Invited Review

The neuropathological changes associated with normal brain aging

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Summary. Neurofibrillary tangles and senile plaques are common neuropathological features in both normal brain aging and Alzheimer's disease. In order to examine the patterns of lesion distribution in cerebral aging, we review the clinicopathological analysis of 1144 non-demented cases comparing their neuropathologic features to that reported in cases with mild cognitive impairment and cases with Alzheimer's disease. Regardless of cognitive status, layer II of the entorhinal cortex is involved with neurofibrillary tangle formation in all of the cases, while the CA1 field of the hippocampus and the subiculum are less consistently affected. Neocortical area 20 is particularly prone to develop neurofibrillary tangles in intellectually preserved elders, whereas other neocortical areas are relatively spared. Substantial senile plaque formation is seen in the neocortex of non-demented cases. Quantitatively, mild cognitive impairment is correlated with neurofibrillary tangle densities in layer II of the entorhinal cortex, and clinically overt Alzheimer's disease with neurofibrillary tangle densities in area 20. In non-demented centenarians, there is an early development of neurofibrillary tangles in areas usually spared in the course of the degenerative process in younger individuals. These observations demonstrate that mesial and inferior temporal lobe structures are affected more frequently than originally thought in normal brain aging. In this respect, neurofibrillary tangle formation in area 20 may represent a crucial step of the degenerative process, because it may precede the emergence of the neuropsychological deficits characteristic of Alzheimer's disease. In addition, this study reveals age-related heterogeneity in the regional vulnerability of the cerebral cortex during normal brain aging.

Key words: Aging, Cerebral cortex, Dementia, Neurofibrillary tangle, Senile plaque

Introduction

Cerebral aging or normal brain aging is characterized by the presence of neuropathological lesions that are similar to those observed in Alzheimer's disease (AD). However, these changes are generally much fewer than in AD, and occur in restricted regions of the cerebral cortex, in the absence of significant cognitive decline. It is now well established that neurofibrillary tangles (NFT), senile plaques (SP) and synaptic loss, the main pathological hallmarks of AD, can often be found in the brains of non-demented elderly individuals (Tomlinson, 1972; Ball, 1977; Mountjoy et al., 1983; Ulrich, 1985; Terry et al., 1991). Neurofibrillary tangles represent the accumulation and abnormal biochemical modification of components of the neuronal cytoskeleton that form paired helical filaments. A great variety of cytoskeletal proteins such as the microtubule-associated protein tau, ubiquitin and neurofilament triplet protein are involved in paired helical filaments formation (Brion, 1990; Vickers et al., 1992; Trojanowski et al., 1993). The microtubule-associated tau protein are the main component of paired helical filaments. The cloning and sequencing of the tau gene demonstrates that a total of six different tau isoforms can be generated from a single tau gene by alternative splicing. The expression of tau is developmentally regulated, since all six tau isoforms are found in the adult human brain, and only the smallest isoform, known as fetal tau, is present in the fetal human brain (Bramblett et al., 1929; Matsuo et al., 1994). In AD, abnormally phosphorylated tau proteins are observed in the somatodendritic domain of the degenerating cortical neurons. In fact, immunoblot analysis in homogenates of post-mortem AD brain show that absorbed anti-paired helical filaments recognizes an abnormally phosphorylated tau protein triplet, known as the tau 55, 64, 69 triplet (Delacourte et al., 1990; Buée-Scherrer, 1996). Although it has been suggested that paired helical filaments-tau are hyperphosphorylated in comparison to normal adult tau (Bramblett et al., 1992),

recent studies indicate that normal adult human tau obtained from biopsy specimens is phosphorylated *in vivo* on sites that are similar to those found in paired helical filaments-tau (Matsuo et al., 1994). It is thus possible that the down-regulation of phosphatases in the AD brain could induce the generation of maximally phosphorylated paired helical filaments-tau that does not bind microtubules and aggregates as paired helical filaments in NFT.

Senile plaques are composed of dystrophic neurites and glial elements with or without a central amyloid core (Terry and Wisniewski, 1970; Brion, 1990). There are several types of SP. The classic SP consist of large amyloid deposits intensely labeled by the histochemical stain thioflavine S, surrounded by dystrophic neurites representing degenerating axon terminals and dendritic arborization. In some SP, there is only a core amyloid without dystrophic neurites, whereas in others the whole SP is formed by dystrophic neurites and glial elements. Finally, diffuse amyloid deposits are often observed, especially in the oldest-old AD patients (Hauw et al., 1986). A prominent component of amyloid deposits is a polypeptide (Aß) of 40-42 amino acids derived through proteolytic cleavage from a set of larger protein isoforms collectively referred to as the amyloid precursor protein (APP), which encoded by a single gene on chromosome 21 (Brion, 1990). Missense mutations on the APP gene have been demonstrated in several cases of familial Alzheimer's disease. Three major isoforms of APP are generated from this gene by alternative splicing (APP 770, 751, 695). All of these isoforms are found in the human brain (Iversen et al., 1995). Structurally, the APP is a protein of 110-135 kDa with a large extracellular N-terminal domain, a cell-surface domain and a small intracellular C-terminal domain. The proteolytic cleavage of this molecule is the Aß domain is catalyzed by a group of enzymes, known as α, ß and γ secretases (Checler, 1995). It is thought that the cleavage of the APP out of the Aß domain could generate large C-terminal fragments containing the Aß sequence which are possibly amyloidogenic.

Recent studies have shown that the majority of elderly people displayed NFT formation in the hippocampal formation even in absence of cognitive impairment or with very mild memory impairment (Price et al., 1991; Arriagada et al., 1992a,b; Hof et al., 1992; West et al., 1994; Bierer et al., 1995). Moreover, SP may appear early in the neocortex of intellectually preserved individuals, whereas the hippocampus is relatively spared by SP formation and the onset of the degenerative process (Pearson et al., 1985; Lewis et al., 1987; Hof and Morrison, 1990; Hof et al., 1990, 1992). Synaptic alterations and neuronal loss have been found in the neocortex of elderly non-demented individuals, suggesting an age-dependent mechanism for the loss of synapses in the neocortex (Terry et al., 1991; Masliah et al., 1993; Masliah, 1995). In addition, several quantitative reports have indicated that NFT and SP formation are not only common features of brain aging,

but are also found in certain regions of the cerebral cortex in densities that would qualify for a neuropathological diagnosis of AD (Crystal et al., 1988; Katzman et al., 1988; Hubbard et al., 1990; Morris et al., 1991; Price et al., 1991; Arriagada et al., 1992a,b; Hof et al., 1992; Berg et al., 1993; West et al., 1994; Bierer et al., 1995). For instance, the hippocampal formation consistently displayed a moderate to severe involvement by NFT in both demented and non-demented cases, and abundant SP were seen in several neocortical areas of non-demented people (Price et al., 1991; Arriagada et al., 1992a; Hof et al., 1992; Berg et al., 1993). The spreading of NFT from the hippocampal structures to the neocortex is considered to be a crucial step for the generalization of the dementing process (Price et al., 1991; Hof et al., 1992; Berg et al., 1993; Bouras et al., 1993, 1994; Bierer et al., 1995). However, numerous neuropathological analyses of non-demented people have demonstrated the presence of NFT confined to the temporal neocortex, implying that the progression in NFT density within adjacent components of the medial and inferior aspects of the temporal cortex may take place in cognitively intact individuals (Hubbard et al., 1990; Arriagada et al., 1992b; Hof et al., 1992; Bouras et al., 1993, 1994; Bierer et al., 1995). Most of the clinico-pathologic studies of brain aging have been based on relative small patient samples consisting of selected cases or small series of non-selected cases (Crystal et al., 1988; Katzman et al., 1988; Hubbard et al., 1990; Morris et al., 1991; Price et al., 1991; Arriagada et al., 1992a,b; Hof et al., 1992; Berg et al., 1993; Bierer et al., 1995). In order to evaluate the age-related factors leading to NFT and SP formation in a non-demented population and to define the differences in hierarchical patterns of lesion distribution between elderly subjects without cognitive deficits and patients with AD, we performed in recent years several comparative analyses of over 1300 unselected cases from a non-psychiatric hospital. In this review we provide an overview of these studies of the neuropathology of cerebral aging.

