Acta Neuropathol (1993) 85: 602 - 610

Acta Neuropathologica

Quantitative immunohistochemical analysis of the distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of nonagenarians and centenarians*

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Received October 23, 1992/Revised, accepted January 5, 1993

Summary. To investigate the neuropathological differences between normal aging and senile dementia of the Alzheimer type (SDAT) in very old people and to see how they compare with a younger population of demented elderly people, we performed an immunohistochemical quantitative analysis of the topography of senile plaques and neurofibrillary tangles in a series of 31 elderly patients aged from 96 to 102 years. According to the medical records, two groups were considered: 7 patients presenting with clinically documented SDAT and 24 patients with no or very mild cognitive impairment. The densities of senile plaques were comparable in both groups. Extensive neurofibrillary tangle formation was restricted to the CA1 hippocampal field of demented subjects, whereas the superior frontal cortex showed rare neurofibrillary tangles, independently of the clinical diagnosis. These results indicate an absence of direct correlation between the number of senile plaques and the clinical manifestation of SDAT. Furthermore, they suggest that the dementing process may involve different cortical structures in nonagenarians and centenarians than in younger demented individuals where a widespread cortical involvement is generally observed. Thus, the neurofibrillary tangle density in the CA1 field may be critical for the neuropathological diagnosis of SDAT in this particular group of very old patients.

Key words: Senile plaques – Neurofibrillary tangles – Centenarians – Immunohistochemistry – Quantitative neuropathology

The neuropathology of aging has been studied in great details during the two last decades. However, many crucial points remain unclear, in particular regarding correlations between the clinical diagnosis of cognitive impairment and the neuropathological findings. It is well known that the age-related evolution and localization of degenerative cerebral changes such as senile plaques (SP) and neurofibrillary tangles (NFT) differ according to the clinical history [7, 35]. Since deterioration of cognitive abilities appears to be a continuous process [33], a quantitative assessment of the pathological alterations in various cerebral regions and at different ages is needed to establish a precise neuropathological diagnosis.

Numerous studies have shown that SP and NFT are common features of brain aging, and that they are concentrated in certain regions of the cerebral cortex with densities sometimes comparable to those observed in senile dementia of the Alzheimer type (SDAT) [2, 3, 5, 17, 20, 30, 33]. In particular, the hippocampal formation of non-demented elderly people consistently displays moderate-to-high lesion densities. In addition, select cortical areas in the medial and inferior aspects of the temporal lobe are likely to play a crucial role in the progression of SDAT, in that they may represent an interface between severely affected regions in the hippocampal formation and less involved neocortical regions [5, 17].

The neuropathological evaluation of brains of nonagenarians and centenarians could provide informations on the correlations between final stages of cerebral aging and SDAT [23]. While there have been several contributions on the visceral pathology of oldest old people, the neuropathological studies of nonagenarian and centenarian brains are still scarce [14, 18, 29]. We had the opportunity to study the brains of 31 patients aged from 96 to 102 years. For such a population of very old people, classical neuropathological diagnostic criteria of degenerative dementia [24] appear difficult to apply. For instance, it has been shown that cortical SP counts do not distinguish between demented and non-demented subjects [29]. Using immunohistochemicel methods, the present study attempted to clarify the quantitative differences of SP and NFT between patients in their

* Supported by grants from the American Health Assistance Foundation and the Brookdale Foundation (to P.R.H.)

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tenth and eleventh decade presenting with either SDAT or very mild cognitive decline.

Materials and methods

The sample included 9 men (97.0 ± 1.4 years old) and 22 women (97.5 ± 1.7 years old) who died and were autopsied at the Geriatric Hospital of the University of Geneva during the years 1972–1991. Most patients were admitted to the hospital with symptoms of chronic vascular or pulmonary diseases. One patient presented with severe mental confusion at the time of admission. Twenty four subjects had no or very mild signs of cognitive deterioration (7 men, 97.28 ± 1.49 years old, 17 women, 97.70 ± 1.82 years old). These patients form the no dementia /mild involvement (ND/MI) group. Before the final admission they lived at home and showed good ability in dealing with daily activities. Observations in the hospital confirmed the global preservation of cognitive functions. No further neuropsychological tests were obtained for these cases. Seven patients (2 men, 96 years old, 5 women, 97.0 ± 1.0 years old) presented with considerable intellectual impairment and were classified clinically as SDAT according to the evaluation made by the medical and nursing staff. Neuropsychological evaluation was performed during the hospitalization for these 7 cases (Mini Mental State [12], Blessed information-memory-concentration test [4]).

