of disorders of the central nervous system. Brain Pathol. 3, 77-85.

Masliah E. and Terry R. (1994). The role of synaptic pathology in the mechanisms of dementia in Alzheimer's disease. Clin. Neurosci. 1, 192-198.

Masliah E., Terry R.D., DeTeresa R.M. and Hasen L.A. (1989). Immunohistochemical quantificaction of the synapse-related protein synaptophysin in Alzheimer disease. Neurosci. Lett. 103, 234-239.

Masliah E., Fagan A.M., Terry R.D., DeTeresa R., Mallory M. and Gage F.H. (1991a). Reactive synaptogenesis assessed by synaptophysin immunoreactivity is associated with GAP-43 in the dentate gyrus of the adult rat. Exp. Neurol. 113, 131-142.

Masliah E., Hansen L., Albright T., Mallory M. and Terry R.D. (1991b). Immunoelectron microscopic study of synaptic pathology in Alzheimer disease. Acta Neuropathol. 81, 428-433.

Masliah E., Hansen L., Mallory M., Albright T. and Terry R.D. (1991c). Abnormal brain spectrin immunoreactivity in sprouting neurons in Alzheimer disease. Neurosci. Lett. 129, 1-5.

Masliah E., Terry R.D., Alford M., DeTeresa R.M. and Hansen L.A. (1991d). Cortical and subcortical patterns of synaptophysin-like immunoreactivity in Alzheimer disease. Am. J. Pathol. 138, 235-246.

Masliah E., Mallory M., Hansen L., Alford M., Albright T., DeTeresa R., Terry R.D., Baudier J. and Saitoh T. (1991e). Patterns of aberrant sprouting in Alzheimer disease. Neuron 6, 729-739.

Masliah E., Mallory M., Ge N. and Saitoh T. (1992a). Amyloid precursor protein is localized in growing neurites of neonatal rat brain. Brain Res. 593, 323-328.

Masliah E., Mallory M., Ge N. and Saitoh T. (1992b). Protein kinases and growth associated proteins in plaque formation in Alzheimer's disease. Rev. Neurosci. 3, 99-107.

Maliash E., Mallory M., Hansen L., Alford M., DeTeresa R., Terry R., Baudier J. and Saitoh T. (1992c). Localization of amyloid precursor protein in GAP43-immunoreactive aberrant sprouting neurites in Alzheimer's disease. Brain Res. 574, 312-316.

Masliah E., Ellisman M., Carragher B., Mallory M., Young S., Hansen L., DeTeresa R. and Terry R.D. (1992d). Three-dimensional analysis of the relationship between synaptic pathology and neuropil threads in Alzheimer disease. J. Neuropathol. Exp. Neurol. 51, 404-414.

Masliah E., Mallory M., Hansen L., Alford M., DeTeresa R. and Terry R. (1993a). An antibody against phosphorylated neurofilaments identifies a subset of damaged association axons in Alzheimer's disease. Am. J. Pathol. 142, 871-882.

Masliah E., Mallory M., Deerinck T., DeTeresa R., Lamont S., Miller A., Terry R.D., Carragher B. and Ellisman M. (1993b). Re-evaluation of the structural organization of neuritic plaques in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 52, 135-142.

Masliah E., Mallory M., DeTeresa R., Alford M. and Hansen L. (1993c). Differing patterns of aberrant neuronal sprouting in Alzheimer's disease with an without Lewy bodies. Brain Res. 617, 258-266.

Masliah E., Mallory M., Ge N., Godson C. and Saitoh T. (1993d). Phorbol ester-induced neuritic alterations in the rat neocortex. Structural and immunocytochemical studies. Mol. Chem. Neuropathol. 20, 125-145.

Masliah E., Mallory M., Hansen L., DeTeresa R. and Terry R.D. (1993e). Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43, 192-197.

Masliah E., Mallory M., Alford M., Hansen L.A. and Saitoh T. (1993f). Immunoreactivity of the nuclear antigen p105 is associated with plaques and tangles in Alzheimer's disease. Lab. Invest. 69, 562-569.

Masliah E., Honer W.G., Mallory M., Voigt M., Krushner P. and Terry R.D. (1994a). Topographical distribution of synaptic-associated proteins in the neuritic plaques of Alzheimer disease hippocampus. Acta Neuropathol. 87, 135-142.

Masliah E., Mallory M., Alford M. and Mucke L. (1994b). Anormal synaptic regeneration in hAPP transgenic and APOE-knockout mice. Neurobiol. Aging 15, S11.