Overview of the population sample

We surveyed the entire autopsy population of the decade 1982-1992, form the Geriatrics Hospital of the University of Geneva School of Medicine, Switzerland. This autopsy series includes 1144 non-demented cases (484 men, 73.7±11.2 years old; 647 women, 76.5±12.3 years old). All of the patients were referred to the Geriatrics Hospital from the area of the City of Geneva (about 400,000 inhabitants). These patients had no or only very mild signs of cognitive deterioration, and showed good ability in dealing with daily activities before they were admitted to the hospital for their terminal illness. Observations in the hospital confirmed the global preservation of cognitive functions. In addition, they had been neuropsychologically evaluated using the Mini-Mental State Examination (MMSE; Folstein et al., 1975) performed at least once during the

last 3 months prior to death. The mean MMSE score for this population was 28.5±1.5, well within the normal range. Cases were also retrospectively evaluated using Clinical Dementia Rating (CDR; Hughes et al., 1982; Heyman et al., 1987) scores and neuropathologically assessed using the consortium to Establish a Registry of Alzheimer's Disease ratings (CERAD; Mirra et al., 1991). No cases were rated CDR scores higher than 1.5, demonstrating the absence of over dementia in this sample.

Additionally, three specific subgroups of the entire autopsy population were extensively analyzed. First, a quantitative evaluation of sixty-one randomly selected patients (age range: 49-101; mean age: 75.3±11.8 years) was performed to evaluate the degree to which areas of the cerebral cortex that are consisted to be the primary site of degeneration in AD are affected during aging (Bouras et al., 1993). Second, a detailed immunohisto-chemical analysis of the entire autopsy population of non-demented cases for the year 1989 (43 men, 79.6±8.2 years old; 59 women, 83.3±7.5 years old) was performed to investigate further the quantitative distribution of lesions associated with aging. This subgroup was compared to 33 patients with dysmnesia and temporo-spatial disorientation and 10 AD cases autopsied in the same hospital for the same year (Bouras et al., 1994). Third, we performed a quantitative immunohisto-chemical analysis of 24 non-demented nonagenarians and centenarians included in the whole series (7 men, 97.3±1.5 years old, 17 women, 97±1.8 years old) to examine the relationship between brain aging and AD in very old people. The distribution of NFT and SP in these cases were compared to that observed in younger non-demented cases and in 19 centenarians with AD (Giannakopoulos et al., 1993, 1994, 1995).

The brains were obtained at autopsy (post-mortem delay: 3-20 hours), fixed in a 10% formalin solution for at least 6 weeks, and cut into 1 cm thick coronal slices. After macroscopic examination, tissue blocks were taken from the mid portion of the hippocampal formation, the inferior temporal (Brodmann's area 20), superior frontal (area 9) and occipital (areas 17/18) cortices of the left hemisphere. In centenarians, additional blocks were taken from the superior temporal (area 22), superior parietal (area 7), and anterior and posterior cingulate (areas 24 and 23) cortices of the left hemisphere. For routine neuropathological evaluation 20 μm thick sections were prepared from the selected areas and were stained with hematoxylin-eosin and cresyl violet. Modified Gallyas and Globus silver impregnation techniques or modified thioflavine S were used for the quantitative assessment of the distribution of NFT and SP (Vallet et al., 1992). For immunohistochemical analyses, additional sections were processed with highly specific and fully characterized antibodies to the microtubule-associated protein tau (Delacourte et al., 1990; Flament et al., 1990; Vermersch et al., 1995; Buée-Scherrer et al., 1996) and to the core-amyloid Aß protein (Kim et al., 1988; Permanne et al., 1995).

Quantitative analysis was performed in the CA1 fields of the hippocampus, subiculum, entorhinal cortex, and in layers II-III and V-VI of areas 9, 17, and 20. In centenarians, quantitative evaluation included also layers II-III and V-VI of areas 7, 18, 22, 24 and 23. The number of NFT and SP was determined in 10 different slides (7 measured cortical fields per slide) for each area, and the mean number per mm^2 was evaluated on a computer-assisted image analysis system consisting of a Zeiss Axioplan microscope, a high sensitivity LH-4036 camera (LHESA Electronic), a COMPAQ Deskpro 386/20 microcomputer and a SAMBA™ 2005 software system developed by TITN Inc. (ALCATEL, Grenoble, France; Vallet et al., 1992). The statistical correlations between the density and distribution of NFT and SP were studied by Spearman rank correlation coefficient (r_s), and the relationship between NFT and SP densities and the clinical diagnosis was assessed by analysis of covariance controlling for age at the time of death (Bierer et al., 1995; Giannakopoulos et al., 1995).

The prevalence and density of NFT and SP increase with age

The survey of the general sample of 1144 non-demented autopsy cases indicates that the density and distribution of NFT and SP with age vary in function to the areas studied (Tables 1, 2). The entorhinal cortex was involved by NFT in all of the cases. In the CA1 field, the number of cases with NFT increased with age (r_s=0.19, p<0.001; Fig. 1). Low NFT densities (2-10 per mm^2) were observed in only 2.2% of the patients younger than 70 years of age, but in 46% of the patients older than 95 years. Area 20 displayed NFT density and distribution correlation with age comparable to that observed in the CA1 fields (r_s=0.27, p<0.001; Fig. 1). However, patients younger than 70 years had low NFT densities in 17.4% of cases in this area. Moderate to high NFT densities were occasionally seen in all age groups in this area. Areas 9 and 17 were rarely involved by NFT. For instance, less than 1% of cases had NFT densities higher than 2 per mm^2 in any of these areas. Moreover, there was no case with moderate to high NFT densities in any age group. In neocortical regions, SP were more frequently seen in these neocortical fields than in the CA1 field of the hippocampus. For instance, 32.8% of cases had SP in the CA1 field, whereas area 20 was involved in 46.3%, area 9 in 45.3% and area 17 in 40.6% of the cases studied. In the CA1 field, the number of cases with SP increased with age (r_s=0.19, p<0.001; Fig. 1). The percent of cases with low SP counts increased below 85 years. In older age groups, the number of patients presenting with moderate to high SP densities augmented, especially in the centenarians group. In areas 20 (r_s=0.23), 9 (r_s=0.23), and 17 (r_s=0.24), there was a positive correlation between age and SP prevalence (p<0.001; Fig. 1). These results demonstrate that both NFT and SP formation are age-related phenomena in non-demented people. The frequent development of

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Table 1. Density and distribution of neurofibrillary tangles in non-demented old people.