Postmortem pathological examination revealed that cardiopulmonary diseases were the most common cause of death; 10 cases presented with pneumonia, 6 with pulmonary embolism, 3 with myocardial infarcts and 4 with global heart failure. Cancer was the direct cause of death only in one case. Interestingly, there was no case with a cerebral cause of death such as tumor or hemorrhage. All causes of death were pathologically confirmed. In one case, the cause of death remained unknown after the autopsy. Among the underlying pathological features, severe atheromatosis was present in 17 cases and valvular diseases in only 2 cases. Three patients had active tuberculosis. Cancer was found in 10 cases. It was occult in 2 subjects.

The brains, obtained at autopsy (postmortem delay: 3–14 h), were fixed in 15% formalin for more than 6 weeks and cut into 1-cm-thick coronal slices. After macroscopic examination, tissue blocks were taken from the anterior part of the hippocampal formation, the inferior temporal, superior frontal and occipital cortex in the left hemisphere. For microscopic purposes paraffin-embedded blocks were cut into 10-μm thick sections. For routine neuropathological evaluation only, tissues were stained with hematoxylin-eosin, cresyl violet, Globus silver impregnation and modified S-thioflavine staining [37]. The quantitative assessment of the localization and the distribution of NFT and SP was made using immunohistochemistry according to Guntern et al. [13]. Highly specific and previously fully characterized antibodies to the microtubule-associated protein tau [9, 11, 38] and to the core-amyloid β protein A4 [25] were incubated at 1:4000 overnight after pretreatment of the tissues [13]. Following incubation, the section were processed by the peroxidase-antiperoxidase (PAP) method with 3,3′-diaminobenzidine used as a chromogen.

For all brains, analyses were performed in the CA1 field of the hippocampus, in layers II and V of the entorhinal cortex and in layers II–III and V–VI of the inferior temporal and superior frontal cortex. The number of NFT and SP was determined in ten different slides for each area and the mean number/mm^2 was calculated 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). Given the normal distribution of NFT and SP numbers in the series studied, statistical analysis was performed by one-way analysis of variance.

Table 1. Distribution of macroscopic cerebral infarcts in the present series

ND/MI group	FC	PC	TC	OC	Basal ganglia	Pons/ cerebellum
1	–	–	–	–	–	–/–
2	–	–	–	–	+	–/–
3	–	–	–	–	–	–/–
4	–	+	–	–	–	–/–
5	–	–	–	–	–	–/–
6	–	–	–	–	+	–/–
7	–	–	–	–	–	–/–
8	–	–	–	–	–	–/–
9	–	–	–	–	+	–/–
10	+	–	–	+	–	–/–
11	–	–	–	–	–	–/–
12	–	–	–	–	–	–/–
13	+	+	–	–	–	+/–
14	–	–	–	–	–	–/–
15	–	–	–	–	–	–/–
16	–	–	–	–	–	–/–
17	–	–	–	–	–	–/–
18	–	–	–	–	–	–/–
19	–	–	–	–	–	–/–
20	–	–	–	–	–	–/–
21	–	–	–	–	–	–/–
22	–	–	–	–	–	–/–
23	–	–	–	–	–	–/–
24	–	–	–	–	–	–/–
SDAT group						
25	–	–	–	–	–	–/+
26	–	–	–	–	–	–/–
27	–	–	–	–	–	–/–
28	–	–	–	–	–	–/–
29	–	–	–	–	+	–/–
30	–	–	–	–	+	–/–
31	–	–	–	–	–	–/–

FC, Frontal cortex; PC, parietal cortex; TC, temporal cortex; OC, occipital cortex; ND/MI, cases with no or minimal cognitive decline; SDAT, cases with senile dementia of the Alzheimer type; +, present; –, absent
Note the relative rarity of vascular alterations in this series

Table 2. Senile plaque (SP) and neurofibrillary tangle (NFT) counts in the cerebral cortex in ND/MI and SDAT groups