Masliah E., Mallory M., Hansen L., DeTeresa R., Alford M. and Terry R. (1994c). Synaptic and neuritic alterations during the progression of Alzheimer's disease. Neurosci. Lett. 174, 67-72.

Masliah E., Ueda K. and Mucke L. (1994d). Pathophysiological basis of Alzheimer's disease. Proceedings of the XVII World congress of anatomic and clinical pathology. Monduzzi Editore. Bologna.

Mattson M.P., Barger S.W., Cheng B., Lieberburg I., Smith-Swintosky V.L. and Rydel R.E. (1993a). ß-amyloid precursor protein metabolites and loss of neuronal $Ca^{2+}$ homeostasis in Alzheimer's disease. TINS 16, 409-414.

Mattson M.P., Cheng B., Culwell A.R., Esch F.S., Lieberburg I. and Rydel R.E. (1993b). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the ß-amyloid precursor protein. Neuron 10, 243-254.

Mattson M.P., Cheng B. and Smith-Swintosky V.L. (1993c). Mechanisms of neurotrophic factor protection against calcium- and free radical-mediated excitotoxic injury: implications for trating neurodegenerative dissorders. Exp. Neurol. 124, 89-95.

McKee A.C., Kosik K.S. and Kowall N.W. (1991). Neuritic pathology and dementia in Alzheimer's disease. Ann. Neurol. 30, 156-165.

Miller A., Alford M., Katzman R., Thal L. and Masliah E. (1994). The expression of the ApoE4 allele in Alzheimer's disease accentuates the synaptic loss and the severity of the dementia. Ann. Neurol. 36, 268-269.

Milward E.A., Papadopulous R., Fuller S.J., Moir R.D., Small D., Beyreuther K. and Masters C.L. (1992). The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. Neuron 9, 129-137.

Mirra S.S., Hart M.N. and Terry R.D. (1993). Making the diagnosis of Alzheimer's disease. A primer for practicing pathologists. Arch. Pathol. Lab. Med. 117, 132-144.

Morrison J.H., Lewis D.A. and Campbell M.J. (1987). Distribution of neurofibrillary tangles and nonphosphorylated neurofilament protein-immunoreactive neurons in cerebral cortex: implications for loss of corticocortical circuits in Alzheimer's disease. In: Molecular neuropathology of aging. Branbury report. Davies P., Finch C.E. (eds). Cold Springs Harbor Laboratory. New York. pp 109-124.

Mucke L., Masliah E., Johnson W.B., Ruppe M.D., Alford M.,

Rockenstein E.M., Forss-Petter S., Pietropaolo M., Mallory M. and Abraham C.R. (1994). Synaptotrophic effects of human amyloide ß protein precursors in the cortex of transgenic mice. Brain Res. 666, 151-167.

Murphy G.M., Forno L.S., Higgins L., Scardina J.M., Engl L.F. and Cordell B. (1994). Development of a monoclonal antibody specific for the COOH-terminal of ß-amyloid 142 and its immunohistochemical reactivity in Alzheimer's disease and related disorders. Am. J. Pathol. 144, 1082-1088.

Nathan B.P., Bellosta S., Sanan D.A., Weisgraber K.H., Mahley R.W. and Pitas R.E. (1994). Differential effects of apiloproteins E3 and E4 on neuronal growth in vitro. Science 264, 850-852.

Neary D., Snowden J.S., Mann D.M.A., Bown D.M., Sims N.R., Northen B., Yates P.O. and Davison A.N. (1986). Alzheimer's disease: a correlative study. J. Neurol. Neurosurg. Psych. 49, 229-237.

Nishiguchi S., Maeda S., Araki S. and Shimada K. (1988). Structure of the mouse serum amyloid P component gene. Biochem. Biophys Res. Commun. 155, 1366-1373.

Peacock M.L., Warren J.T. Jr., Roses A.D. and Fink J.K. (1993). Novel polymorphism in the A4 region of the amyloid precursor protein gene in a patient without Alzheimer's disease. Neurology 43, 1254-1256.

Poirier J., Bacchichet A., Dea D. and Gauthier S. (1993). Cholesterol synthesis and lipoprotein reuptake during synaptic remodeling in hippocampus in adult rats. Neuroscience 55, 81-90.

Probst A., Langui D., Ipsen S., Robakis N. and Ulrich J. (1991). Deposition of b/A4 protein along neuronal plasma membranes in diffuse senile plaques. Acta Neuropathol. 83, 21-29.

