Distinct Patterns of Neuronal Loss and Alzheimer's Disease Lesion Distribution in Elderly Individuals Older than 90 Years

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Abstract. To explore the characteristics of brain aging in very old individuals, we performed a quantitative analysis of neurofibrillary tangle (NFT) and senile plaque (SP) distribution and neuron densities in 13 nondemented patients, 15 patients with very mild cognitive impairment, and 22 patients with Alzheimer's disease (AD), all older than 90 years of age. Nondemented cases displayed substantial NFT formation in the CA1 field and entorhinal cortex only. Very mild cognitive impairment cases were characterized by the presence of high NFT densities in layers V and VI of area 20, and AD cases had very high NFT densities in the CA1 field compared to nondemented cases. Moreover, high SP densities were found in areas 9 and 20 in AD, but not in cases with very mild cognitive impairment and nondemented cases. In contrast to previous reports concerning younger demented patients, neuron densities were preserved in the CA1 field, dentate hilus, and subiculum in centenarians with AD. In these cases, there was a marked neuronal loss in layers II and V of the entorhinal cortex, and in areas 9 and 20. In the present series, no correlation was found between neurofibrillary tangle and neuron densities in the areas studied, whereas there was a negative correlation between senile plaque and neuron densities in area 20. The comparison between the present data and those reported previously concerning younger cohorts suggests that there is a differential cortical vulnerability to the degenerative process near the upper age-limit of life.

Key Words: Alzheimer's disease; Brain aging; Centenarians; Neurofibrillary tangle; Neuronal loss; Senile plaque.

INTRODUCTION

Epidemiological and clinical studies have indicated that the characteristics of Alzheimer's disease (AD) in very old individuals differ significantly from those observed in patients younger than 90 years of age, and have suggested that the "oldest-old" population may represent a genetically distinct group (1–5). To date, most of the studies of neuropathological changes associated with brain aging have been performed in series of subjects younger than 90 years. Neurofibrillary tangles (NFT), senile plaques (SP), and neuronal and synaptic loss are common pathological features in both cognitively intact individuals and patients with AD (6–11). In nondemented cases, NFT are usually restricted to the hippocampal formation, whereas the progressive involvement of the association areas in the temporal neocortex parallels the development of overt clinical signs of dementia (6, 9, 10, 12–16). In contrast, severe SP formation may take place in several neocortical areas in the presence of very mild cognitive impairment (17, 18), and there is no correlation between the quantitative distribution of SP and severity of AD (6, 12, 13, 15, 16, 19–21). With regard to neuronal loss, stereological analyses have revealed age-related decreases in total neuron number of 30% and 50% in the dentate hilus of the hippocampus and subiculum, respectively, in nondemented individuals between ages 13 and 85. Conversely, no neuronal loss has been found in CA1–3 fields and entorhinal cortex where AD lesions were also observed (22–24). These studies also showed that in AD, there is an additional depletion of neurons in the dentate hilus and subiculum, as well as a massive reduction in the numbers of pyramidal neurons in the CA1 field and layers II and V of the entorhinal cortex (22–25). Moreover, recent studies have shown a neuronal reduction in temporal, inferior and superior parietal and frontal cortices of AD cases (25, 26).

Several neuropathological analyses have demonstrated consistent differences in the distribution and densities of NFT and SP in the cerebral cortex between very old people and younger elderly individuals (27–34). For instance, centenarians with AD display substantial NFT formation within the CA fields, whereas the inferior temporal and frontal association areas are relatively spared. In addition, SP formation is not associated with the development of mild AD, but appears to be correlated with the duration and severity of dementia in this age group (27–34). In these latter studies, no distinction was made between nondemented individuals and patients with very mild cognitive impairment, and NFT densities assessment was performed using nonstereological counting methods which are subject to several biases (27–30). Moreover, there is no study to date of neuronal loss in the cerebral cortex of AD centenarians. To address these issues, we report a quantitative analysis of NFT and SP distribution.
as well as estimates of neuronal loss in the hippocampal formation, and superior frontal and inferior temporal cortices in a large series of nonagenarians and centenarians including nondemented cases, cases with very mild cognitive impairment, and AD cases.