ND/MI group	Sex	Age	NFT-CA1	NFT-Sub	NFT-Ent/II	NFT-Ent/V	NFT-ITC/II–III	NFT-ITC/V–VI	NFT-SFC/II–III	NFT-SFC/V–VI	SP-CA1	SP-Sub	SP-Ent/II	SP-Ent/V	SP-ITC/II–III	SP-ITC/V–VI	SP-SFC/II–III	SP-SFC/V–VI
1	F	102	9.3	16.7	20.4	21.3	6.5	5.6	0	0	0	0	0	0	0	0	0.2	0.2
2	M	100	41.7	13	29.6	11.1	25	17.6	0	0.9	0	0	0	0	0	0	0	0
3	F	100	21.3	13	23.1	1.9	15.7	1.9	1.9	1.9	4.4	2.7	6.4	0.9	10.4	9.3	10.9	9.1
4	F	100	67.6	17.6	79.6	29.6	21.3	18.5	0	0	11.3	2.7	13.1	3.1	23.6	18.2	33.3	26.7
5	F	99	5.6	1.9	15.7	13.9	11.1	12	12.9	10.2	0.4	0.2	4	0.7	11.6	3.3	4	2.9
6	F	99	37	24.1	29.6	9.3	20.4	11.1	0	0	0.4	0	0	0	0.4	0.7	0	0
7	F	98	79.6	47.2	37.9	16.7	43.5	25	0.9	0.9	4.4	12	10.2	2	13.6	9.1	7.8	8.7
8	M	98	75	24.1	67.6	20.4	23.1	27.8	0	0	6.7	6.4	8.4	2.9	6.2	10.2	•	6
9	F	98	13.9	15.8	14.8	0.9	0	0	0	0	2.7	2.2	2.2	0.2	•	•	2.7	1.6
10	M	98	27.8	17.4	73.1	20.4	8.3	8.3	0.9	1.9	1.3	0.9	13.8	5.8	12.2	10	•	4.2
11	F	98	24.1	16.7	27.8	4.6	10.2	8.3	4.6	2.8	3.3	0.2	4.7	0.9	7.3	0.4	10.2	5.1
12	F	98	47.2	30.6	•	•	14.8	8.3	27.8	8.3	1.3	2.9	1.1	0	9.8	8.2	7.8	4.7
13	M	97	3.7	0	23.1	0.9	2.8	0.9	0	0	0	0	0	0	0	0	0	0
14	F	97	23.1	13.9	50.9	40.7	17.6	5.6	0.9	0	0	0	0	0	0.2	0.2	3.8	0.7
15	F	96	81.5	27.8	75	36.1	28.7	21.3	12	9.3	2.7	3.1	10.4	4.2	17.1	4.4	3.8	3.3
16	F	96	36.1	12.9	10.2	2.8	20.4	7.4	1.9	0.9	0	0	4.9	0.4	6.9	2.4	7.3	2.4
17	F	96	2.8	0	4	2.8	3.7	0	0	0	0	0	0	0	0	0	0.7	0
18	F	96	13	9.3	30.6	•	•	•	0	0	4.7	1.3	1.1	2.9	•	•	4.7	3.8
19	F	96	14.8	2.4	•	•	•	•	0	0	3.3	1.3	8.2	0.2	7.3	6.9	7.3	2.7
20	M	96	41.2	14.8	63	35.2	36.1	25.9	0	0	6.4	2.2	8.4	6.4	18.4	0	9.6	6
21	M	96	25.9	13	15.7	13.9	10.2	2.8	0	0	0	0	0	0	•	•	0	0
22	F	96	36.1	16.7	24.1	12.9	23.1	19.4	9.3	0.6	0.2	0	0	0	0	0	0.4	0
23	M	96	32.4	19.4	25	25.9	7.4	12	18.2	0.9	2	2	4.2	4.2	12.2	5.6	3.6	3.1
24	F	96	56.5	54.6	40.7	25	•	•	13.8	0.9	4	4	6.4	2.2	•	•	8.2	3.3
SDAT group																		
25	F	98	67.6	19.4	27.8	17.6	25.9	21.3	1.9	0.9	0.9	2.9	6.4	3.6	9.1	4.9	10.2	3.6
26	F	98	79.6	8.3	46.3	9.3	21.3	13.9	3.7	1.9	2	1.3	3.6	0.7	6.9	3.8	11.8	5.1
27	F	97	25.9	18.5	50	17.6	22.2	13.9	0	0	0	0	0	0	0	0	0	0
28	F	96	88.9	16.7	22.2	29.6	10.2	24.1	2.8	10.2	2.9	1.6	4.2	3.8	7.8	3.1	•	7.8
29	M	96	57.4	30.6	37.9	11.1	55.6	25	3.7	0.9	2.2	4.4	3.3	0.9	12	4.2	9.1	7.6
30	F	96	46.3	27.8	11.1	4.6	9.3	0.9	3.7	0.9	2.4	2.7	3.6	0.2	4	0.7	7.8	1.6
31	M	96	91.7	12	9	2.7	0.9	5.6	32.4	17.6	8.4	2	9.1	1.8	16.4	7.3	7.8	5.1

