ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1994, 54: 47-53

The second layer neurones of the entorhinal cortex and the perforant path in physiological ageing and Alzheimer's disease

Janusz Moryś[1], Marcin Sadowski[1], Maria Barcikowska[2], Beata Maciejewska[1] and Olgierd Narkiewicz[1]

[1]Department of Anatomy, School of Medicine, 1 Dębinki St., 80-211 Gdansk; [2]Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa St., 00-784 Warsaw, Poland

Abstract. The hippocampal formation was studied in 5 brains of younger (29 to 52 years of age) and 6 brains of elderly (61 to 89 years of age) subjects without signs of dementia, as well as in 11 brains of patients with Alzheimer's disease (65 to 91 years of age). The 8-μm-thick sections were stained either with cresyl violet, Weil method or with immunocytochemical methods for amyloid (4G8) and neurofibrillary tangles (Tau-1). Cell bodies, senile plaques and tangles were counted in all brains. In brains of patients with Alzheimer's disease a significant neuronal loss (about 56 %) was observed in the second layer of the entorhinal cortex. The tangles/neurones ratio was very high (62.79.1%) in this layer. A great number of senile plaques were present in the whole hippocampal formation, especially in the molecular layer of the dentate gyrus (22.91.5 plaques/mm^2) which is the termination zone of the perforant path. It seems therefore, that pathological alterations in Alzheimer's disease disrupt the main input to the hippocampal formation. In "physiological" ageing we did not observe changes in the density of neurones, although single tangles and plaques were found in all hippocampal areas. In elderly individuals 3.81.3% of neurones of the second layer revealed neurofibrillary pathology; a few plaques were found in various areas of the hippocampal formation. These observations may suggest only a slight decrease in number of neurones in the hippocampal formation. However, these changes cause a slight impairment of memory and learning often found in elderly individuals without dementia.

Address for correspondence:
Janusz Moryś
Department of Anatomy
School of Medicine
1 Dębinki St.
80-211 Gdansk, Poland

Key words: entorhinal cortex, perforant path, Alzheimer's disease, ageing, neuronal loss, neurofibrillary tangles, senile plaques, morphometry

INTRODUCTION

In the human brain the entorhinal region spreads over both the ambient gyrus and the anterior portion of the parahippocampal gyrus (Braak 1972, 1980). The cardinal feature of this area is the presence of the second layer formed by large multipolar neurones whose dendritic arborization creates a star-like or stellate appearance in Golgi preparations (Braak 1980, Van Hoesen et al. 1991). The axons arising from these neurones form an important neuronal system called the perforant path (Van Hoesen and Pandya 1975, Witter et al. 1989). Fibres of the perforant path run through deeper layers of the entorhinal cortex and form the angular bundle. After perforation of the subicular complex they terminate in outer two-thirds of the molecular layer of the dentate gyrus. Some fibres cross the closed portion of the hippocampal fissure and enter the dentate gyrus directly from the subiculum while other fibres travel through the stratum lacunoso-moleculare of CA1-CA3 sectors of the cornu Ammonis. Most of the perforant path fibres reach dendrites of the granule cells of the dentate gyrus, but some of them terminate on the apical dendrites of CA1, CA2 and CA3 sectors (Amaral 1987, Amaral et al. 1987, Amaral and Witter 1989, Witter et al. 1989, Amaral and Insausti 1990, Witter and Amaral 1991). According to anatomical and physiological data, the most widely accepted and long-lived proposal of hippocampal function relates to its role in memory (Amaral 1987, Squire 1992, McDonald and White 1993, Squire et al. 1993, Zola-Morgan and Squire 1993).