AREA	N	AGE							MEAN
		65-69	70-74	75-79	80-84	85-89	90-94	95-105	
CA1									
0	873	97.8	92.0	84.7	76.9	73.6	69.2	54.0	77.2
+	258	2.2	8.0	15.3	23.1	26.4	30.8	46.0	22.8
++	0	0	0	0	0	0	0	0	0
20									
0	793	80.3	80.8	78.7	71.8	71.6	66.0	54.1	70.5
+	332	17.4	18.4	21.2	26.4	28.4	34.0	43.2	29.4
++	6	2.3	0.8	0.1	1.8	0	0	2.7	0.1
9									
0	1126	99.3	98.3	100	100	100	100	100	99.9
+	5	0.7	1.7	0	0	0	0	0	0.1
++	0	0	0	0	0	0	0	0	0
17									
0	1131	100	100	100	100	100	100	100	100
+	0	0	0	0	0	0	0	0	0
++	0	0	0	0	0	0	0	0	0

Results represent the percentage of cases displaying no (0), low (+) or moderate-to-high (++) NFT densities in the different cortical areas. Mean represents the average from the whole population. Cases are separated into 5-years age categories. N: number of cases.

Table 2. Density and distribution of senile plaques in non-demented old people.

AREA	N	AGE							MEAN
		65-69	70-74	75-79	80-84	85-89	90-94	95-105	
CA1									
0	760	93.1	79.0	73.0	64.0	65.5	65.5	54.1	67.2
+	323	6.2	15.8	21.2	31.4	29.5	28.9	27.0	28.6
++	48	0.7	5.2	5.8	4.6	5.0	5.6	18.9	4.2
20									
0	607	80.8	67.6	62.8	52.6	51.8	51.6	37.8	53.7
+	291	13.9	21.1	19.3	28.1	25.3	24.5	27.0	25.7
++	233	5.3	11.3	17.9	21.3	22.9	23.9	35.2	20.6
9									
0	619	80.8	65.9	64.7	55.0	52.9	53.5	45.9	54.7
+	329	13.9	23.7	23.1	27.5	29.5	27.7	24.3	28.9
++	186	5.3	10.4	12.2	17.5	17.6	18.8	29.8	16.4
17									
0	672	85.4	74.6	70.2	57.7	54.0	56.4	48.6	59.4
+	285	7.7	17.5	19.4	25.9	25.0	29.3	29.7	25.2
++	174	6.9	7.9	10.4	16.4	21.0	14.3	21.7	15.4

Results represent the percentage of cases displaying no (0), low (+) or moderate-to-high (++) SP densities in the different cortical areas. Mean represents the average from the whole population. Cases are separated into 5-years age categories. N: number of cases.

NFT in mesial and inferior temporal structures contrast with the sparing of other neocortical areas, suggesting that the damage of these areas is not necessarily associated to cognitive deterioration in the elderly. Moreover, the substantial SP formation in the neocortex of more than 40% of our cases confirms previous observations on the presence of NFT confined to the entorhinal cortex and many SP in the neocortex in intellectually preserved individuals (Hof et al., 1992; Bouras et al., 1994). In the immunohistochemical study of one-year autopsy population, non-demented cases displayed significantly fewer SP than cases with dysmnesia and temporospatial disorientation or AD, but

no difference was observed in SP densities between cases with dysmnesia and temporospatial disorientation, and AD cases (Fig. 2). Also, it should be noted that despite the relationship between SP densities in the neocortex and dementia, some demented cases were devoid of amyloid deposition in the cerebral cortex (Bouras et al., 1994; Giannakopoulos et al., 1994). These findings parallel several earlier reports showing the early and generalized development of SP in the neocortex (Katzman and Terry, 1983; Crystal et al., 1988; Dickson et al., 1991; Morris et al., 1991; Price et al., 1991; Arriagada et al., 1992b; Hof et al., 1992). These studies all demonstrate a lack of correlation between amyloid

deposition and deterioration of cognitive abilities, and suggest that amyloid deposits are an epiphenomenon of aging rather than a specific marker of AD.

The hippocampal formation is a key region in the early stages of brain aging

The quantitative evaluation of 61 randomly selected non-demented patients revealed that NFT are consistently present in layer II of the entorhinal cortex in higher densities than the other regions studied (Bouras et al., 1993). It is also worth noting that in the seven youngest cases of these groups (aged 49 to 59 years), this area was always affected, suggesting that the neuropathological changes characteristic of AD may be present even in young adults (Vickers et al., 1992, 1994). Eight cases (13.1%) were characterized by higher numbers of neurons affected by NFT higher than one standard deviation form the mean of the total population. For instance, as many as 22.6% of the total number of

neurons contained NFT in layer II of the entorhinal cortex, 8% in the CA1 field and 10% in the subiculum. In the remaining 53 cases, these values were 5.1% in the entorhinal cortex, 1% in the CA1 field, and 1.5% in the subiculum. Despite the absence of signs of cognitive impairment and a mean MMSE score comparable to that of the whole group, these patients may represent a group at risk for the development of AD. In this respect, the average percentage of neurons containing NFT in the entorhinal cortex could be used as a pathologic indicator for this group (Bouras et al., 1993). This possibility is supported further by the results of the immunohisto-chemical analysis of one-year autopsy population (Bouras et al., 1994). Besides the consistent presence of NFT in the entorhinal cortex of non-demented individuals, this study revealed a strong correlation between the densities of NFT in this region and the onset of cognitive decline in patients with isolated memory and temporospatial orientation problems (Fig. 3). Furthermore, the CA1 field and subiculum had variable
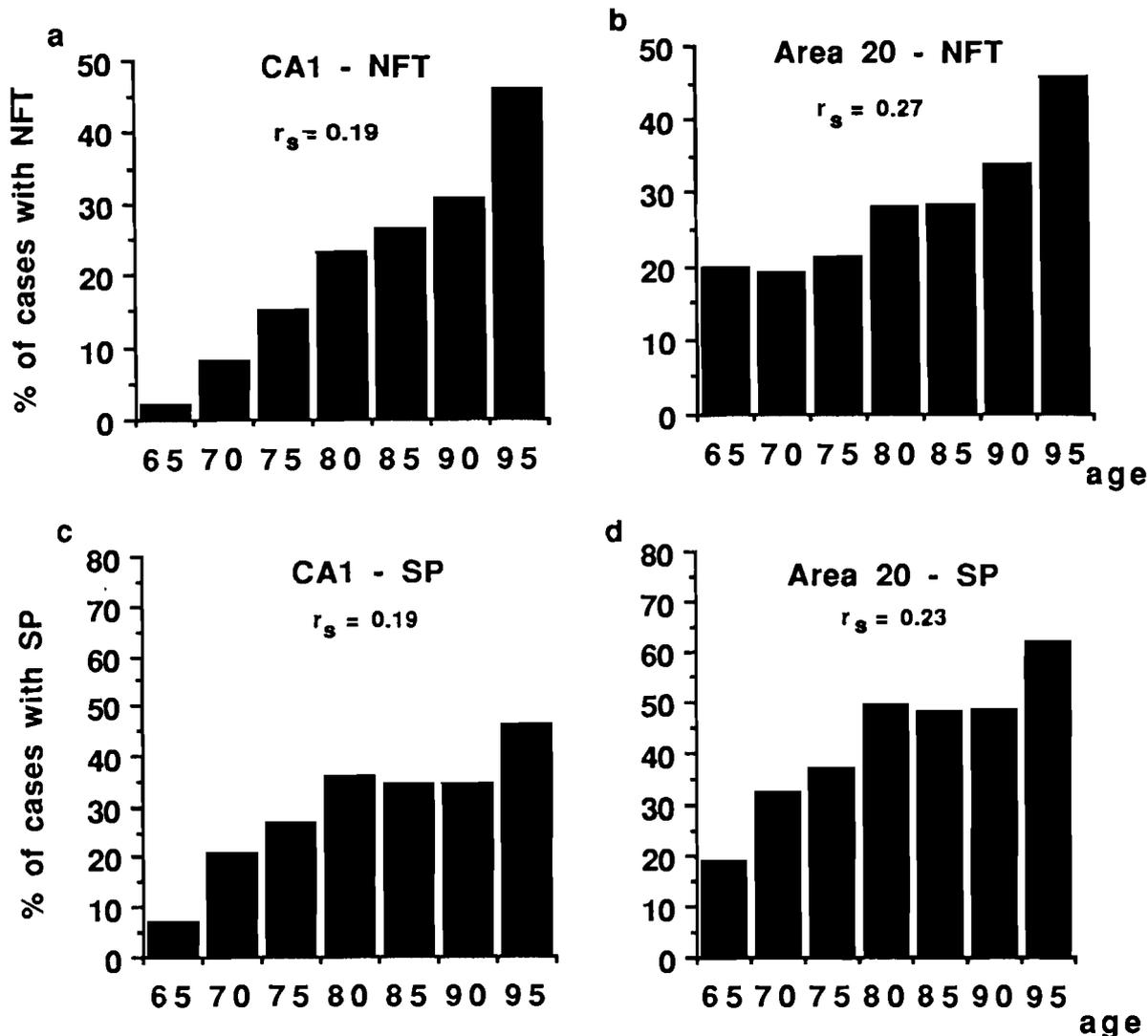
Fig. 1. Cases showing NFT and SP (percent of the total population) as a function of age in the CA1 field of the hippocampus and area 20 of 1144 non-demented cases. Note the positive correlation between NFT and SP prevalence and age in both regions. R_s: Spearman's rank correlation coefficient. See text for details.