Results represent counts/mm^2 (± SEM) in each cortical layer. Missing values mean that tissue was not available for examination. Cases are classified by age in each diagnostic group
Sub, subiculum; Ent, entorhinal cortex; ITC, inferior temporal cortex; SFC, superior frontal cortex. Layers are indicated by Roman numerals

Table 3. Severity assessment of NFT and SP counts in the ND/MI groupe

NFT/mm²	CA1	Subiculum	Entorhinal II	Entorhinal V	ITC II–III	ITC V–VI	SFC II–III	SFC V–VI
0	0	7	0	0	5	10	50	50
1–10	17	10	4	30	23	40	30	44
11–20	14	58	20	25	31	30	17	3
21–30	21	10	32	28	32	20	3	3
31–40	17	5	10	11	5	0	0	0
>40	31	10	34	6	4	0	0	0
SP/mm²								
0	30	39	33	39	25	30	18	25
1–5	64	55	28	55	13	38	45	50
6–10	3	3	28	6	22	17	23	21
11–15	3	3	11	0	25	10	9	0
16–20	0	0	0	0	10	5	0	0
>20	0	0	0	0	5	0	5	4

Values represent percentages of cases displaying NFT and SP. Note that NFT were present in all of the cases in the CA1 field and entorhinal cortex. Conversely, NFT were absent in 50% of cases in the superior frontal cortex. The majority of cases had less than 10 SP/mm² in the CA1, entorhinal cortex and subiculum. Layers are indicated by Roman numerals.
ITC, Inferior temporal cortex; SFC, superior frontal cortex

Results

From both the ND/MI and the SDAT group two brains showed marked frontal atrophy. In 1 case of SDAT, there was a pronounced frontal and temporal atrophy. The remaining 26 cases had no obvious cerebral atrophy. Cerebral infarcts were present in 9 cases; in 6 they were limited to the basal ganglia. Pontine or cerebellar infarcts were found in 2 cases. The exact distribution of infarcts in relation to clinical diagnosis is shown in Table 1. Cortical infarcts were not seen in the SDAT group. There was no case with cerebral hemorrhage. Only in 1 case (no. 13) an old subdural hematoma was observed.

Senile changes in this series of oldest old people are summarized in Table 2. In the ND/MI group, 2 cases (nos. 13, 17) displayed very few NFT, localized in the CA1 field of the hippocampus, entorhinal and inferior temporal cortex, and no SP. In the same group, case 21 showed no SP but higher NFT densities. In these cases the superior frontal cortex was entirely free of lesions. According to the medical and nursing records, these patients were intellectually preserved and well adapted to their daily living. The majority of the remaining ND/MI cases had numerous NFT in the CA1 field, subiculum, entorhinal and inferior temporal cortex. There were no cases without NFT formation in the CA1 field and entorhinal cortex. In 2 cases (nos. 13, 17) NFT were absent in the subiculum and in only one case (no. 9) inferior temporal cortex was not involved. Conversely, in 50% of ND/MI cases there were no NFT in the superior frontal cortex. High NFT densities (> 10 NFT/mm²) were observed in this area only in 5 cases (nos. 5, 12, 15, 23, 24). There were large interindividual variations in NFT counts. This variability was more pronounced in the CA1 field and in layer II of the entorhinal cortex (Table 3).