The entorhinal cortex receives numerous afferent connections from the allocortex as well as from isocortical association areas (Krettek and Price 1977, Berger et al. 1981, Wyss 1981, Hyman et al. 1990, White et al.1990, Caballerobleda and Witter 1993). The basolateral complex of the amygdala, ventral part of the claustrum and some thalamic nuclei also send a strong input to the entorhinal cortex. Thus, the entorhinal cortex receives information from many sources and relays it via the perforant path to the hippocampus. As the perforant path is the main cortical input to the hippocampus, its damage may be a cause of memory and learning impairment observed in the Alzheimer's disease (Hyman et al. 1984, 1986, 1990).

The main aim of this study was to evaluate pathological changes of neurones of the second layer of the entorhinal cortex as well as those in the terminal zone of the perforant path in "physiological" ageing in comparison with Alzheimer's disease.

METHODS

Five brains of individuals aged from 29 to 52 years (38.6±3.4, mean±SEM), six brains of elderly individuals aged from 61 to 89 years (76±3.9) both groups without signs of dementia, and 11 brains of Alzheimer's patients (AD) aged from 65 to 91 years (81.1±2.1) were studied. Within a few months before death the AD patients were rated using the Global Deterioration Scale (GDS; (Reisberg et al. 1982, Reisberg, 1983). All patients from the latter group were rated as severely demented. For the control patients, the clinical records written during hospitalization by neurologists, psychiatrists or specialists in internal medicine, revealed no indication of cognitive impairments.

Brains were fixed in 10% solution of buffered formalin for three months. None of the brains had macroscopically detectable infarcts or other gross pathological changes. The hippocampal head and adjacent entorhinal cortex were removed and cut into 8-m-thick paraffin sections. Sections were stained either with cresyl violet, Weil method or with immunocytochemical methods for amyloid (4G8) and for neurofibrillary tangles (Tau-1) using the monoclonal antibody (mAb) and standard peroxidase-antiperoxidase method (Kim et al. 1988, Bancher et al. 1989b, Wisniewski et al. 1989a). Clinical diagnosis of AD was confirmed by neuropathological examination, with criteria recommended by the NIH (Khachaturian 1985). The control brains on neuropathological examination did not meet conventional criteria for diagnosis of AD.

In our considerations we used the classification of the neurofibrillary tangles proposed by Bancher et al. (Bancher et al. 1989a, 1989b) and the classification of senile plaques proposed by Wisniewski et al. (Wisniewski et al. 1989a). Morphometric studies were performed in each layer of the entorhinal cortex and hippocampus. The number of neurones, plaques and tangles for all studied cases were taken from each section using an image analysis system consisting of a projection microscope (Pictoval, Zeiss Jena), a digitising tablet and a PC-software system (Sigma-Scan, Jandel Scientific Corp.). An image of the section was projected onto the digitising tablet at final magnification x560 for neurones and tangles, and x165 for plaques. The outline of each neurone, tangle and plaque in the defined test area was traced using a cursor and the resulting x, y co-ordinates converted into the number of objects per square millimetre by a computer. The statistic analysis was performed by means of Mann-Whitney U-test.

RESULTS

Neuronal loss

In the brains of subjects without dementia we did not notice any striking differences between younger and older individuals in the cytoarchitecture of the entorhinal cortex. However, the morphometric analysis showed in layer II a non-significant decrease

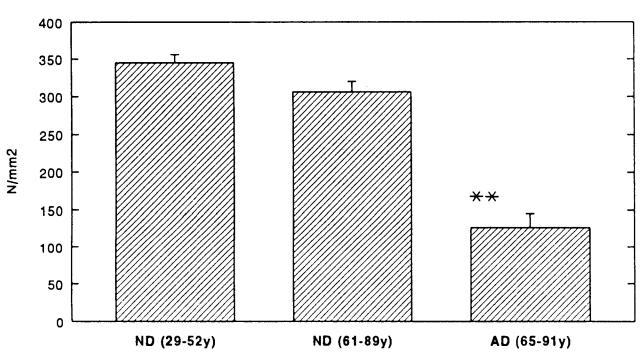


Fig. 1. The number of neurones per mm^2 (mean±SEM) in layer II of the entorhinal cortex. Marked neuronal loss in AD patients. ND, non-demented subjects. **P<0.001.