Roch J-M., Shapiro P., Sundsmo M.P., Otero D.A.C., Refolo L.M., Robakis N.K. and Saitoh T. (1992). Bacterial expression, purification, and functional mapping of the amyloid b/A4 protein precursor. J. Biol. Chem. 267, 2214-2221.

Roch J-M., Masliah E., Roch-Levecq A-C., Sundsomo M.P., Otero D.A.C., Veinberg I. and Saitoh T. (1994). Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid b/A4 protein precursor. Proc. Natl. Acad. Sci. USA 91, 7650-7654.

Saitoh T., Roch J-M., Jin L-W., Ninomiya H., Otero D.A.C., Yamamoto K. and Masliah E. (1994). The biological function of amyloid b/A4 protein precursor. In: Amyloid protein precursor in development, aging and Alzheimer's disease. Masters C.L. (ed). Springer-Verlag. Berlin. pp 90-99.

Salmon D.P., Kwo-on-Yuen P.F., Heindel W.C., Butters N. and Thal L.J. (1989). Differentiation of Alzheimer's disease and Huntington's disease with the Dementia Rating Scale. Arch. Neurol. 46, 1204-1208.

Samuel W., Terry R.D., DeTeresa R., Butters N. and Masliah E. (1994). Clinical correlates of cortical and nucleus basalis pathology in Alzheimer dementia. Arch. Neurol. 51, 772-778.

Saunders A.M., Strittmatter W.J., Schmechel D., St.George-Hyslop P.H., Pericak-Vance M.A., Joo S.H., Rosi B.L., Gusella J.F., Crapper-MachLachlan D.R., Alberts M.J., Hulette C., Crain B., Goldgaber D. and Roses A.D. (1993). Association of apolipoprotein E allele E4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43, 1467-1472.

Scheff S.W., DeKosky S.T. and Price D.A. (1990). Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol. Aging 11, 29-37.

Scheff S.W. and Price D.A. (1993). Synapse loss in the temporal lobe in Alzheimer's disease. Ann. Neurol. 33, 190-199.

Schellenberg G.D., Bird T.D., Wijsman E.M., Orr H.T., Anderson L., Nemens E., White J.A., Bonnycastle L., Weber J.L., Alonso M.E., Potter H., Heston L.L. and Martin G.M. (1992). Genetic linkage evidence for a familial Alzheimer's disese locus on chromosome 14. Science 258, 668-671.

Schmechel D.E., Saunders A.M., Strittmatter W.J., Crain B.J., Hulettte C.M., Joo S.H., Pericak-Vance M.A., Goldgaber D. and Roses A.D. (1993). Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc. Natl. Acad. Sci. USA 90, 9649-9653.

Schubert W., Prior R., Weidemann A., Dircksen H., Multhaup G., Masters C.L. and Beyreuther K. (1991). Localization of Alzheiemr beta A4 amyloid precursor protein at central and periperal synaptic sites. Brain Res. 563, 184-194.

Selkoe D.J. (1989). Amyloid protein precursor and the pathogenesis of Alzheimer's disease. Cell 58, 611-612.

Sisodia S.S., Koo E.H., Beyreuther K., Unterbeck A. and Price D.L. (1990). Evidence that ß-amyloid protein in Alzheimer's disease is not derived by normal processing. Science 248, 492-494.

Slack B.E., Nitsch R.M., Livneh E., Kunz G.M. Jr., Breu J., Eldar H. and Wurtman R.J. (1993a). Regulation by phorbol esters of amyloid precursor protein release from Swiss 3T3 fibroblasts overexpressing protein kinase C alpha. J. Biol. Chem. 268, 21097-21101.

Slack B.E., Nitsch R.M., Livneh E., Kunz G.M. Jr., Eldar H. and Wurtman R.J. (1993b). Regulation of amyloid precursor protein release by protein kinase c in Swiss 3T3 fibroblasts. Ann. NY Acad. Sci. 695, 128-131.

Small D.H., Nurcombe V., Reed G., Clarris H., Moir R., Beyreuther K. and Masters C.L. (1994). A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. J. Neurosci. 14, 2117-2127.

Strittmatter W.J., Saunders A.M., Schmechel D., Pericak-Vance M., Enghild J., Salvesen G.S. and Roses A.D. (1993a). Apolipoprotein E: high avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc. Natl. Acad. Sci. USA 90, 1977-1981.

