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Alzheimer Neuropathologic Alterations in Aged Cognitively Normal Subjects

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Abstract. The histopathologic changes distinguishing early Alzheimer disease (AD) from normal or pathologic aging are not clearly defined. This report describes the autopsy findings of 59 elderly, well-educated, volunteers. They were examined longitudinally with mental status testing, some for up to 8 years, as part of our normal aging study. This study reveals that (1) the brains of many subjects who did not show cognitive impairment on neuropsychologic testing contain abundant senile plaques (SP) and/or neurofibrillary tangles (NFT); (2) 29 subjects met Khachaturian criteria for AD, 15 met CERAD and 7 met National Institute on Aging-Reagan Institute guidelines; (3) Braak and Braak staging method included 9 in stage IV subjects, 4 in stage V, and 1 in stage VI; (4) there was a progression of NFT from entorhinal cortex to hippocampus and amygdala as a function of age; (5) 2 subjects met criteria for a diagnosis of dementia with Lewy bodies but were not demented; (6) cerebral amyloid angiopathy was present in leptomeningeal vessels in 75% of subjects and in parenchymal vessels in 62% of subjects; (7) only 10 of 59 subjects (17%) had no or few degenerative brain changes. Our study demonstrates that the brains of a large percentage of cognitively normal, relatively well-educated individuals contain numerous degenerative changes and only a small percentage are relatively free of these changes.

Key Words: Alzheimer; Argyrophilic grains; Cognitively normal; Dementia; Neuropathology; Neuropsychologic; Vascular lesions.

INTRODUCTION

Distinguishing the histopathologic alterations that differentiate Alzheimer disease (AD) from normal brain aging is critical to understanding AD and the aging process. In AD, there are abundant neurofibrillary tangles (NFT) and senile plaques (SP) in the entorhinal cortex, hippocampus, and cerebral neocortex. Two histopathologic guidelines for the diagnosis of AD are presently used (1, 2); both emphasize the presence of neuritic plaques (NP) and 1 places emphasis on NFT in medial temporal lobe structures and neocortex (2). Numerous autopsy studies demonstrate that NFT and SP of variable densities are present in nondemented subjects in brain regions most involved in AD, although the true significance of these findings to aging and AD are unresolved (3–17). These changes in nondemented individuals have been referred to by some as pathologic aging (6) or presymptomatic AD (10).

Only a modest number of autopsy studies of normal elderly subjects who were prospectively studied have been published (3–5, 8–10, 17). One study of 21 longitudinally followed elderly subjects found that 9 subjects had high neocortical SP density and hippocampal and entorhinal NFT (10). Seven of the subjects had a Clinical Dementia Rating Scale score of 0.5 and were thought to have presymptomatic or incipient AD. Three other studies of prospectively evaluated nondemented subjects described the presence of variable numbers of neocortical NP (8, 17, 18). Entorhinal and hippocampal NFT have been described in variable numbers in these studies of prospectively evaluated nondemented subjects (8, 17). Some elderly nondemented subjects have few or no SP or NFT. Thus, there is a spectrum of change in nondemented elderly from none or few to abundant neocortical diffuse SP, a few with rare or moderate numbers of neocortical NP, and others with moderately abundant entorhinal and hippocampal NFT. However, the question of whether NFT in medial temporal lobe structures are related to normal aging or represent presymptomatic AD is not clear. This report describes the neuropathologic features of 59 elderly individuals who were followed, some for up to 8 years, with longitudinal mental status testing as part of our normal aging study.