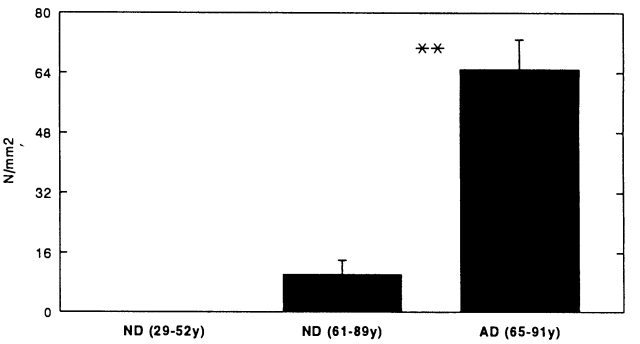
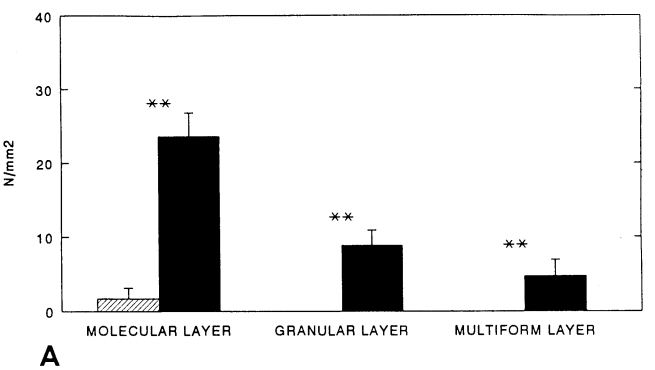


Fig. 2. The number of neurofibrillary tangles per mm^2 (mean±SEM) in layer II of the entorhinal cortex. Large number of neurofibrillary tangles were found in AD. ND, non-demented subjects. **P<0.001.



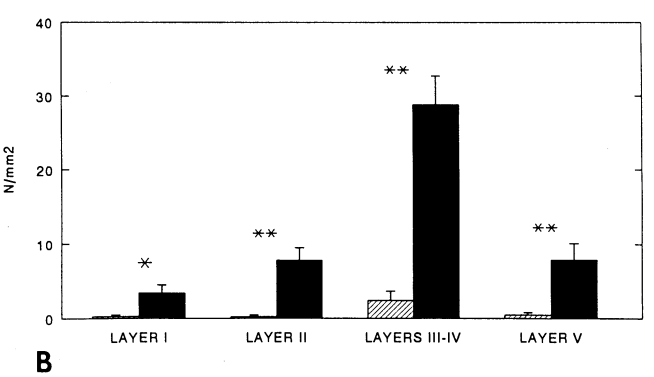


Fig. 3. Distribution of senile plaques in various layers of the dentate gyrus (A) and entorhinal cortex (B) in non-demented older individuals (dashed bar) and in Alzheimer's patients (solid bar). Significant number of senile plaques in AD was noticed in the molecular layer of the dentate gyrus (terminal zone of the perforant path) and in layers III-IV of the entorhinal cortex. Values given as mean±SEM. *P<0.05; **P<0.001.