In 4 cases (nos. 2, 13, 17, 21) of the ND/MI group no SP were observed. Among them, 1 case (no 2) had numerous NFT in all cortical areas with the exception of the superior frontal cortex. In 3 additional cases (nos. 1, 6, 22) scarce SP were present in the hippocampal formation and neocortical areas. The 17 other ND/MI cases showed a significantly higher number of SP in all regions, comparable to that observed in the cases with SDAT. However, these cases could not be considered as a neuropathologically distinct group since their NFT densities were not statistically different to those found in the SDAT cases. In the CA1 field, SP were seen in 70% of the ND/MI cases. The subiculum and the entorhinal cortex displayed the lowest frequency of SP. Layers II, III, V and VI of the inferior temporal and superior frontal cortex were involved in 75% of ND/MI cases. Senile plaques were more frequently seen in layers II and III of the superior frontal cortex (about 82% of ND/MI cases; Table 3). The SP densities showed few interindividual variations. With the exception of layers II and III of the inferior temporal and superior

Table 4. Comparison of NFT counts between ND/MI and SDAT cases

Area	NFT/mm²	
	ND/MI	SDAT
CA1	34.05 ± 4.84	65.34 ± 9.04*
Subiculum	17.62 ± 2.65	19.04 ± 3.01
Entorhinal II	35.52 ± 4.82	29.18 ± 6.14
Entorhinal V	16.49 ± 2.68	13.21 ± 3.49
ITC II–III	16.66 ± 2.42	20.77 ± 6.69
ITC V–VI	11.41 ± 1.92	14.95 ± 3.49
SFC II–III	4.37 ± 1.51	6.88 ± 4.28
SFC V–VI	1.64 ± 0.62	4.62 ± 2.53

The mean NFT density in the CA1 field in patients with SDAT was significantly higher than in ND/MI cases (* $P < 0.01$). The NFT densities in layers II and III were higher than in layers V and VI of the inferior temporal and superior frontal cortex
ITC, Inferior temporal cortex; SFC, superior frontal cortex