NFT counts in most of the cases.

The marked vulnerability of the entorhinal cortex to the degenerative process has been reported by Braak and Braak (1985, 1990, 1991) who have demonstrated that the transition zone between the allo- and neocortex is consistently involved early in the course of brain aging and have proposed a neuropathological staging of dementia based on the progressive alteration of the hippocampal formation. Our observations agree with other reports of AD-related pathologic changes in the brain of patients with no signs of neurologic or psychiatric disorders (Tomlinson et al., 1968; Crystal et al., 1988; Katzman et al., 1988; Braak and Braak, 1990; Hubbard et al., 1990; Morris et al., 1991; Price et al., 1991; Arriagada et al., 1992a,b; Vickers et al., 1992, 1994), and suggest that NFT formation in the hippocampal structures may take place long before the emergence of cognitive deterioration. The presence of high NFT densities in the hippocampal formation is clearly insufficient to cause dementia, but may represent the neuropathological basis for the age-associated memory impairment syndrome (Crook et al., 1986;

Gordon, 1992; Koivisto et al., 1995). These are middle-aged as well as elderly subjects with marked forgetfulness, whereas their daily functioning and judgement capacities are preserved. It has been proposed that a small percentage of these patients can evolve to AD (Koivisto et al., 1995).

The inferior temporal cortex is the most vulnerable neocortical region in brain aging

The neuropathological evaluation of our autopsy population revealed that area 20 is particularly prone to develop NFT in cognitively intact elders (Giannakopoulos et al., 1994). This finding was confirmed further by the quantitative analysis of one-year autopsy population which showed that NFT densities in this area were correlated with the onset of AD (Bouras et al., 1994; Fig. 4). Thus, the massive involvement of the inferior temporal cortex appears to be a necessary condition for the clinical expression of AD. This strengthens the hypothesis that this region may represent an area of transition between the hippocampal
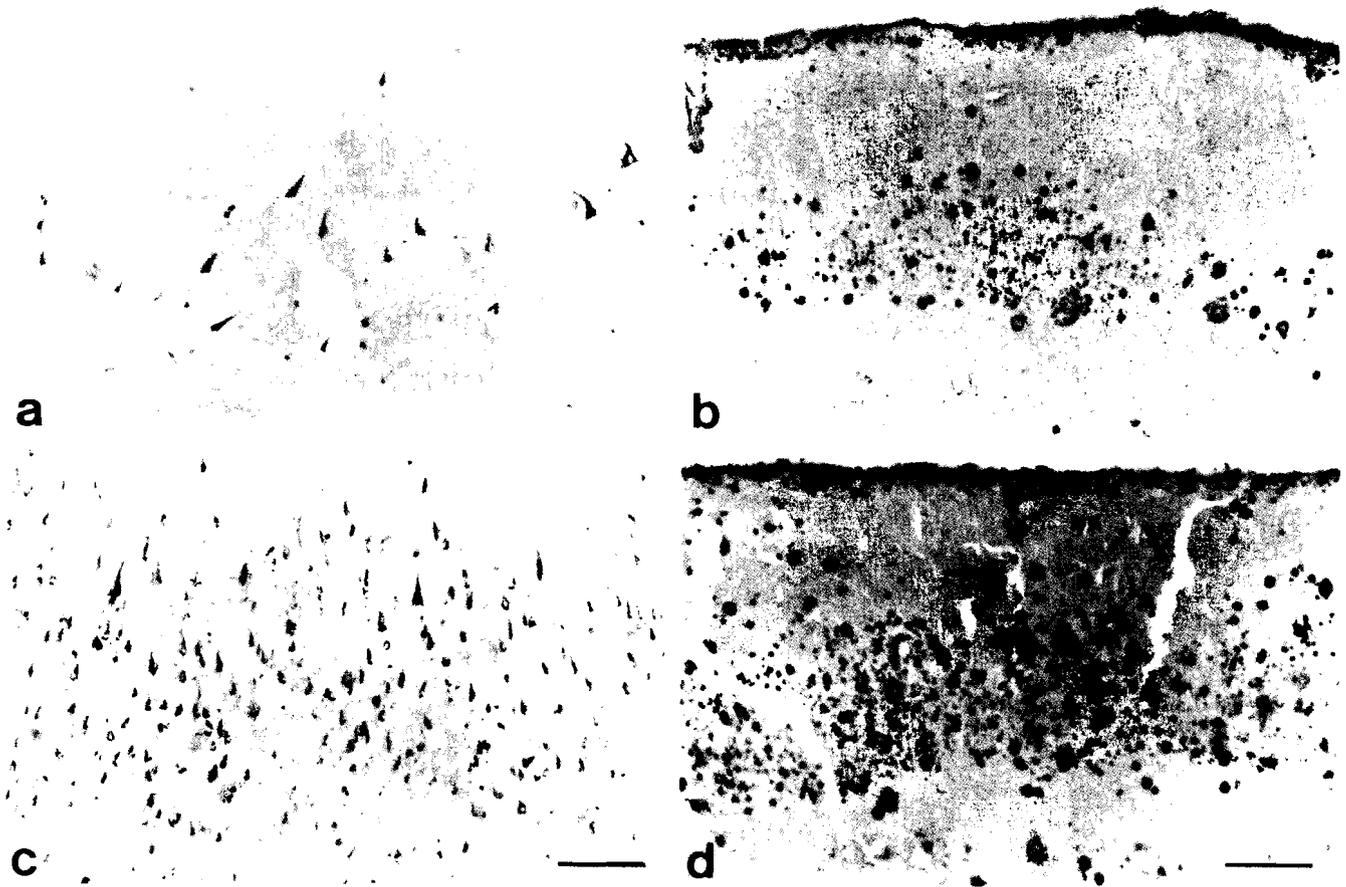
Fig. 2. Neurofibrillary tangles in the CA1 field **(a, c)** and senile plaques in layer II of the entorhinal cortex **(b, d)** in a non-demented case (a, b) compared to an AD case (c, d). Note the very high NFT densities in the CA1 field and the numerous SP in the entorhinal cortex of the AD case. Materials were stained with antibodies to the microtubule-associated protein tau (a, c) or to the amyloid Aβ protein (b, d). Scale bar: 250 μm (a, c), 500 μm (b, d).