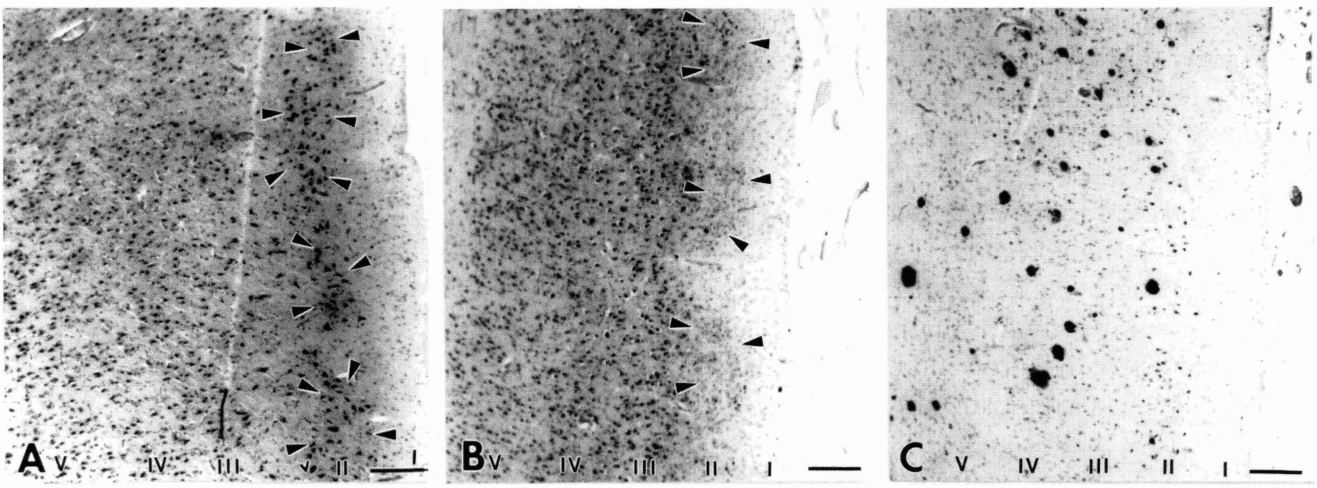


Fig. 4. Pathological changes in the entorhinal cortex in AD affected brains. A, neuronal islands (arrowheads) of layer II of the entorhinal cortex in control brains (A). Severe loss of neurones in this layer in AD (B). Arrowheads, almost neurone free islands. Cresyl violet; C, numerous senile plaques in layers III-IV. Immunostaining for β-amyloid (4G8). Scale bar, 200 μm.

in neuronal density from 344.6±11.2 to 306.3± 13.9 neurones per mm^2 (11.25%). In brains of patients with Alzheimer's disease in comparison to age-matched control marked alterations in layer II of the entorhinal cortex were observed. There was a significant decrease in neuronal density from 306.3±13.9 to 126.3±18.7 neurones per mm^2 (58.7%; Z=-3.32; *P*=0.001). The characteristic neuronal islands were severely reduced in size and contained numerous glial cells and only sporadic neurones (Fig. 4A,B). The loss of neurones in other layers was much less evident. Layers III and IV showed it statistically non significant either (about 11.5%). The lamina dissecans was more prominent. Layer V did not present alterations in cytoarchitectonic.

Neurofibrillary pathology

We did not observe neurofibrillary tangles in younger individuals without dementia, whereas in elder ones 3.5±1.3% of neurones (10.1±3.9 tangles per mm^2) in layer II revealed neurofibrillary tangles (Fig. 2); they were mostly in stage 0 or 1 of maturation. In AD brains in layer II 62.7±9.1% (65±7.95 tangles per mm^2) of neurones revealed neurofibrillary pathology. Most of tangles presented stage 2 or 1 of maturation; tangles in stage 0 and ghost tangles (stage 3) were seen occasionally only.

Neurofibrillary threads, which express the presence of paired helical filaments (PHF) in dendrites and axons of the pathologically changed neurones, were numerous. They were especially abundant in the molecular layer of the dentate gyrus (Fig. 5C), CA1 sectors and layers I-IV of the entorhinal cortex .