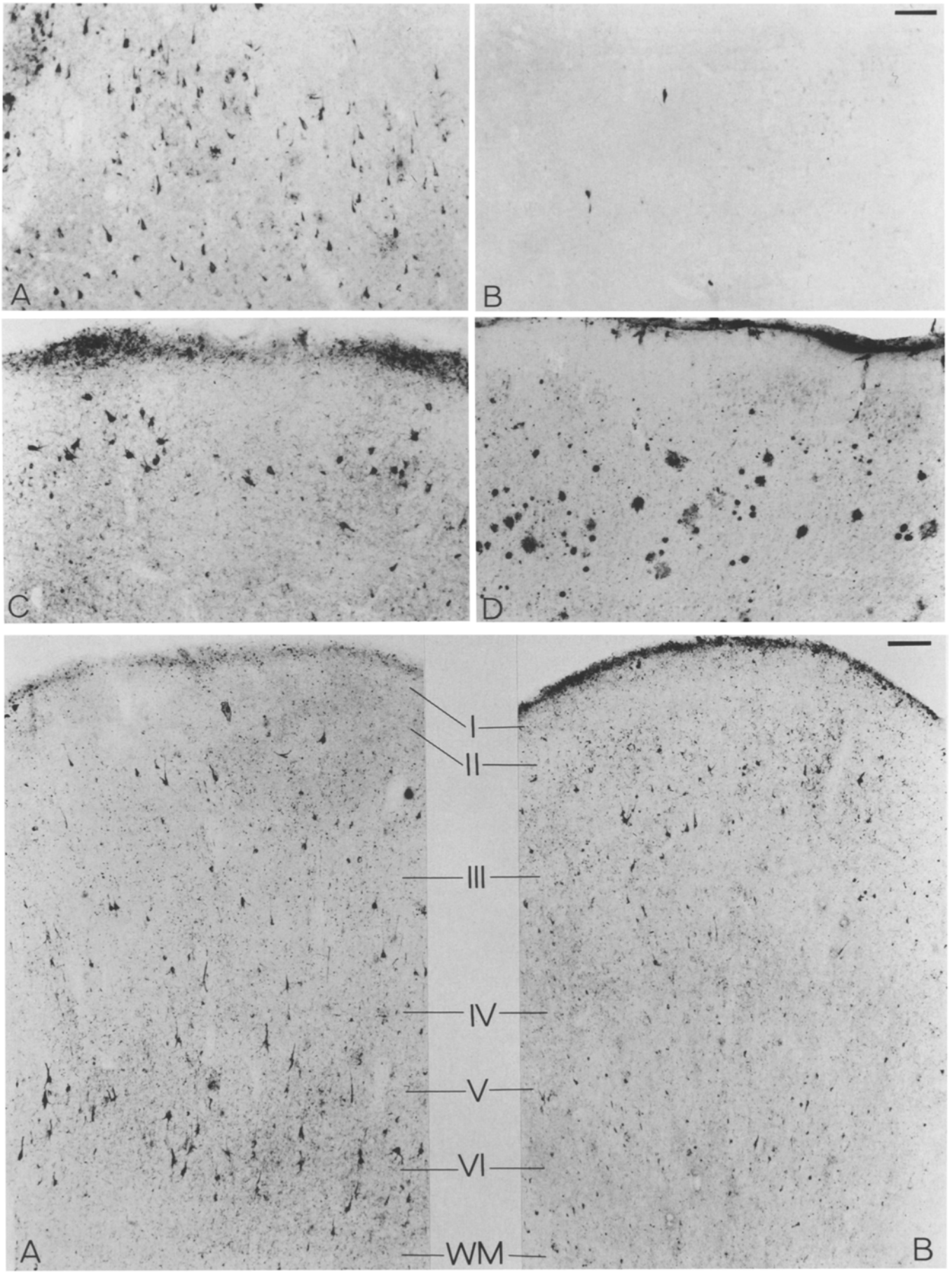
A
B
C
D
I
II
III
IV
V
VI
WM
A
B

Fig. 1. A,B CA1 field of the hippocampus in two centenarian cases. Note the higher density of neurofibrillary tangles (NFT) in a patient with senile dementia of the Alzheimer type (SDAT; **A**) in comparison to a non-demented case (**B**). **C,D** NFT and senile plaques (SP) in layers II and III of the entorhinal cortex in a demented centenarian (**C**) and in a non-demented case (**D**). Note the severe involvement of layer II in the SDAT case and the very high density of SP in the non-demented case. Materials were stained with an anti-tau protein antibody (**A–C**) or with an anti-βA4 protein antibody (**D**). *Scale bar* = 100 μm

frontal cortex, most cases had less than 10 SP/mm^2. There was no correlation between the laminar distribution of SP and NFT.

In the cases with SDAT, NFT and SP were always present in all areas examined, except in case 27 who showed no NFT in the superior frontal cortex and no SP in all areas studies. Six cases presented with severe NFT formation (> 40 NFT/mm^2) in the CA1 field. The mean NFT density in this region was significantly higher than in the ND/MI cases ($P < 0.01$; Table 4, Fig. 1A,B). Conversely, in the subiculum, the entorhinal cortex and the inferior temporal cortex, SDAT cases displayed comparable NFT densities comparable to those observed in the ND/MI cases (Fig. 1C). Involvement of the superior frontal cortex was more frequent in the SDAT cases (85.7 %). Nevertheless, there was no significant difference in NFT counts in this region between the SDAT and ND/MI groups. Neocortical regions exhibited a higher density of SP in comparison to limbic structures. SP densities in all areas examined were comparable between SDAT and ND/MI cases (Fig. 1D, Table 5).

Table 5. Comparison of SP counts between ND/MI and SDAT cases

	SP/mm^2	
Area	ND/MI	SDAT
CA1	2.47 ± 0.58	2.68 ± 1.02
Subiculum	1.83 ± 0.55	2.12 ± 0.52
Entorhinal II	4.47 ± 0.92	4.31 ± 1.06
Entorhinal V	1.54 ± 0.40	1.57 ± 0.59
ITC II–III	7.86 ± 1.59	8.02 ± 2.00
ITC V–VI	4.44 ± 1.13	3.42 ± 0.94
SFC II–III	5.74 ± 1.73	7.78 ± 1.67
SFC V–VI	3.93 ± 1.13	4.40 ± 1.09

There was no statistically significant difference between the two groups
ITC, inferior temporal cortex; SFC, superior frontal cortex