Strittmatter W.J., Weisgraber K.H., Huang D.Y., Dong L.M., Salvesen G.S., Pericak-Vance M., Schemechel D., Saunders A.M., Goldgaber D. and Rosas A.D. (1993b). Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc. Natl. Acad. Sci. USA 90, 8098-8102.

Suzuki N., Cheung T.T., Cai X-D., Odaka A., Otvos L. Jr., Eckman C., Golde T.E. and Younkin S.G. (1994). An increased percentage of long amyloid ß protein secreted by familial amyloid ß protein precursor ($\beta APP_{717}$) mutants. Science 264, 1336-1340.

Tagawa K., Kunishita T., Maruyama K., Yoshikawa K., Kominami E., Tsuchiya T., Suzuki K., Tabira T., Sugita H. and Ishiura S. (1991). Alzheimer's disease amyloid ß-clipping enzyme (APP secretase): Identification, purification, and characterization of the enzyme. Biochem. Biophys. Res. Commun. 177, 377-387.

Terry R.D. and Wisniewski H.M. (1970). The ultrastructure of the neurofibrillary tangle and the senile plaque. In: Ciba Foundation Symposium on Alzheimer's Disease and Related Conditions. J.A. Churchill. London. pp 145-168.

Terry R.D., Gonatas N.K. and Weiss M. (1964). Ultrastructural studies in Alzheimer's presenile dementia. Am. J. Pathol. 44, 269-297.

Terry R.D., Peck A., DeTeresa R., Schechter R. and Horoupian D.S.

(1981). Some morphometric aspects of the brain in senile dementia of the Alzheimer type. Ann. Neurol. 10, 184-192.

Terry R.D., DeTeresa R. and Hansen L.A. (1987a). Neocortical cell counts in normal human adult aging. Ann. Neurol. 21, 530-539.

Terry R.D., Hansen L.A., DeTeresa R., Davies P., Tobias H. and Katzman R. (1987b). Senile dementia of the Alzheimer type without neocortical neurofibrillary tangle. J. Neuropathol. Exp. Neurol. 46, 262-268.

Terry R.D., Masliah E., Salmon D., Butters N., DeTeresa R., Hansen L. and Katzman R. (1990). Structure function corelations in Alzheimer diseases. J. Neuropathol. Exp. Neurol. 49, 318 (Abstract).

Terry R.D., Masliah E., Salmon D., Butters N., DeTeresa R., Hansen L. and Katzman R. (1991). Physical basis of cognitive alterations in Alzheimer disease: synapse loss is the major correlate of cognitive impairment. Ann. Neurol. 39, 572-580.

Terry R.D., Hansen L. and Masliah E. (1994). Structural alterations in Alzheimer disease. In: Alzheimer disease. Terry R.D. and Katzman R. (eds). Raven Press. New York. pp 179-196.

Trojanowski J.Q., Schmidt M.L., Shin R-W., Bramblett G.T., Rao D. and Lee V.M.-Y. (1993). Altered tau and neurofilament proteins in neurodegenerative diseases; diagnostic implication for Alzheimer's disease and Lewy body dementias. Brain Pathol. 3, 45-54.

Uchida Y., Takio K., Titani K., Ihara Y. and Tomonaga M. (1991). The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. Neuron 7, 337-347.

Whitson J.S., Selkoe D.J. and Cotman C.W. (1989). Amyloid a protein enhances survival of hippocampal neurons *in vitro*. Science 243, 1488-1490.

Wisniewski T., Golabek A., Matsubara E., Ghiso J. and Frangione B. (1993). Apoliporotein E: binding to soluble Alzheimer's ß-amyloid. Biochem. Res. Commun. 192, 359-365.

Yamaguchi H., Hirai S., Morimatso M., Shoji M. and Ihara Y. (1988). A variety of cerebral amyloid deposits in the brains of Alzheimer-type dementia demonstrated by ß-protein immunostaining. Acta Neuropathol. 76, 541-549.

Yankner B.A., Duffy L.K. and Kirschner D.A. (1990). Neurotrophic and neurotoxic effects of amyloid ß protein: reversal by tachykinin neuropeptides. Science 250, 279-282.

Zhan S.S., Beyreuther K. and Schmitt H.P. (1993). Quantitative assessment of the synaptophysin immuno-reactivity of the cortical neuropil in various neurodegenerative disorders with dementia. Dementia 4, 66-74.

Zong Z., Quon D., Higgins L.S., Higaki J. and Cordell B. (1994). Increased amyloid production from aberrant ß-amyloid precursor proteins. J. Biol. Chem. 269, 12179-12184.