MATERIALS AND METHODS

Clinical Characteristics of the Series

The brains of 50 nonagenarians and centenarians (37 women, 98.8 ± 2.2 years old; 13 men, 99.5 ± 1.7 years old; age range: 96-103 years) who died and were autopsied in the Hospitals of the University of Geneva School of Medicine were included in the present study. Among them, 13 patients (3 men, 99.0 ± 2.6 years old; 10 women, 99.0 ± 1.2 years old; 7 cases previously reported [29, 30] and 6 new cases) had no signs of cognitive impairment. Their mean Mini-Mental State Examination (MMSE) score (35) at admission was 29.5 ± 0.5. All of these cases were assessed retrospectively using extended Clinical Dementia Rating (CDR) scale (36) and were CDR 0. Neuropsychological testing of memory, language skills, psychomotor performance and visuospatial abilities revealed a good preservation of these functions. These patients form the nondemented (ND) group of the present study. Fifteen patients (7 men, 97.7 ± 1.2 years old; 8 women, 98.6 ± 1.2 years old; 10 cases previously reported [29, 30] and 5 new cases) showed very mild cognitive impairment characterized by mild deficits in immediate memory and temporal disorientation. Old memory, spatial orientation, language, psychomotor performance and visuospatial abilities were consistently spared. These patients form the very mild cognitive impairment (VMCI) group. Their mean MMSE score at admission was 28.5 ± 1.5, and their extended CDR score was 0.8 ± 0.2. Most of ND and VMCI patients were admitted to the hospital with symptoms of cardiac failure, pulmonary insufficiency or chronic vascular diseases. The remaining 22 patients (3 men, both 99.2 ± 2.5 years old; 19 women, 98.0 ± 2.1 years old; 13 cases previously reported [28–30] and 9 new cases) showed severe cognitive deterioration and were classified clinically as AD according to DSM-III-R criteria. Their admission was motivated by the presence of major behavioral disturbances such as psychomotor agitation, feeding difficulties, marked aggressiveness, delusions of persecution, and suicidal thoughts. Neuropsychological examination performed at least twice during the 6 months prior to death revealed a global decline of higher cortical functions characterized by severe memory impairment, temporal and spatial disorientation, language impoverishment, apraxia, and agnosia in all of the cases. These patients form the AD group of this study. The mean MMSE score of this group at admission was 20.0 ± 1.2, and extended CDR score was 3.6 ± 0.3.

Staining Procedures

The brains were obtained at autopsy (postmortem delay: 3 to 12 hours), fixed in 10% formalin for more than 6 weeks, and cut into 1-cm-thick coronal slices. Following macroscopic examination, tissue blocks were taken from the hippocampal formation including entorhinal cortex, the superior and middle frontal cortex, superior, middle and inferior temporal cortex, and the inferior parietal cortex and midbrain including substantia nigra. For microscopic purposes, paraffin-embedded blocks were cut into 50-µm-thick sections. For diagnostic purposes, tissues were stained with cresyl violet, hematoxylin-eosin, Globus silver impregnation, and modified thioflavine S (37). Subsequently, all cases were evaluated using the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) criteria (38, 39). Estimates of neuron densities were performed on cresyl-violet-stained sections, while the quantitative assessment of NFT and SP was made on adjacent sections stained with highly specific and fully characterized antibodies to the microtubule-associated protein tau and to the amyloid Aβ protein as previously described (28). The anti-tau antibody used in the present study was a polyclonal antibody (961-S28T) raised against a synthetic peptide corresponding to a sequence located in the carboxy terminal of tau protein (serine 400-threonine 429). This sequence contains two putative sites of phosphorylation serine-proline at serine residues 404 and 422 that may be found in paired helical filaments. However, by Western blotting, the immunoreaction labels both normal and abnormally phosphorylated tau proteins. This antibody detects both intracellular and extracellular NFT (40).