Fig. 2. A,B Laminar distribution of NFT in the inferior temporal cortex of a SDAT case younger than 80 years of age (**A**), as compared to a demented centenarian case (**B**). Note the predominance of NFT in layers V and VI in the younger SDAT case (**A**), and in layers II and III in a centenarian (**B**). Materials were stained with an anti-tau protein antibody. *Scale bar* = 200 μm

Finally, the laminar distribution of pathological changes in very old SDAT cases differed from those previously reported in younger demented subjects [3, 31] since the NFT predominated in layers II and III but not in layers V and VI of the inferior temporal cortex and superior frontal cortex (Fig. 2A,B).

Discussion

The results of the present study indicate that the degenerative process in the brains of oldest old patients shows some particular features in respect to the localization and quantification of SP and NFT that might differ from the usual distribution observed in SDAT. In spite of an increasing number of publications on neurobiology of aging, neuroanatomical analysis of cases over 95 years of age are very limited. Peress et al. [32] and Matsuyama and Nakamura [27] found a decrease in the frequency of senile lesions after 90 years of age. Based on these observations, these authors postulated that from a neuropathological standpoint, nonagenarian and centenarian patients may constitute a distinct subpopulation. More recently, Hauw et al. [14] and Mizutani and Shimada [29] performed detailed studies of centenarian brains. In both studies classic histological staining methods were used (hematoxylin-eosin, modified Bielschowsky method, Bodian silver impregnation), whereas the present analysis relied on immunohistochemical staining for a better visualization of the neuropathological lesions for quantitative purposes.

Three patients in the present study displayed a low density of NFT in the hippocampal formation and neocortex areas and a total absence of SP. A review of the clinical records of these patients revealed a striking preservation of the cognitive functions and maintained social independence. This observation strengthens the hypothesis proposed by Karasawa [22] and Mizutani and Shimada [28] concerning the existence of a group of "supernormal centenarians". However, it is difficult at the present time to define the precise causes of the relative resistance to cerebral aging in these cases. Interestingly, estimates of mild, moderate and severe cognitive impairment in a large population study has revealed that among the oldest patients (older than 85 years of age), the prevalence was up to 38.1 % [10]. Also, there was a more striking increase in the number of affected patients with mild or moderate impairment than in patients with severe cognitive dysfunction [10]. This could be related to the fact that in these oldest old individuals the hippocampal formation appears to be more severely affected than the neocortex, and suggests that clinically overt dementia might be more related to neocortical damage, at least within the inferior temporal cortex, than to the occurrence of pathological lesions restricted to the hippocampal formation [5, 17].

NFT were present in all the cases in the present study. The hippocampal and parahippocampal areas were the most affected in both patients with SDAT and those with no or minimal cognitive impairment. Quantitatively the only statistically significant difference was in the density

of NFT in the CA1 field. These observations are in agreement with those of Hauw et al. [14], who examined the NFT distribution in the brains of 12 centenarians (1 case with SDAT). The NFT density in the CA1 field was higher in the patient suffering from dementia than in intellectually normal centenarians. The authors did not assess NFT counts in the entorhinal cortex and subiculum. Similar observations have been reported by Mizutani and Shimada [29]. These authors studied the quantitative topography of NFT, by classical histological methods, in the brains of 27 non-demented centenarians. The results obtained were then compared with the results from a younger group of patients presenting with SDAT. In the demented patients, the number of NFT was significantly higher in the CA4 field of the hippocampus. No difference was detected in the entorhinal cortex and the subiculum. These observations point to the primordial role played by the Ammon's horn in the neuropathological diagnosis of SDAT in centenarians and almost-centenarians. In addition, vascular damage is unlikely to play a major role as en etiopathogenic factor for dementia in these very old patients, given that in the present series the demented cases were generally devoid of such lesions. Also, it is worth noting that in the SDAT cases the role of vascular lesions in the cognitive changes may be relatively limited since they were observed only in subcortical structures.