Senile plaques

In younger individuals we did not observe amyloid deposits in the hippocampal formation; in the group of elder non-demented subjects single senile plaques were noticed in various areas, especially in the molecular layer of the dentate gyrus (1.7±1.4 plaques per mm^2) and in layers III and IV of the entorhinal cortex (2.4±1.3 plaques per mm^2). In all brains of patients with Alzheimer's disease a large number of senile plaques were found. Most of them were classified as primitive plaques, which were characterised by tufts of amyloid "threads" and ranged between 10 to 30 μm in diameter. Both classical plaques (with typical "halos" which express a neuritic response) and diffuse amyloid deposits were noticed occasionally. The highest concentration of senile plaques was observed in layers III and IV of the entorhinal cortex (28.8±3.9/mm2; Figs. 3 and 4C) and in the molecu-

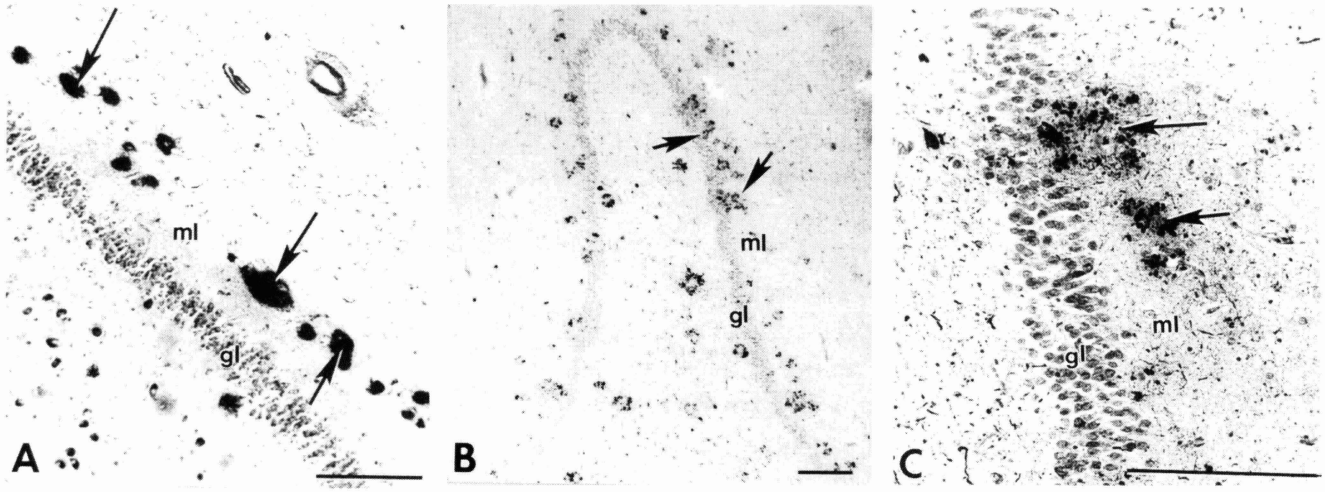


Fig. 5. Pathological changes in the dentate gyrus in AD affected brains. Numerous senile plaques (arrows) in the molecular layer (ml). gl, granular layer; Immunostaining for (A) β-amyloid (4G8) and for (B, C) neurofibrillary pathology (Tau-1). Scale bar, 210 μm.

lar layer of the dentate gyrus (23.6±3.2/mm^2; Figs. 3 and 5A,B). In the particular sectors of the cornu Ammonis and in the subiculum the number of plaques ranged from 2.8±0.9/mm^2 in CA2 to 9.8±2.1/mm^2 in CA1.

Myelin

In the preparations stained by Weil method we did not notice any marked difference between younger and older individuals without dementia; the size of myelin fibre bundles of the parahippocampal gyrus and perforant path was similar in both groups. In the brains of patients with Alzheimer's disease a marked thinning of the white matter of the parahippocampal gyrus along the course of the perforant path was found. We observed a severe loss of myelin sheaths, axons and oligodendroglial cells, especially in the molecular layer of the entorhinal cortex, subicular complex and dentate gyrus.

DISCUSSION

Severe pathological changes in the entorhinal cortex have been described by various authors (Hyman et al. 1984, 1986, 1988, Van Hoesen et al. 1986, Hyman and Van Hoesen 1989, Van Hoesen and Hyman 1990, Van Hoesen et al. 1991, Cabalka et al. 1992). According to them these changes cause a decrease in glutamate level (neurotransmitter of the perforant path) by over 80% (Hyman et al. 1988) and a marked synaptic loss (Hamos et al. 1989) in the main components of this path.