Counting Procedures

In order to evaluate the tissue shrinkage effect on neuron, NFT and SP densities, we determined the whole brain weights and measured the cortical thickness in the CA1 field, subiculum, entorhinal cortex, and areas 9 and 20 in all of the cases according to previously described methods (41). Additional measures of laminar thickness were performed in the entorhinal cortex and areas 9 and 20 to see if changes in total cortical width reflect those of individual layers (41). In all of the brains, NFT, SP, and neuron counting was performed in the anterior CA1-3 fields and dentate hilus of the hippocampus, subiculum, layers II and V of the entorhinal cortex, and layers II and III and V and VI of areas 9 and 20. Neuron and NFT densities were estimated using the optical dissector, an unbiased stereological counting method implying that all regions within the structure of interest have an equal chance of being analyzed (i.e. there is no bias in sampling), and that counts do not depend on variables such as the size and shape of neurons (22-24, 42). The technique relies on a three-dimensional counting box located entirely within the tissue section, and objects are quantified by focusing in the section depth (i.e. in the z axis). The fact that the three-dimensional counting box is located within the section and the existence of exclusion (forbidden) planes guarantee that each neuron is counted only once (22-24, 42). Total cell and NFT numbers were not obtained in the present study due to the fact that only the anterior portion of the hippocampus and topographically equivalent samples of areas 9 and 20 were available for analysis. The volume of these samples was variable, rendering comparison of total cell numbers impossible from case to case. For this reason, neuron and NFT densities per mm² were counted in a 1 in 10 series of sections, 500 µm apart, using a Zeiss 63× Plan-Neofluar objective (numerical aperture 1.4). Ten optical dissectors were placed on a random grid covering the area of interest in a manner to provide a systematic sampling paradigm for each region or layer of interest. The numbers of SP were determined for each area and
TABLE 1

Comparison of Cortical Thickness in ND, VMCI and AD Centenarians

<table>
<thead>
<tr>
<th>Area/layers</th>
<th>ND</th>
<th>VMCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>1.60 ± 0.07</td>
<td>1.70 ± 0.08</td>
<td>1.55 ± 0.08</td>
</tr>
<tr>
<td>Subiculum</td>
<td>2.05 ± 0.11</td>
<td>1.97 ± 0.15</td>
<td>2.01 ± 0.11</td>
</tr>
<tr>
<td>Entorhinal</td>
<td>2.34 ± 0.13</td>
<td>2.14 ± 0.10</td>
<td>1.95 ± 0.07*</td>
</tr>
<tr>
<td>20</td>
<td>2.43 ± 0.10</td>
<td>2.57 ± 0.12</td>
<td>2.44 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td>2.24 ± 0.13</td>
<td>2.27 ± 0.13</td>
<td>2.17 ± 0.06</td>
</tr>
</tbody>
</table>

Results represent cortical thickness (mm) in each area. There was a statistically significant difference in cortical thickness between ND and AD cases in the entorhinal cortex. Statistical analysis was performed by analysis of variance with Bonferroni post-hoc correction; * p < 0.05 compared to ND cases.

the average densities per mm² were calculated. Since the number of diffuse BA4 deposits is not correlated with the severity of AD (6, 12), only neuritic plaques as defined in CERAD guidelines were considered in the present study. All analyses were performed using 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). To assess the relationships among NFT and SP densities, neuron counts, and clinical diagnosis, analysis of variance was performed on the 3 diagnosis groups when the global difference was statistically significant. Subsequently, the differences between each pair of groups were assessed by analysis of variance with post-hoc Bonferroni correction. Relationships between neuronal loss and NFT and SP formation in each area and among different cortical areas were evaluated by correlation analysis using Spearman’s coefficient (r).

In addition, a multiple regression analysis model with neuron density as a dependent variable and NFT and SP densities as independent variables was used to adjust the effect of each independent variable with the other included in the model.