In the present study the inferior temporal cortex was severely affected but still comparable in all cases, regardless of the clinical diagnosis. This agrees with the observations of Hauw et al. [14]. Mizutani and Shimada [29] reported a significant difference between the density of NFT in the temporal neocortex in patients with and without SDAT. However, the SDAT group was much younger (mean age: 86.5 years) in comparison to the group of patients without SDAT (mean age: 102 years). Our results suggest that the strong involvement of the inferior temporal cortex by NFT is a frequent phenomenon even in non-demented or very mildly demented individuals. As Hubbard et al. [20] and Hof et al. [17] have suggested, it appears that numerous NFT in the inferior temporal cortex is a neuropathological finding characteristic of a possible preclinical stage of SDAT.

The involvement of the superior frontal cortex was more frequent among patients with SDAT. The proportion of ND/MI cases with NFT in this area was, nevertheless, quite high (50 %) as compared to the rare occurrence of these lesions reported by Hauw et al. [14] and Mizutani and Shimada [29]. Improved lesion detection using immunohistochemical methods may be a possible explanation for this discrepancy. No difference was observed in this age group between the density of NFT in the superior frontal cortex of demented and non-demented patients. This observation does not parallel previous observations by Mizutani and Shimada [29] in centenarians and those by Hof et al. [17] in a younger case, showing that the clinical manifestation of SDAT in both centenarians and younger patients may be associated with a widespread involvement of the neocortex. The mild involvement of the superior frontal cortex in the very old demented patients suggests that this region, usually severely affected in SDAT [1, 15, 16] is less affected in the oldest age group. According to this observation, the neuropathological profile of centenarians and almost-centenarians with SDAT may differ considerably from that described in younger persons [17, 33]. Another arguement in favor of this possibility is the laminar distribution of NFT in the present study. NFT predominated in layers II and III of the inferior temporal cortex and superior frontal cortex as opposed to observations in younger SDAT cases in which NFT densities were usually higher in layers V and VI [15, 16, 26, 31, 34].

With respect to the distribution of SP, the results of the present study confirm that they are not consistently present and that their density is not correlated with the severity of the cognitive changes in these very old people. Senile plaque counts in all areas studied were lower than in demented octogenarians [5]. Their density was comparable in demented and non-demented cases. Similar observations have been reported by other authors [17, 21] in elderly people. Mizutani and Shimada [29], distinguished a subgroup of 6 patients, out of 27 cases studied, with a high number of neocortical and hippocampal SP along with a minimal density of neocortical NFT, notably lower than the number observed in younger demented patients. In view of these findings, the authors postulated that this subgroup could represent the upper limit of normal aging. The results of the present study do not support such a possibility. No neuropathologically distinct group as far as number of SP is concerned could be defined. This lack of agreement could be explained by the fact that patients with SDAT in our study (mean age: 96.5 years) had much lower neocortical NFT densities than those reported in younger patients [1, 15–17, 21, 26, 31, 34].

In conclusion, even if it is possible to define the lower limit of normal brain aging in very old patients (diffuse but minimal presence of NFT, absence of SP), the idea of an upper limit defined in function of the SP densities remains vague. The development of SP in the present series seems to be an epiphenomenon of aging with no direct correlation to the occurrence of SDAT. As demonstrated in other studies of brain aging, NFT are closely related to the dementing process [8, 17, 19, 21, 35, 36]. The very old people with SDAT show a marked decrease in the NFT density in the subiculum, entorhinal cortex and neocortical areas as compared to younger patients with the same clinical diagnosis. Thus, it is possible that in demented patients the presence of a high density of NFT in the cerebral cortex is not compatible with extended life span [6]. In any case, neuropathological criteria for SDAT in the oldest old population should be revised. It is likely that quantitative assessment of NFT in the hippocampal CA_1 field is crucial to establish this diagnosis. Furthermore, NFT involvement of the neocortex, although very frequent in younger patients with SDAT, appears to be a less reliable measure in this group of nonagenarian and centenarian patients. The differences between demented and non-demented very old people and elderly patients younger than 90 suggest that the development

of the dementing process might involve different cortical structures in this age group. Functionally, prospective neuropsychological studies of very old individuals are necessary to clarify the exact correlations between neuroanatomical findings and impairment of specific cognitive functions.

Acknowledgements. We wish to thank Drs. A. Delacourte and N. K. Robakis for generous provision of the antibodies to the tau and βA4 proteins, Dr. J. H. Morrison for helpful discussion and Mr. P.Y. Vallon for skillful technical assistance.

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