In our observation the most severe neuronal loss and the presence of neurofibrillary tangles concerned neurones of the second layer of the entorhinal cortex. In these brains the number of neurones in the second layer was decreased by 59%, whereas neurofibrillary tangles were found in 63% of remaining neurones. The white matter was severely reduced in the parahippocampal gyrus in the areas containing the perforant path. The molecular layer of the dentate gyrus - the main termination zone of the perforant path - was strongly affected and revealed a very high concentration of senile plaques. Most of them contained degenerating neuronal processes (neurofibrillary threads) visible in Tau-1 immunostaining. These changes may isolate the hippocampus from cortical input and lead to a memory dysfunction.

The single plaques and neurofibrillary tangles in the hippocampal formations in "physiological" ageing have been observed by various authors (Dayan 1970, Ball 1976, Ulrich 1982, 1985, Miller et al. 1984, Mann et al. 1990, Arnold et al. 1991, Fewster et al. 1991, Arriagada et al. 1992, Trillo and Gonzalo 1992, Roberts et al. 1993). Ulrich (1982, 1985) and Miller et al. (1984) found that more than

12% of cases above 55 years of age had a various number of tangles and plaques. According to some authors (Dayan 1970, Morimatsu et al. 1975, Ball 1976) these changes are positively correlated with age.

In "physiological" ageing we did not notice either a severe neuronal loss or a high concentration of neurofibrillary tangles in the entorhinal cortex. The intensity of pathological alteration, including the neuronal loss, was much less expressed than in the AD patients. However, single senile plaques were found in various areas, especially in layer III and IV of the entorhinal cortex and in the molecular layer of the dentate gyrus. Their localisation correspond with the course of the perforant path. Taking into account that most of plaques in deeper layer of the entorhinal cortex contained the neuritic component, we suppose that in these cases as in AD brains, the amyloid deposits disrupt some fibres of the perforant path, which may be a cause of a slight impairment of memory and learning often observed in elderly individuals without dementia.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Wojciech Łojek M.Sc., for copy-editing the manuscript and Mrs. I. Krupska and G. Świątek for skilful technical assistance. This research was supported by funds from Committee of Scientific Research, Grant No. A-1.

REFERENCES

Amaral D.G. (1987) Memory: anatomical organization of candidate brain regions. In: The nervous system. Vol. V. Higher functions of the brain, Part I. (Eds. V.B. Mountcastle, F. Plum and S.R. Geiger). Am. Physiol. Soc. Bethesda, Maryland, p. 211-294.

Amaral D.G., Insausti R., Cowan W.M. (1987) The entorhinal cortex of the monkey: I. Cytoarchitectonic organization. J. Comp. Neurol. 264: 326-355.

Amaral D.G., Insausti R. (1990) Hippocampal formation. In: The human nervous system (Ed. G. Paxinos). Academic Press, Inc., San Diego, p. 711-755.

Amaral D.G., Witter M.P. (1989) The threedimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31: 571-591.

Arnold S.E., Hyman B.T., Flory J., Damasio A.R., Van Hoesen G.W. (1991) The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients in Alzheimer's disease. Cerebral Cortex 1: 103-116.

Arriagada P.V., Marzloff K., Hyman B.T. (1992) Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 42: 1681-1688.

Ball M.J. (1976) Neurofibrillary tangles and the pathogenesis of dementia: a quantitative study. Neuropathol. Appl. Neurobiol. 2: 395-410.

Bancher C., Brunner C., Lassmann H., Budka H., Jellinger K., Seitelberger F., GrundkeIqbal I., Iqbal K., Wisniewski H.M. (1989a) Tau and ubiquitin immunoreactivity at different stages of formation of Alzheimer neurofibrillary tangles. In: Alzheimer's disease and related Disorders. Alan R. Liss, Inc.,New York, p. 837-848.