structure, severely affected by NFT yet with only mild repercussions on the daily functioning of the individual and the other neocortical areas less involved in brain aging (Hof et al., 1992; Bouras et al., 1993, 1994; Bierer et al., 1995). In this context, some of the non-demented cases with relatively high NFT densities in the inferior temporal cortex could represent a preclinical stage of the AD, as Hubbard and colleagues (1990) and Hof, Bouras and collaborators (1992, 1993, 1994; Giannakopoulos et al., 1994) suggested. This is also supported by a recent biochemical analysis of brains of non-demented elderly subjects (Vermersch et al., 1995), that demonstrated using Western blot analysis that an abnormal hyperphosphorylated tau protein triplet (tau 55, 64, 69) a biochemical marker of neurofibrillary degeneration typically observed in AD cases, was detected in the hippocampal formation of most of the cases, whereas it was present only occasionally in the inferior temporal cortex along with numerous SP. These authors suggested that non-demented cases with tau protein alterations in the temporal lobe and numerous SP in the neocortex

were likely to represent subclinical stages of AD. However, the high percentage of non-demented cases with mild NFT formation in this area imply that the involvement of the inferior temporal cortex is not necessarily accompanied by overt clinical signs of AD, including severe memory impairment, aphasia, apraxia and agnosia, and that the widespread damage of neocortical areas is critical to develop neuropsychological deficits incompatible with independent daily living. It should be kept in mind that, in absence of an extensive investigation of the cerebral cortex in non-demented people, it is still possible that other cortical areas may contain significant numbers of NFT early in the course of the degenerative process. In this respect, the distribution of lesions in cortical regions related to the limbic system such as the orbitofrontal and cingulate cortex is of particular interest. Although our comprehensive quantitative study of a non-demented individual with minimal signs of cognitive impairment does not support this notion (Hof et al., 1992), detailed, prospective studies on large series of cognitively
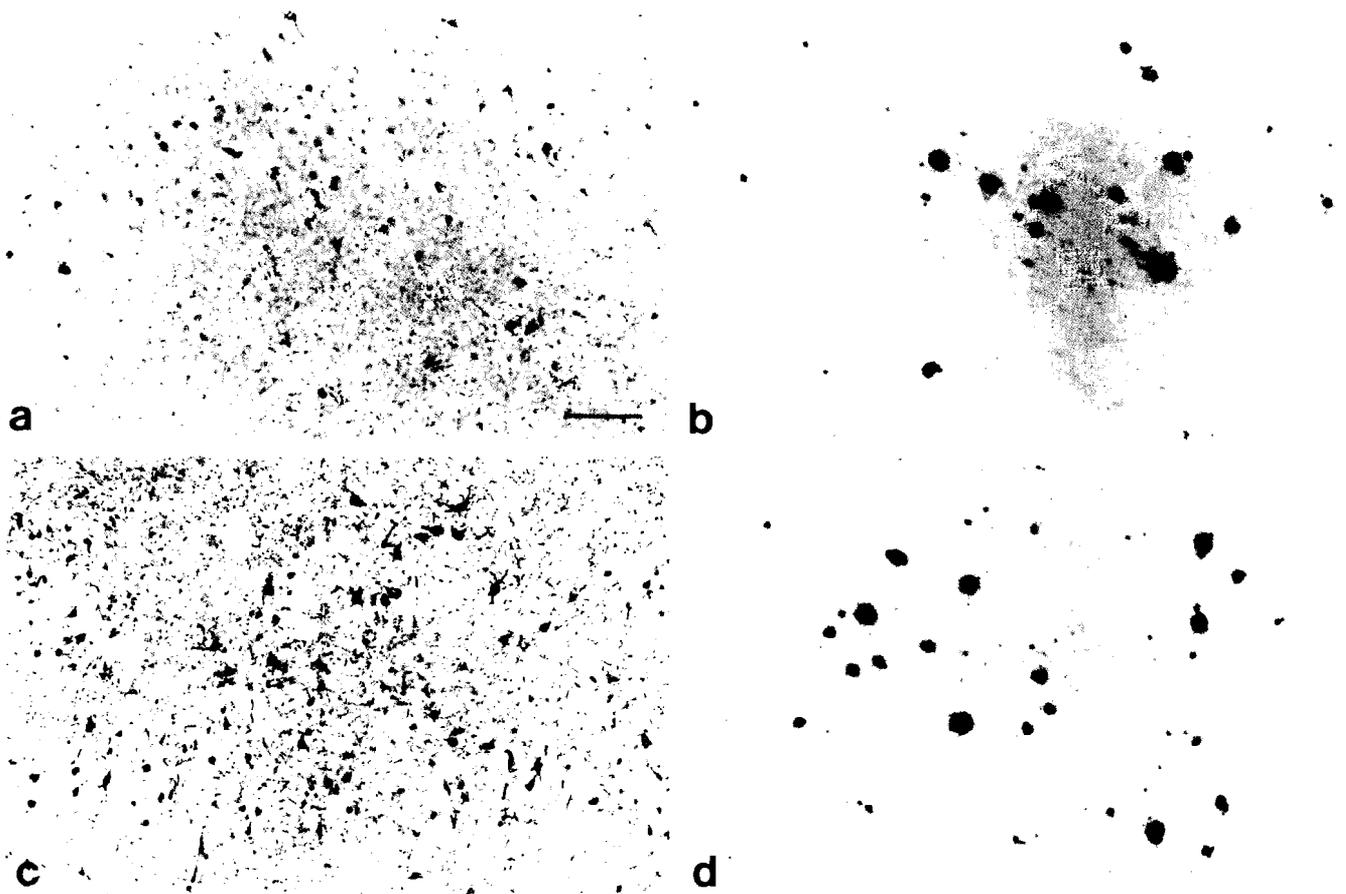
Fig. 3. Neurofibrillary tangles in layer II of the entorhinal cortex **(a, c)** and senile plaques in area 9 **(b, d)** in a non-demented cases (a, b) compared to a case with mild cognitive impairment (c, d). Higher NFT and SP densities were observed in he case with mild cognitive impairment. Materials were stained with antibodies to the microtubule-associate protein tau (a, c) or to the amyloid Aβ protein (b, d). scale bar: 250 μm.

preserved individuals are needed to resolve this issue.

Lesion distribution in very old people: neuropathologic findings in centenarians

An additional problem in the filed of cerebral aging is that correlations between the topography of senile lesions and cognitive decline change in the «oldest-old» population. The definition of normal brain aging in this segment of the aging population is of particular interest, since it may provide informations on the correlations between the final stages of normal cerebral aging and AD. Our group as well as other authors performed several quantitative analysis of senile lesion distribution in nonagenarians and centenarians (Hauw et al., 1986; Mizutani and Shimada, 1990, 1992; Delaère et al., 1993; Giannakopoulos et al., 1993, 1994, 1995). Classical and immunohistochemical studies of the cerebral cortex revealed the existence of certain oldest-old persons characterized by a low density of NFT in the hippocampal formation, a total absence of SP, and a striking preservation of the cognitive functions. These individuals may represent the lower limit of normal brain aging, and have been referred to as «supernormal centenarians» (Mizutani and Shimada, 1990; Karasawa, 1992; Giannakopoulos et al., 1993). In our studies, the regional patterns of NFT and SP distribution in non-demented centenarians were comparable to that reported in younger non-demented cases, in that NFT were found in all of the cases in the hippocampal formation, whereas the involvement of the neocortex was less frequent (Bouras et al., 1993, 1994; Giannakopoulos et al., 1994). However, numerous cases had some NFT in areas 22, and 23 and 24, suggesting an early NFT formation in these neocortical areas. This finding does not confirm our previous observations of a younger (82 years old) non-demented individual (Hof et al., 1992). In this case, the cingulate and superior temporal cortex was essentially devoid of NFT. It is thus possible that the degenerative process progresses within different cortical regions in centenarians than in younger subjects (Giannakopoulos et al., 1993, 1994).