TABLE 2

Comparison of Neurofibrillary Tangle Densities in ND, VMCI and AD Centenarians

<table>
<thead>
<tr>
<th>Area/layers</th>
<th>ND</th>
<th>VMCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>1,642 ± 132</td>
<td>1,645 ± 170</td>
<td>3,879 ± 495**</td>
</tr>
<tr>
<td>CA2-3</td>
<td>1,076 ± 280</td>
<td>1,050 ± 211</td>
<td>1,075 ± 356</td>
</tr>
<tr>
<td>Hilus</td>
<td>119 ± 12</td>
<td>123 ± 19</td>
<td>127 ± 23</td>
</tr>
<tr>
<td>Subiculum</td>
<td>811 ± 150</td>
<td>963 ± 165</td>
<td>1,020 ± 180</td>
</tr>
<tr>
<td>Entorhinal II</td>
<td>1,544 ± 106</td>
<td>1,585 ± 117</td>
<td>1,650 ± 122</td>
</tr>
<tr>
<td>Entorhinal V</td>
<td>665 ± 58</td>
<td>735 ± 111</td>
<td>737 ± 103</td>
</tr>
<tr>
<td>20 II-III</td>
<td>640 ± 150</td>
<td>835 ± 198</td>
<td>980 ± 240</td>
</tr>
<tr>
<td>V-VI</td>
<td>300 ± 87</td>
<td>728 ± 137*</td>
<td>835 ± 154**</td>
</tr>
<tr>
<td>9 II-III</td>
<td>117 ± 23</td>
<td>137 ± 55</td>
<td>184 ± 96</td>
</tr>
<tr>
<td>V-VI</td>
<td>123 ± 18</td>
<td>146 ± 22</td>
<td>188 ± 87</td>
</tr>
</tbody>
</table>

Results represent NFT counts/mm²(± SEM) in each area. AD cases had significantly higher NFT densities in the CA1 field compared to both ND and VMCI cases. Moreover, VMCI and AD cases showed significantly higher NFT densities in layers V and VI of area 20 than ND cases. Layers are indicated by Roman numerals. Statistical analysis was performed by analysis of variance with Bonferroni post-hoc correction; * p < 0.05, ** p < 0.01.

RESULTS

Neuropathological Diagnosis

All ND cases were classified as normal, according to the CERAD criteria (38, 39). In the VMCI group, 75% of the cases were characterized as normal, while 25% of cases were judged as having possible AD (age-related plaque score of B and absence of clinical history of dementia). In the AD group, 55% of the cases had an age-related plaque score of B in the most severely affected neocortical area, and were classified as neuropathologically probable AD. The remaining AD cases fulfilled the CERAD criteria of neuropathologically definite AD (age-related plaque score of C and history of dementia). No coexisting pathologic lesions such as Lewy bodies or cerebral infarcts were encountered in the present series.

Brain Weights and Cortical Thicknesses

There was no statistically significant difference in brain weights between ND (1202.7 ± 48.3 g), VMCI (1142.7 ± 31.4 g), and AD (1113.1 ± 22.3 g) cases. In the entorhinal cortex, significant cortical narrowing was present in AD compared to ND cases. Measures of laminar width in layers II and V in this area showed that total cortical thinning reflects that of each of these layers in AD cases. Conversely, no statistically significant difference was found in the other cortical areas between the 3 diagnosis groups (Table 1).

Distribution and Densities of NFT and SP

In the ND group, NFT were found in 100% of cases in the CA1 field and in layer II of the entorhinal cortex, in 90% of cases in layer V of the entorhinal cortex, and in 80% of cases in the subiculum. High NFT densities (> 1000 per mm²) were observed in 60% of cases in the CA1 field, and in 66% of cases in layer II of the entorhinal cortex. All ND cases showed NFT in layers II and...
III of area 20, while 30% of cases had low NFT densities (< 500 per mm²) in area 9 (Table 2). All VMCI cases displayed NFT formation in the CA1 field, subiculum, layers II and V of the entorhinal cortex, and area 20. High NFT densities were found in 72% of the cases in the CA1 field, in 82% of the cases in layers II of the entorhinal cortex, and in 54% of the cases in layers II and III of area 20. In area 9, low NFT densities were seen in 45% of the cases (Table 2). All of the AD cases had NFT in all of the hippocampal subdivisions and in area 20. High NFT densities were found in 90% of the cases in the CA1 field, in 40% in the subiculum, in 70% in layer II, in 28% in layer V of the entorhinal cortex, and in 54% in layer II and III and V and VI of area 20. Area 9 was involved in only 55% of the cases and showed NFT densities lower than 250 per mm². Quantitatively, the number of NFT in the CA1 field was statistically correlated with AD, and VMCI was correlated with the involvement of layers II and III of area 20 (Table 2; Fig. 1).