Bancher C., Brunner C., Lassmann H., Budka H., Jellinger K., Wiche G., Seitelberger F., GrundkeIqbal I., Iqbal K., Wisniewski H.M. (1989b) Accumulation of abnormally phosphorylated τ precedes the formation of neurofibrillary tangles in Alzheimer's disease. Brain Res. 477:90-99.

Berger T.W., Bassett J.L., SempleRowland S.L. (1981) Neocortical input to entorhinal and cingulate cortices is relayed by the claustrum. Soc. Neurosci.7: 886.

Braak H. (1972) Zur Pigmentarchitektonik der Großhirnrinde des Menschen. I. Regio entorhinalis. Z. Zellforsch. 127: 407-438.

Braak H. (1980) Architectonics of the human telencephalic cortex. In: Studies of brain function (Ed. V. Braitenberg). Springer-Verlag, Berlin, p. 37-39.

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

Cabalka L.M., Hyman B.T., Goodlett C.R., Ritchie T.C., Van-Hoesen G.W. (1992) Alteration in the pattern of nerve terminal protein immunoreactivity in the perforant path in Alzheimer's disease and in rats after entorhinal lesions. Neurobiol. Aging 13: 283-291.

Caballerobleda M., Witter M.P. (1993) Regional and laminar organization of projections from the presubiculum and parasubiculum to the entorhinal cortex An anterograde tracing study in the rat. J. Comp. Neurol. 328: 115-129.

Dayan A.D. (1970) Quantitative histological studies on the aged human brain. Acta Neuropathol. 16: 85-94.

Fewster P.H., Griffin-Brooks S., McGregor J., Ojalvo-Rose E., Ball M.J. (1991) A topographical path by which histopathological lesions disseminate through the brain of patients with Alzheimer's disease. Dementia 2: 121-132.

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

Hoesen G.W., Hyman B.T. (1990) Hippocampal formation: anatomy and the patterns of pathology in Alzheimer's disease. Prog. Brain Res. 83: 445-457.

Hyman B.T., Kromer L.J., Van Hoesen G.W. (1988) A direct demonstration of the perforant path terminal zone in Alz-

heimer's disease using the monoclonal antibody Alz50. Brain Res. 450: 392-397.

Hyman B.T., Van Hoesen G.W. (1989) Hippocampal and entorhinal cortex cellular pathology in Alzheimer's disease. In: The hippocampus. New vistas. (Eds. V. ChanPalay and C. Khler). Alan R. Liss, Inc., New York, p. 499-512.

Hyman B.T., Van Hoesen G.W., Damasio A.R. (1990) Memory-related neural systems in Alzheimer's disease: an anatomic study. Neurology 40: 1721-1730.

Hyman B.T., Van Hoesen G.W., Damasio A.R., Barnes C.L. (1984) Alzheimer's disease: cellspecific pathology isolates the hippocampal formation. Science 225: 1168-1170.

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

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

Kim K.S., Miller D.L., Sapienza V.J., Cheng C.-M.J., Bai C., Grundke-Iqbal I., Currie J.R., Wisniewski H.M. (1988) Production and characterization of monoclonal antibodies reactive to synthetic cerebrovascular amyloid peptide. Neurosci. Res. Commun. 2: 121-130.

Krettek J.E., Price J.L. (1977) Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. J. Comp. Neurol. 172: 723-752.

Mann D.M.A., Brown A.M.T., Pringa D., Jones D., Davies C.A. (1990) A morphological analysis of senile plaques in the brains of nondemented persons of different ages using silver, immunocytochemical, and lectin histochemical staining techniques. Neuropathol. Appl. Neurobiol. 16: 17-25.

Marimatsu M., Hirai S., Muramatsu A., Yoshikawa M. (1975) Senile degenerative brain lesions and dementia. J. Am. Geriatr. Soc. 23: 390-406.