MATERIALS AND METHODS

Since 1989, 499 aged, cognitively and neurologically normal volunteer subjects have been prospectively followed by the Sanders-Brown Center on Aging at the University of Kentucky. The control volunteers were recruited from a pool of 4,500 community residents over 60 years of age. Most were contacted using an introductory letter followed by visitation with a center staff member. Other subjects came to the program following articles in the press and broadcast news media. The participants agreed to annual mental status testing, telephone interviews every 6 months, biennial physical and neurological examination, Apo-E testing, and donation of their brain at death. The group consists of 317 (63.5%) females and 182 (36.5%) males. The age range at recruitment was 60–98 years and the average age was 75.1 years. Seventy subjects have died and 60 were autopsied. One had clinically and pathologically proven AD and was excluded from the analysis. The annual mental status tests administered in the study include the following: Iowa Dementia

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TABLE 1
Mean (SD) Mental Status Scores for Control Subjects

Measure	Mean (SD)	Measure	Mean (SD)
Age at autopsy	83.9 (7.4)	Mini-Mental State Exam (MMSE)	28.0 (1.9)
Education (years)	16.3 (2.6)	*Memory*:	
Test to autopsy interval (months)	8.0 (5.2)	List learning (max 30)	18.6 (4.5)
Postmortem interval (hours)	3.5 (3.3)	Delayed recall (max 10)	5.8 (2.3)
Brain weight (gm)	1,227.8 (97.7)	Memory savings	90.9% (29.0)
		Wechsler logical memory	26.1 (7.8)
Language:		*Other*:	
Animal naming	14.6 (4.7)	Trailmaking A (sec)	58.1 (38.9)
Verbal fluency	34.6 (11.5)	Temporal orientation	0.9 (2.5)
Short Boston naming (max 15)	14.1 (2.1)	Wechsler mental control	6.7 (1.5)

Number of assessments	Number of cases	Number of sample
1	7	12%
2	9	15%
3	4	7%
4	10	17%
5	14	24%
6	8	14%
7	4	7%
8	3	5%

Clinical data, mental status scores and frequency of neuropsychologic evaluations in control subjects.

Screening Battery (Temporal Orientation, Benton Visual Retention, and Verbal Fluency); The Washington University Battery (Wechsler Memory, Logical Memory and Mental Control, Verbal Fluency, Trailmaking); Mini Mental State Examination (MMSE); Memory Information Test (MIT); Boston Naming Test; abbreviated Alzheimer Disease Assessment Scale (word list learning); and Memory Savings. A summary of the mental status scores and the number of evaluations is presented in Table 1. For some analyses, subjects were separated into 3 age groups by years: < 79 ($n = 19$), 80 through 90 ($n = 28$), and > 90 ($n = 12$). A clinical chart review examining subjects for a history of heart disease, hypertension, diabetes, endocrine abnormalities, head trauma, malignancy, and other chronic disease states is summarized in Table 2. None of our subjects had epilepsy or acquired immunodeficiency disease. Educational level attained for the 59 autopsied subjects ranged from 8–20 years (mean = 16.34 ± 2.58 years). Twenty-eight males and 32 females, whose ages ranged from 69–100 years (mean 84 years), died during the study interval. Full postmortem examinations were available for only a small subset of the group. Postmortem interval before autopsy ranged from 1.7–23.4 hours with an average of 3.5 hours and a median of 2.6 hours (only 2 subjects had postmortem intervals greater than 6 hours). The brains were removed in a standard manner after ventricular cerebrospinal fluid sampling. Brain weights ranged from 1020–1500 g (mean

TABLE 2
Medical Information Summary on Control Subjects

Disorder	Number of cases	Percent of cases
Heart disease		
Valvular disease	1	1.6%
Hypertension	15	25%
Myocardial infarction	11	18%
Angina w/o cardiac infarct	14	23%
Congestive failure	5	8%
Diabetes	4	6%
Hypothyroidism	11	18%
Head trauma	3	5%
Lymphoma	1	1.6%
Leukemia	1	1.6%
Multiple myeloma	2	3%
Carcinoma	5	8%
No chronic disease	9	15%

Note: Subjects often manifested more than 1 disorder.

Medical Information Summary. Ischemic cardiac disease and hypertension present in a large proportion of the subjects. No subjects had epilepsy or acquired immunodeficiency syndrome (AIDS).