In the ND group, the CA1 field and subiculum showed mild SP formation (< 10 per mm²) in 50% of the cases, layer II of the entorhinal cortex in 70% of the cases, and layer V of the entorhinal cortex in 90% of the cases. Mild SP formation was also observed in all cases in layers II and III of area 20, and in 80% of the cases in layers II and III of area 9 (Table 3). In the VMCI group, SP were present in all cases in area 20, and in all but two cases in the CA1 field, subiculum, layer II of the entorhinal cortex and area 9 (Table 3). In the AD group, SP were found in 80% of the cases in the CA1 field and subiculum, in 94% of the cases in layer II, and in 70% of the cases in layer V of the entorhinal cortex. They were also consistently present in areas 9 and 20. Quantitatively, layers II and III of areas 9 and 20 showed significantly higher SP densities in AD than in ND and VMCI cases (Table 3; Fig. 2).

**Neuron Densities in the Cerebral Cortex**

Stereological estimation of neuron densities in the present population are summarized in Table 4. No statistically significant difference was found in neuron densities in all areas studied between ND and VMCI cases. In contrast, after correction for cortical narrowing, ND and VMCI centenarians displayed significantly higher neuron densities in layers II and V of the entorhinal cortex and in layers II and III, and V and VI of areas 9 and 20 compared to AD cases (Table 4). In comparison to ND cases, AD cases showed a 30.8% neuronal loss in layer II and a 17.8% loss in layer V of the entorhinal cortex, a 26.7% loss in layers II and III and a 27.5% loss in layers V and VI of area 20, and a 13.7% loss in layers II and III and a 12.1% loss in layers V and VI of area 9. There was no effect of gender on neuron density in the present sample.

**Correlations Between AD Lesions and Neuron Densities**

Paired comparisons revealed no correlation between NFT and neuron densities in each area and among different cortical areas (Fig. 3a, c, e, g). However, SP counts in layers II and III of area 20 were negatively correlated with neuron densities in both layers II and III (r =

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**Fig. 1.** Neurofibrillary tangles in the CA1 field of the hippocampus (a, b, c) and layers V–VI of area 20 (d, e, f) in a nondemented 98-year-old patient (a, d), a 96-year-old patient with very mild cognitive impairment (b, e), and a 99-year-old patient with Alzheimer's disease (c, f). Note the presence of higher neurofibrillary tangle densities in the CA1 field in the patient with AD compared to the other cases. Note also the increase of neurofibrillary tangle density in layers V and VI of area 20 in the case with very mild cognitive impairment compared to the non-demented case. Materials were stained with an antibody against the microtubule-associated tau protein. Scale bar = 50 μm.

**Fig. 2.** Senile plaques in the entorhinal cortex (a, b, c) and layers II and III of area 20 (d, e, f) in a nondemented 99-year-old patient (a, d), a 97-year-old patient with very mild cognitive impairment (b, e), and a 100-year-old patient with Alzheimer's disease (c, f). Note the presence of comparable senile plaque densities in the entorhinal cortex in the three cases. In contrast, the patient with AD displayed a massive senile plaque formation in layers II and III of area 20. Materials were stained with an antibody against the amyloid Aβ protein. Scale bar = 100 μm.