Quantitatively, the highest NFT counts were found in the anterior part of the CA1 field, entorhinal cortex,
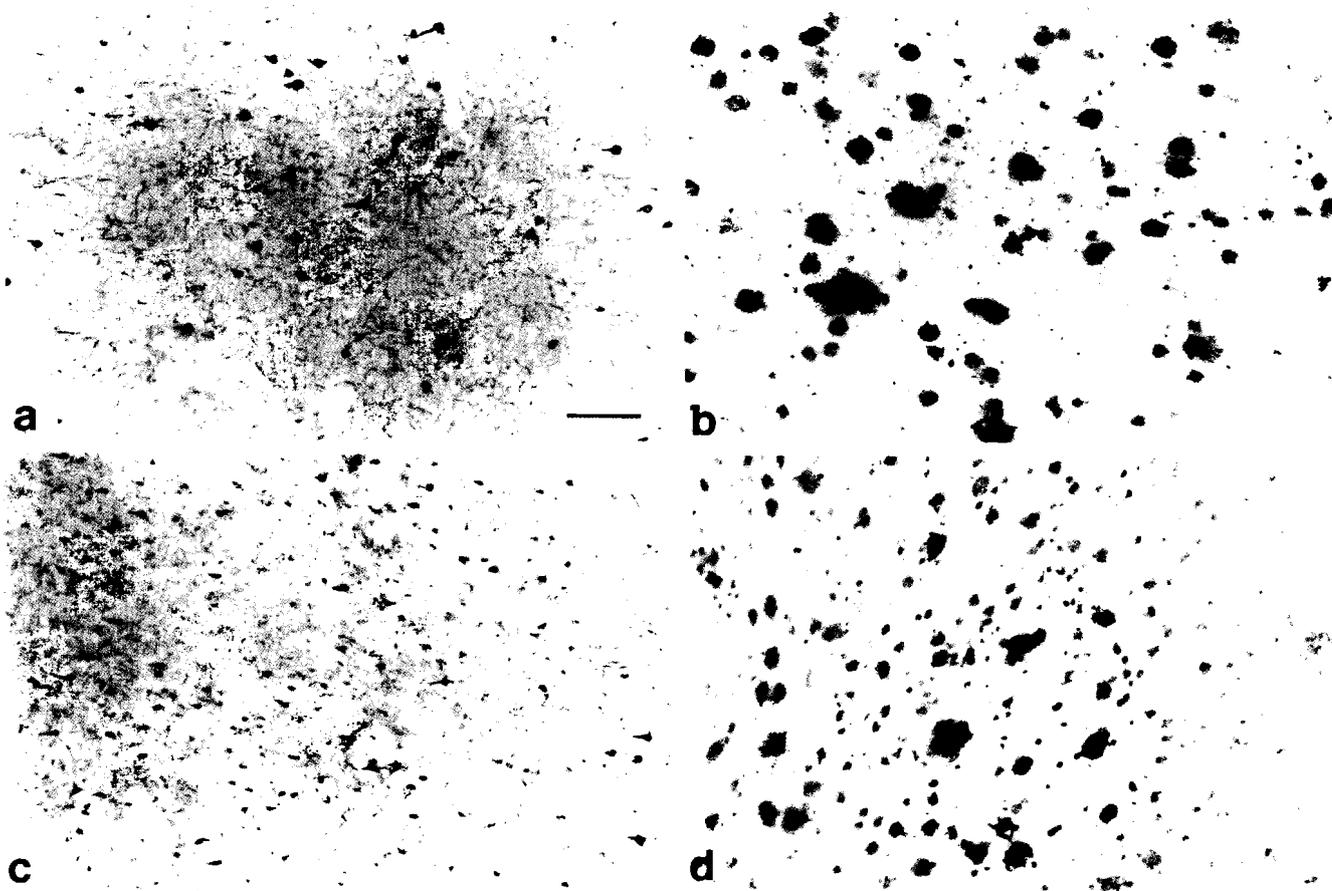
Fig. 4. Neurofibrillary tangle **(a, c)** and senile plaque **(b, d)** distribution in area 20 in a case with mild cognitive impairment (a, b) compared to an AD case (c, d). Note the substantial increase in NFT densities in the AD case. Numerous SP were observed in both cases. Materials were stained with antibodies to the microtubule-associated protein tau (a, c) or to the amyloid Aβ protein (b, d). scale bar: 250 μm.

subiculum, area 20, and amygdala in the centenarians. Conversely, areas 7, 17, 18, 22, 23, and 24 displayed consistently NFT densities lower than 10 per mm^2 (Giannakopoulos et al., 1993, 1995). As in younger non-demented cases, SP were homogeneously distributed in the neocortex in most of non-demented centenarians, while they were less frequently observed in the CA1 field of the hippocampus. High SP densities were found in more than 30% of the cases in layers II and III of areas 7, 22 and 24. The neuropathological profile of non-demented centenarians is comparable to that reported by Katzman and collaborators in their study of a large series of very old nursing home residents (Katzman et al., 1988). These authors distinguished a group of non-demented individuals with moderate number of neuritc plaques and few tangles in the neocortex, and postulated that these persons may represent incipient AD cases but did not express it clinically because of a greater reserve of cortical neurons. Furthermore, the hierarchical patterns of cortical involvement appear to be different in nonagenarians and centenarians than in younger individuals (Hauw et al., 1986; Mizutani and Shimada,