McDonald R.J., White N.M. (1993) A triple dissociation of memory systems - hippocampus, amygdala, and dorsal striatum. Behav. Neurosci 107: 3-22.

Miller F.D., Hicks S.P., D'Amato C.J., Landis J.R. (1984) A descriptive study of neuritic plaques and neurofibrillary tangles sin an autopsy population. Am. J. Epidemiol. 120: 331-341.

Reisberg B. (1983) Clinical presentation, diagnosis, and symptomatology of ageassociated cognitive decline and Alzheimer's disease. In: Alzheimer's disease (Ed. B. Reisberg). The Free Press, New York, p. 173-187.

Reisberg B., Ferris S.H., de Leon M.J., Crook T. (1982) The Global Deterioration Scale (GDS): an instrument for the assessment of primary degenerative dementia (PDD). Am. J. Psychiatry 139: 1136-1139.

Roberts G.W., Nash M., Ince P.G., Royston M.C., Gentleman S.M. (1993) On the origin of Alzheimer's disease: a hypothesis. Neuroreport 4: 7-9.

Squire L.R. (1992) Memory and the hippocampus - A synthesis from findings with rats, monkeys, and humans. Psychol. Rev. 99: 195-231.

Squire L.R., Knowlton B., Musen G. (1993) The structure and organization of memory. Ann. Rev. Psychol. 44: 453-495.

Trillo L., Gonzalo L.M. (1992) Ageing of the human entorhinal cortex and subicular complex. Histol. Histopathol. 7: 17-22.

Ulrich J. (1982) Senile plaques and neurofibrillary tangles of the Alzheimer type in nondemented individuals at presenile age. Gerontology 28: 86-90.

Ulrich J. (1985) Alzheimer changes in nondemented patients younger than sixty five: a possible early stage of Alzheimer's disease and senile dementia of Alzheimer's type. Ann. Neurol. 17: 273-277.

Van Hoesen G.W., Hyman B.T., Damasio A.R. (1986) Cellspecific pathology in neural systems of the temporal lobe in Alzheimer's disease. In: Aging of the brain and Alzheimer's disease. (Eds. D.F. Swaab, E. Fliers, M. Mirmiran, W.A. Van Gool and F. Van Haaren). Elsevier. Amsterdam, p. 321-335.

Van Hoesen G.W., Hyman B.T., Damasio A.R. (1991) Entorhinal cortex pathology in Alzheimer's disease. Hippocampus 1: 1-8.

Van Hoesen G.W., Pandya D.N. (1975) Some connections of the entorhinal (area 28) and perirhinal (area 38) cortices of the rhesus monkey. I. temporal lobe afferents. Brain Res. 95: 1-24.

White T.D., Tan A.M., Finch D.M. (1990) Functional reciprocal connections of the rat entorhinal cortex and subicular complex with the medial frontal cortex: an in vivo intracellular study. Brain Res. 533: 95-106.

Wisniewski H.M., Bancher C., Barcikowska M., Wen G.Y., Currie J. (1989a) Spectrum of morphological appearance of amyloid deposits in Alzheimer disease. Acta Neuropathol. 78: 337-347.

Wisniewski H.M., Wen G.Y., Kim K.S. (1989b) Comparison of four staining methods on the detection of neuritic plaques. Acta Neuropathol. 78: 22-27.

Witter M.P., Amaral D.G. (1991) Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. J. Comp. Neurol. 307: 437-459.

Witter M.P., Hoesen G.W., Amaral D.G. (1989) Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. J. Neurosci. 9: 216-228.

Wyss J.M. (1981) An autoradiographic study of the efferent connections of the entorhinal cortex in the rat. J. Comp. Neurol. 199: 495-512.

Zola-Morgan S., Squire L.R. (1993) Neuroanatomy of memory. Annu. Rev. Neurosci. 16: 547-563.

Received 22 July 1993, accepted 2 December 1993