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**Table 3**

<table>
<thead>
<tr>
<th>Area/layer</th>
<th>ND</th>
<th>VMCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>2.3 ± 0.3</td>
<td>3.5 ± 0.6</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>CA2-3</td>
<td>4.2 ± 1.1</td>
<td>5.1 ± 0.4</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Hilus</td>
<td>1.2 ± 0.3</td>
<td>2.0 ± 0.6</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Subiculum</td>
<td>1.7 ± 0.4</td>
<td>2.4 ± 0.9</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>Entorhinal II</td>
<td>4.8 ± 0.3</td>
<td>5.2 ± 0.9</td>
<td>6.9 ± 1.3</td>
</tr>
<tr>
<td>Entorhinal V</td>
<td>1.0 ± 0.2</td>
<td>2.0 ± 0.7</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>20 II–III</td>
<td>7.6 ± 2.2</td>
<td>9.3 ± 2.5</td>
<td>17.6 ± 2.6*</td>
</tr>
<tr>
<td>V–VI</td>
<td>4.3 ± 1.2</td>
<td>5.2 ± 1.5</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>9 II–III</td>
<td>4.9 ± 1.1</td>
<td>7.3 ± 2.5</td>
<td>13.2 ± 2.4**</td>
</tr>
<tr>
<td>V–VI</td>
<td>4.2 ± 0.9</td>
<td>5.6 ± 1.6</td>
<td>7.2 ± 1.3</td>
</tr>
</tbody>
</table>

Results represent SP counts/mm² (± SEM) in each area. AD cases had statistically significantly higher SP counts in layers II and III of areas 9 and 20 compared to ND cases. No statistically significant differences were found in SP densities between ND and VMCI patients. Layers are indicated by Roman numerals. Statistical analysis was performed by analysis of variance with Bonferroni post-hoc correction; * p < 0.05, ** p < 0.05 compared to ND cases.
TABLE 4
Comparison of Neuron Densities Between ND, VMCI and AD Centenarians

<table>
<thead>
<tr>
<th>Area/layers</th>
<th>ND</th>
<th>VMCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>36,781 ± 1,618</td>
<td>36,394 ± 3,679</td>
<td>32,113 ± 3,238</td>
</tr>
<tr>
<td>CA2–3</td>
<td>51,344 ± 3,977</td>
<td>47,975 ± 3,876</td>
<td>51,295 ± 3,495</td>
</tr>
<tr>
<td>Hilus</td>
<td>25,788 ± 1,234</td>
<td>27,245 ± 1,542</td>
<td>28,462 ± 1,780</td>
</tr>
<tr>
<td>Subiculum</td>
<td>36,733 ± 1,254</td>
<td>37,570 ± 2,113</td>
<td>35,938 ± 1,964</td>
</tr>
<tr>
<td>Entorhinal II</td>
<td>39,223 ± 2,237</td>
<td>39,100 ± 1,236</td>
<td>27,102 ± 1,850**</td>
</tr>
<tr>
<td>Entorhinal V</td>
<td>59,767 ± 2,347</td>
<td>37,456 ± 2,131</td>
<td>49,046 ± 2,283*</td>
</tr>
<tr>
<td>20 II–III</td>
<td>71,457 ± 2,451</td>
<td>70,428 ± 3,440</td>
<td>52,009 ± 3,900***</td>
</tr>
<tr>
<td>V–VI</td>
<td>69,064 ± 2,387</td>
<td>68,247 ± 2,890</td>
<td>50,649 ± 3,900***</td>
</tr>
<tr>
<td>9 II–III</td>
<td>59,710 ± 1,030</td>
<td>58,977 ± 1,221</td>
<td>51,647 ± 3,477*</td>
</tr>
<tr>
<td>V–VI</td>
<td>61,890 ± 1,099</td>
<td>63,800 ± 3,011</td>
<td>54,465 ± 2,399*</td>
</tr>
</tbody>
</table>

Results represent neuron counts/mm² (± SEM) in each area. These densities were estimated using the optical dissector method (22, 23, 42). A statistically significant neuronal loss was observed in layers II and V of the entorhinal cortex and in areas 9 and 20 of AD cases. Layers are indicated by Roman numerals. Statistical analysis was performed by analysis of variance with Bonferroni post-hoc correction. *p < 0.05; **p < 0.01; ***p < 0.005 compared to ND and VMCI cases.

−0.51, p < 0.005; Fig. 3f) and V and VI (r = −0.46, p < 0.005) of area 20. There was no correlation between SP densities and neuron numbers in the other cortical areas (Fig. 3b, d, i). The negative correlation between SP and neuron densities in area 20 was further confirmed using a multiple regression model with neuron densities as a dependent variable and NFT and SP densities as independent variables (layers II and III, regression coefficient = −1188, p < 0.005; layers V and VI, regression coefficient = −993, p < 0.01).