1990, 1992; Delaère et al., 1993; Giannakopoulos et al., 1993, 1994, 1995). For instance, the anterior CA1 field of the hippocampus showed significantly higher NFT densities in demented compared to non-demented centenarians, while NFT densities in the posterior CA1 field are comparable in non-demented and AD centenarians (Table 3, Fig. 5). This suggests that the massive NFT formation in the anterior part of the Ammon's horn may be a crucial step in the development of AD symptomatology in this age group, whereas the posterior part of this area can be severely affected without compromise of the higher cortical functions. In areas 7, 22, 23 and 24, AD cases displayed significantly higher NFT densities compared to non-demented cases, while no significant difference was observed in NFT densities in the anterior temporal, superior frontal cortex, as well as in areas 17 and 18 between AD and non-demented cases. The only statistically significant difference between non-demented and AD cases in the subcortical nuclei was in the density of NFT in the nucleus basalis of Meynert (Table 3). It is conceivable
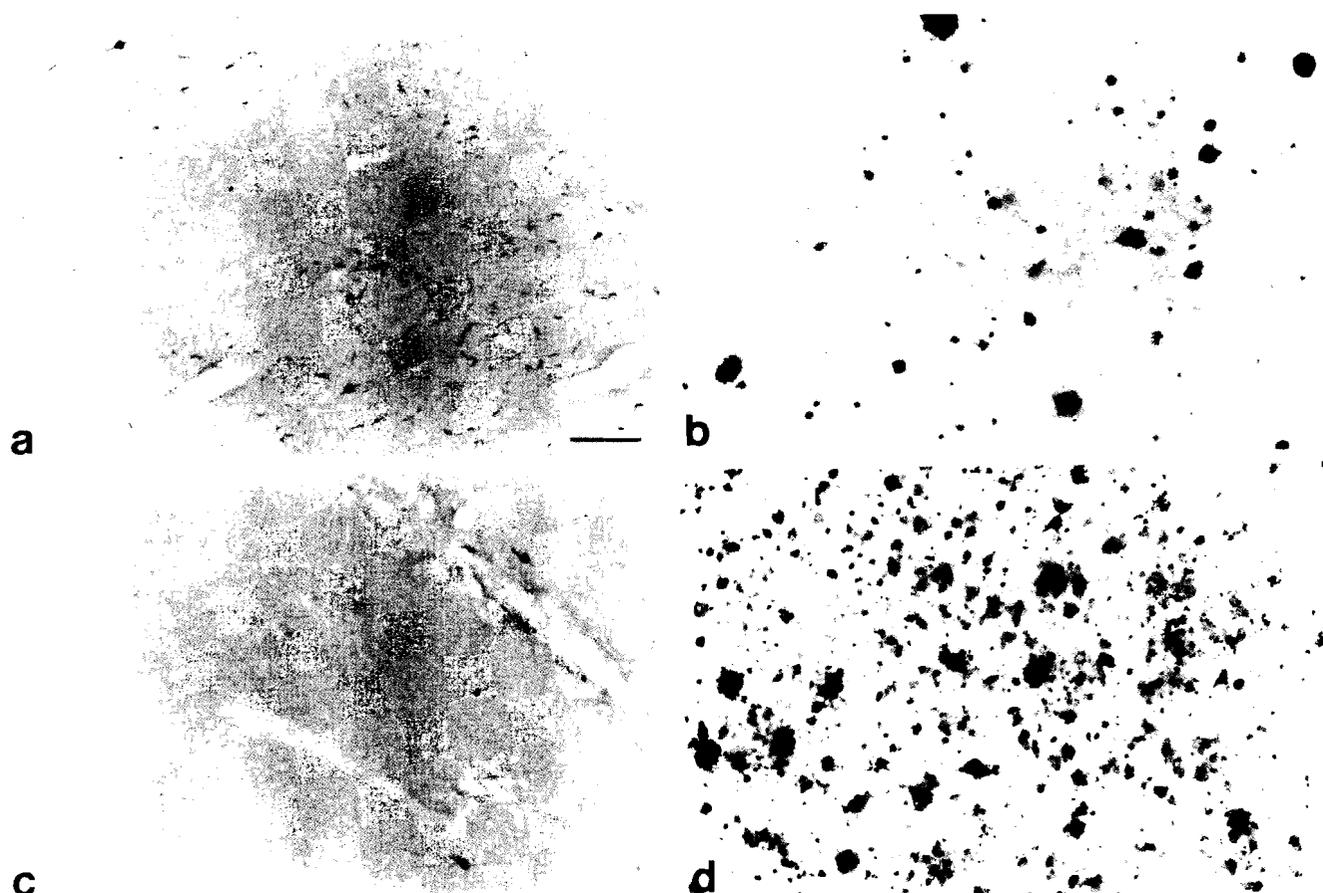
Fig. 5. Neurofibrillary tangles in the CA1 field **(a, c)** and senile plaques in area 18 **(b, d)** in a non-demented centenarian (a, b) compared to a centenarian with mild AD (c, d). Note the higher NFT densities in the demented case, whereas there was no difference in senile plaque density between the two cases. Materials were stained with antibodies to the microtubule-associated protein tau (a, c) or to the amyloid Aβ protein (b, d). Scale bar: 250 μm.

Table 3. Comparison of neurofibrillary tangle counts in centenarians

AREA/LAYERS		ND	AD	p
Ant CA1		34.1±4.8	67.6±12.3	<0.01
Post CA1		59.9±9.5	59.5±7.8	ns
Subiculum		17.6±2.7	19.9±2.8	ns
Entorhinal II		35.5±4.8	35.3±3.7	ns
Entorhinal V		16.5±2.7	16.1±1.9	ns
20	II-III	16.7±2.4	21.5±4.7	ns
	V-VI	11.4±1.9	16.1±3.0	ns
9	II-III	4.4±1.5	6.9±2.6	ns
	V-VI	1.6±0.6	5.3±2.2	ns
7	II-III	1.1±0.3	16.8±5.2	<0.01
	V-VI	0±0	14.4±4.1	<0.005
22	II-III	8.7±3.7	22.8±8.4	<0.05
	V-VI	4.8±2.6	16.7±6.3	<0.01
17	II-III	0.1±0.1	10.7±2.4	ns
	V-VI	0±0	2.0±0.9	ns
18	II-III	0.2±0.1	10.9±2.7	ns
	V-VI	0±0	4.8±2.6	ns
24	II-III	4.5±1.0	23.0±4.0	<0.005
	V-VI	1.5±0.5	11.7±2.6	<0.005
23	II-III	6.2±2.5	17.5±6.4	<0.05
	V-VI	0.7±0.2	8.6±2.4	<0.005
Amygdala		21.8±4.2	23.9±3.5	ns

Results represent NFT counts/mm^2 (±SEM) in each area. Statistical analysis was performed by analysis of covariance controlling for age at death. Ant CA1: anterior part of the CA1 field; Post CA1: posterior part of the CA1 field; ns: not statistically significant; ND: non-demented cases; AD: Alzheimer's disease cases. Layers are indicated by Roman numerals.

Table 4. Comparison of senile plaque counts in centenarians

AREA/LAYERS		ND	AD	p
Ant CA1		2.4±0.5	4.2±0.8	ns
Post CA1		3.4±0.8	3.3±0.3	ns
Subiculum		1.8±0.6	3.7±0.7	ns
Entorhinal II		4.4±0.8	9.0±1.8	ns
Entorhinal V		1.5±0.4	2.8±0.5	ns
20	II-III	10.5±2.0	22.1±2.9	<0.05
	V-VI	4.4±1.1	7.4±1.3	ns
9	II-III	5.7±1.5	12.8±2.3	<0.05
	V-VI	39±1.1	7.7±1.2	ns
7	II-III	13.2±2.3	26.0±3.0	<0.05
	V-VI	4.7±1.0	9.9±1.5	<0.05
22	II-III	21.4±8.6	27.4±5.6	<0.05
	V-VI	6.4±1.9	12.5±3.7	ns
17	II-III	10.0±1.5	11.1±1.5	ns
	V-VI	11.3±2.2	7.1±1.0	ns
18	II-III	9.1±1.5	12.2±1.5	ns
	V-VI	6.6±1.2	8.8±1.8	ns
24	II-III	22.8±3.2	33.8±3.7	<0.05
	V-VI	12.1±3.1	12.1±1.6	ns
23	II-III	13.9±2.1	26.4±2.9	<0.05
	V-VI	4.4±0.8	8.2±0.9	<0.05
Amygdala		9.2±1.7	10.6±1.0	ns

Results represent SP counts/mm^2 (±SEM) in each area. Statistical analysis was performed by analysis of covariance controlling for age at death. Ant CA1: anterior part of the CA1 field; Post CA1: posterior part of the CA1 field; ns: not statistically significant; ND: non-demented cases; AD: Alzheimer's disease cases. Layers are indicated by Roman numerals.

that the regional patterns of the neuronal degeneration differ in several aspects between centenarians and younger subjects. First, the spreading of NFT from the hippocampus to the temporal neocortex may play a less important role in the clinical expression of AD in centenarians compared to younger patients in whom it has been associated to overt dementia symptomatology (Hof et al., 1992; Bouras et al., 1994; Giannakopoulos et al., 1994). In addition, the clinical diagnosis of AD in oldest-old individuals is correlated with the number of NFT in areas 7, 22, 23 and 24 which are thought to be affected at a late stage of the dementing process (Hof et al., 1992). This distribution of NFT can be regarded as a displacement of these lesions, such that parietal and cingulate areas are more affected than is usually the case in AD, whereas superior frontal and inferior temporal association areas are relatively spared.