DISCUSSION

Regional Distribution of NFT and SP in Centenarians

Our data reveal characteristics of AD lesions in very old patients that differ from those reported for younger elderly AD patients. Earlier studies proposed that NFT densities in the CA1 field, but not in the other hippocampal subdivisions, differ significantly between ND and AD centenarians (27, 29, 31–33). For instance, Hauw and collaborators (33) examined the NFT distribution in the cerebral cortex of 12 centenarians (1 case with AD) and found higher NFT densities in the CA1 field in the demented patient than in cognitively intact centenarians. In their study of 27 nondemented centenarians and younger AD cases, Mizutani and Shimada reported that demented patients had dramatically higher NFT densities in the dentate hilus of the hippocampus, whereas no difference was observed in the entorhinal cortex and the subiculum (34). We found a significant difference in NFT counts in the anterior, but not in the posterior, CA1 field between very old demented and nondemented patients and suggested that nonagenarians and centenarians may show a particular subregional distribution of NFT within the Ammon’s horn (27, 29). Our stereological observations confirm that the presence of high NFT densities in the anterior CA1 field represents a reliable quantitative hallmark of AD in this age group (27–31). Furthermore, the absence of significant difference in NFT densities between ND and VMCI patients suggests that overt clinical signs of dementia, but not isolated memory impairment and disorientation, are associated with a severe damage of the CA1 field in centenarians. Very mild cognitive impairment in this series was associated with substantial NFT formation in layers V and VI of area 20. This is consistent with the frequent involvement of the temporal neocortex reported for younger elderly patients with very mild AD (9, 16–18), and implies that accumulation of NFT in this area may represent a preclinical stage of AD (6, 14, 43). However, the relative preservation of areas 9 and 20 in centenarians with AD is in contrast to the more marked damage in these regions reported for younger demented individuals (6, 9, 13, 16). This finding must be interpreted in conjunction with our previous results showing that AD in very old people is characterized by a spreading of NFT from the anterior CA1 field to neocortical areas 7, 22, 23, and 24, which are relatively preserved at the early stages of the degenerative process (27). Altogether these observations suggest that the regional vulnerability to NFT formation changes substantially after 90 years of age in that the involvement of

Fig. 3. Correlation between NFT (per mm²) or SP densities (per mm²) and neuron densities (per mm²) in select cortical areas (a, b, CA1 field; c, d, layer II of the entorhinal cortex; e, f, layers II and III of area 20; g, h, layers II and III of area 9). There was no statistically significant correlation between NFT and neuron densities (a, c, e, g). A statistically significant correlation between SP and neuron densities was observed only in layers II and III of area 20 (f). Note differences in the scale of the histograms. Layers are identified by Roman numerals.

inferior temporal cortex may account for very mild cognitive impairment. AD symptomatology requires both high NFT densities in the anterior CA1 field and progressive damage of several neocortical areas. The neuropathological distinction between very old and younger people is further supported by the dissociation between NFT and neuron densities in all the areas studied in the present series, indicating that neuronal loss unrelated to the presence of NFT is the rule in very old people, in contrast to elderly subjects younger than 85 where a correlation was observed (24, 44).

Unlike earlier findings in younger cohorts (6, 16–18, 20, 21), SP were found in comparable densities in ND and VMCI centenarians, indicating that their formation within the cerebral cortex is not associated with the early stages of cognitive decline in this age group. Conversely, SP density in layers II and III of areas 9 and 20, but not in the CA fields, subiculum and entorhinal cortex, is correlated with overt clinical signs of AD. Moreover, there is a negative relationship between SP densities and neuron counts in area 20, indicating that SP formation may parallel neuronal depletion within certain cortical regions in centenarians. This confirms previous studies demonstrating that SP formation in the neocortex is correlated to the severity of dementia in centenarians (27, 28). These data differ from those reported for younger cases (6, 9, 10, 12, 15, 19) and suggest that SP may be a useful hallmark of the evolution of AD in very old patients. However, it is worth noting that most AD cases displayed only mild to moderate SP scores in neocortical areas. Consequently, although all of our AD cases showed clinical and neuropsychological features of severe dementia, only 45% of them fulfilled the CERAD criteria for definite AD (38, 39). These data show that the distinction between CERAD categories of probable and definite AD is difficult to establish in very old people, and suggest that alternative diagnostic criteria, including NFT densities in the anterior CA1 field and areas 7, 22, 23, and 24 may be valuable in this particular age group.