With respect to SP counts, several studies indicated that there is no relationship between their formation in the neocortex and the early stages of the dementing process, as is the case in younger populations (Delaère et al., 1993; Giannakopoulos et al., 1993; Fig. 5). However, recent evaluations of centenarians form a psychiatric hospital demonstrated a clear correlation between SP densities in areas 7, 9, 20, 22, 23 and 24 and severe AD (Table 4, Fig. 6; Giannakopoulos et al., 1994, 1995). These findings differ from those of previous studies in younger demented and non-demented cases where there is a lack of correlation between the clinical severity of

dementia and SP densities (Arriagada et al., 1992a,b; Berg et al., 1993; Bierer et al., 1995), and support the possiblily that SP formation may not be etiologically linked to the early stages of cognitive impairment but may represent a reliable pathological hallmark of severe AD in this age group.

Conclusion

The distribution of lesions in aging and preclinical stages of dementia suggest that the most vulnerable neurons are certain subpopulations of pyramidal neurons in the hippocampal formation and in the inferior temporal cortex. These neurons are in a position to furnish key hippocampal and neocortical circuits, that link the medial temporal lobe with neocortical association areas and amygdala (Hyman et al., 1990). Thus, all corticocortical systems are not equally vulnerable, with short projections from primary sensory to adjacent secondary sensory areas generally being more resistant to degeneration (Hof and Morrison, 1990, 1991, 1994; Hof et al., 1990, 1991, 1993). It is possible that the specific neurochemical and morphologic phenotype of certain pyramidal neurons may predispose them to degeneration and/or NFT formation (Hof and Morrison, 1994). Conversely, the combination of factors such as high cytoplasmic levels of calcium-binding proteins and GABA, with the morphological features of locally projecting interneurons may render them more

resistant to the degenerative process (Hof and Morrison, 1994). Furthermore, the cells that provide these projections appear to be highly specialized neurons that may be identified by restricted anatomic and neurochemical features, although relationships between etiopathogenesis and specific cellular vulnerability clearly are highly complex. Comprehensive morphologic and biochemical knowledge of neuron topology is needed before the critical features leading to the relative vulnerability or resistance of a particular neuronal subset can be assessed. Many other factors, known to be present in vulnerable neuronal populations and in the lesions of AD, such as amyloid proteins fragments, apolipoprotiens, copper-zinc superoxide dismutase, and iron-binding proteins, may also contribute to the cascade of events that leads to cellular degeneration seen in aging and to a more severe degree in dementia (Hof and Morrison, 1994). It has also been proposed that environmental neurotoxic substances, in particular aluminum and iron (Good et al., 1992; Shin et al., 1995), are involved in the neuronal degeneration process.

Dementia can be considered to represent the consequence of pathologic changes restricted to neocortical areas. It is clear that extensive hippocampal alterations exist in presence of only minor disruptions in daily living (Arriagada et al., 1992a,b; Hof et al., 1992; Bouras et al., 1993). Memory may be highly dependent on hippocampal circuits at certain processing stages, but may be independent of the hippocampal formation, once addressing and retrieval of information relies on neocortical circuitry (Hodges et al., 1992; Zola-Morgan et al., 1992). Thus, dementia may ensue if mechanisms by which pathological changes that are limited to the hippocampal formation and inferior temporal areas at early stages of the disease, spread to other neocortical circuits. This could explain why patients with minimal age-associated memory impairment frequently demonstrate limited neocortical involvement, but sometimes severe degree of hippocampal changes that may directly account for their mild cognitive decline.
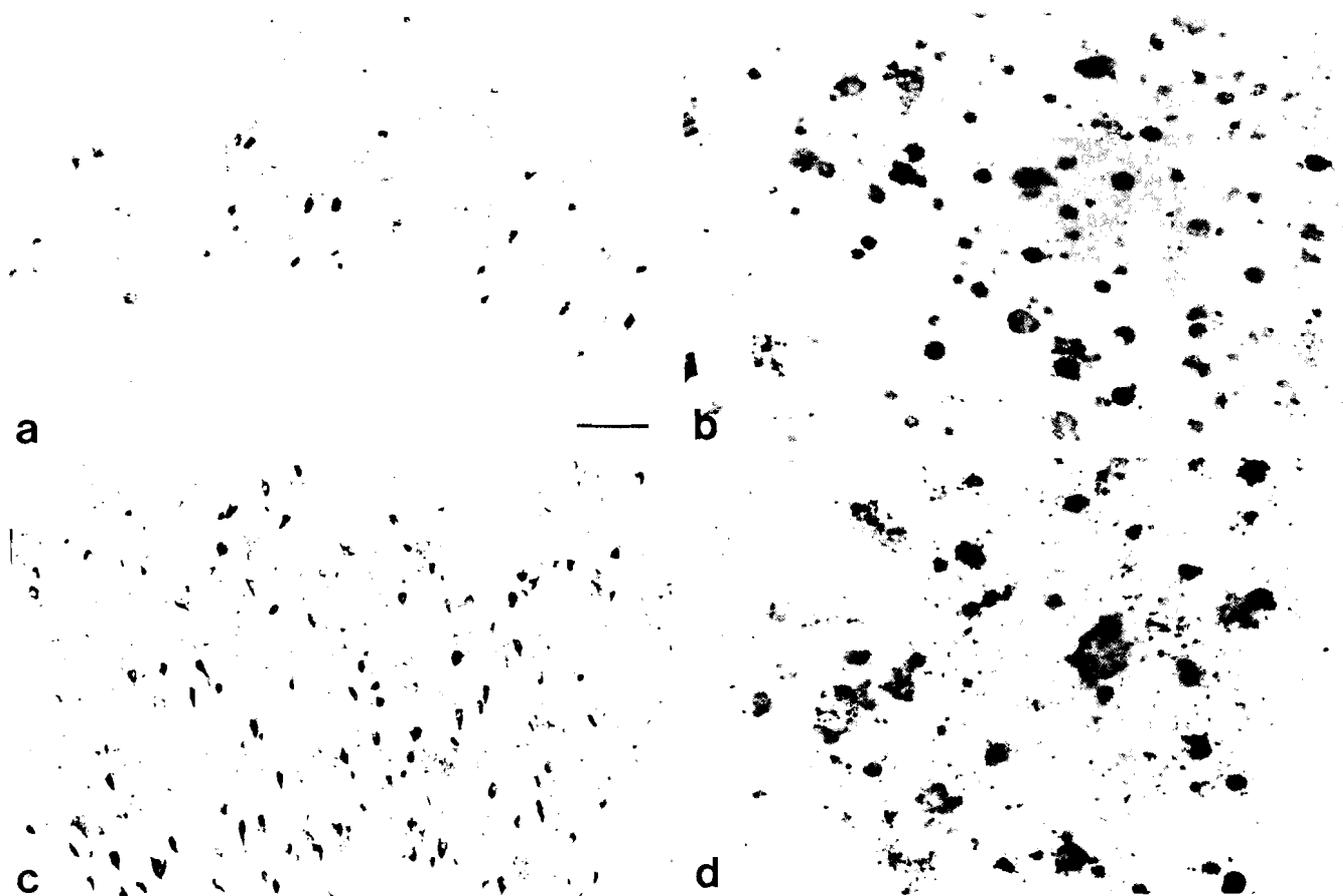
Fig. 6. Neurofibrillary tangle **(a, c)** and senile plaque **(b, d)** distribution in area 9 in a non-demented centenarian (a, b) compared to a centenarian with severe AD (c, d). Note the low NFT counts in both cases and the presence of higher SP densities in the demented case. Materials were stained with antibodies to the microtubule-associated protein tau (a, c) or to the amyloid Aβ protein (b, d). scale bar: 250 μm.

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