Patterns of Neuronal Loss in the Very Old

The absence of AD-related neuronal loss in the CA fields and subiculum of centenarians is the most intriguing finding of the present study. West and collaborators (22) reported a neuronal loss of 68% in the CA1 field and 47% in the subiculum in AD cases younger than 88, and postulated that neuronal depletion in the CA1 field may allow differentiation between normal aging and AD. The present results suggest that this may not be the case in very old people, since our data showed a 7.5% lower cell loss in the CA1 field. Although the relative preservation of neuron densities in this area might not reflect normal total neuron number because of diminished cortical volume, this is a very unlikely scenario since there was no difference in cortical thickness in the CA1 field among our diagnosis groups. The neuronal loss observed in layer II and V of the entorhinal cortex and areas 9 and 20 in AD centenarians parallels earlier observations in younger demented individuals (7, 24–26), and implies that decreasing neuron densities in these areas may be critical to developing AD in any age group (25, 26, 45). In particular, our data for the entorhinal cortex are partly consistent with a recent report by Gómez-Isla and collaborators who estimated, using stereological methods, total neuron numbers in the entorhinal cortex of patients younger than 95 years (24). In their study, the number of neurons in layer II of the entorhinal cortex decreased by 60% in patients with CDR 0.5 and by 90% in severe AD cases. In contrast, we found that VMCI centenarians had preserved neuron densities in the entorhinal cortex, indicating that isolated memory impairment after 95 years is not necessarily associated with neuronal depletion in this area. Moreover, the magnitude of neuronal loss in AD centenarians is significantly lower than that reported in younger AD cases (24, 25), suggesting that a mild decrease in neuron numbers in cortical regions such as the entorhinal cortex and areas 9 and 20 may be sufficient to cause dementia after 95 years of age (46). However, these conclusions must be drawn with caution since the differences observed may be due to the more rigorous counting paradigm used by West, Gómez-Isla, and their collaborators (22, 24), who were able to count total neuron numbers through the hippocampus.

Clinicopathologic Considerations

Our observations strengthen the hypothesis that very old people represent a neuropathologically, clinically, and possibly genetically distinct subgroup (1–5). Ritchie and Kildes (1) showed that the increase in prevalence of dementia levels off at age 95, with a prevalence of only 40%. In their epidemiological study of 1,694 patients who met criteria for probable or definite AD, Lausenschlager and colleagues (2) reported that the risk of AD decreases significantly after age 90, and postulated that AD may not be an inevitable pathology associated with brain aging. Moreover, a lack of association between AD and apolipoprotein E allele e4, a major risk factor for late-onset AD, has been demonstrated in centenarians (3–5). Although these findings could reflect the fact that younger individuals are at higher risk to develop AD, the present observations as well as previous studies of the neuropathology of advanced aging strongly suggest that very old individuals display differential neuronal aging and susceptibility to the degenerative process of AD (27–34). In this respect, the absence of significant neuronal loss in CA1 field as well as the low rate of NFT formation in areas 9 and 20 in centenarians with AD may be related to a genetically determined resistance of these neuronal subpopulations in very old people. Moreover,
the dissociation between neuron and NFT densities suggest that NFT-independent mechanisms of neuronal loss in the neocortex are of major importance in the course of AD in this age group. Molecular genetic and biochemical analyses will be necessary to precisely characterize the features of brain aging in very old people.

ACKNOWLEDGMENTS

We thank Dr E A Nimmichsky for advice on stereology, Drs N K Robakis and A Delacourte for generous provision of antibodies to Aβ and tau proteins, and Drs J-P Michel and J Richard for providing the postmortem materials. We also wish to thank M Surini and P-Y Vallon for expert technical assistance.

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Received May 20, 1996
Revision received August 23, 1996
Accepted September 4, 1996