1228 g, ± 98 g). The left cerebral hemisphere was sectioned and used for biochemical, trace element, and histologic analyses. Gross alterations in the left cerebral hemisphere were determined at the time the brain was sectioned at autopsy. Sections adjacent to regions used for experimental studies were taken as below. The right cerebral hemisphere, brainstem, and remaining cerebellum were fixed in 4% neutral buffered formalin for 7–10 days before sectioning. The following sections were taken for microscopic examination: frontal pole (Brodmann areas 10–11); middle frontal gyrus (area 9); temporal pole (area 38); superior and middle temporal gyri (areas 21–22); inferior parietal lobule (areas 39–40); anterior and posterior cingulate gyrus; occipital association area (areas 18–19); hippocampus; entorhinal cortex; amygdala; neostriatum and nucleus basalis of Meynert; thalamus; midbrain; pons; medulla; cerebellar vermis; and dentate nucleus. All sections were examined using hematoxylin and eosin. Neocortical, hippocampal, entorhinal, and amygdala sections were examined using the Bielschowsky stain and 10D-5 (Athena Neurosciences, San Francisco, CA) immunohistochemistry for β-amyloid. In addition, hippocampus, entorhinal cortex, and amygdala were examined using Gallyas silver stained sections. Meningovascular and intraparenchymal vascular amyloid (VA) was graded by the method of Vonsattel et al (19) and a VA score was obtained using manual counts of blood vessels displaying β-amyloid as follows: no affected vessels = 0; 1–2 affected vessels = sparse; 3–5 affected vessels = moderate; 6 or more affected vessels = frequent. Neocortical sections were examined with ubiquitin immunohistochemistry if Lewy (LB) or pale bodies were identified in substantia nigra or locus ceruleus. Vascular lesions were divided into gross (infarcts visible to naked eye) and/or microscopic, acute (less than 2 months of age), or remote, and counted in each section. Diffuse neuron loss and gliosis were semiquantitatively graded as zero, mild, moderate, or severe in degree. Neurofibrillary tangles and SP, which were separated as diffuse plaques (DP) or neuritic plaques (NP), were quantitated by manual counts of 5 microscopic fields in the most severely affected cortical region. Neurofibrillary tangles were counted using a 20x objective and plaques using a 10x objective. The presence or absence of LB, balloon cells, Pick bodies, and argyrophilic grains was recorded. Braak and Braak histopathologic staging (20, 21) (hereafter referred to as Braak staging) was performed on all cases using Gallyas-stained sections of the entorhinal cortex, hippocampus, and amygdala in conjunction with the Bielschowsky silver-stained sections. The subjects were divided into 4 Braak stages (0, I-II, III-IV, and V-VI) for statistical comparison with mental status test scores. Each case was classified using the AD diagnostic guidelines of Khachaturian (22), CERAD (1), and National Institute on Aging-Reagan Institute (NIA-RI) (2). Data were compiled into an electronic database (Access® Microsoft) and spreadsheet (Excel®, Microsoft). Statistical analysis was performed using Abstat® 7.01 and SAS® (SAS Institute). The following statistical methods were used: one-way analysis of variance with Newman-Keuls post hoc tests, independent *t*-test, regression, Chi-square, and correlations. Significance was set at $p < 0.05$. Bonferroni correction was applied to multiple comparisons.

RESULTS

One of the 60 subjects developed progressive cognitive decline and the clinical evaluation led to a diagnosis of AD. At autopsy the histopathologic changes in the brain met all the major guidelines for the diagnosis of AD. This subject was excluded from the analysis. A broad spectrum of SP and NFT densities was present in the remainder of study subjects. Brain weight at autopsy was mildly correlated with age at death ($r = -0.29$, $p < 0.05$) and MMSE score ($r = 0.29$, $p < 0.05$). However, no other measures were associated with brain weight and the correlations between brain weight and the MMSE as well as age at death were not significant once Bonferroni corrections were applied for the multiple comparisons.

Effect of Systemic or Chronic Disease and Head Trauma

Forty-two percent of the subjects (n = 25) had a clinical history of ischemic heart disease with or without myocardial infarction. In addition, 25% (n = 15) of subjects had a clinical history of hypertension. Statistical evaluation used *t*-test, regression and Chi-square examination of these subgroups and revealed no predictive value to the presence or absence of hypertension or ischemic cardiovascular disease related to NFT or SP. The scant incidence of the remaining medical conditions including head trauma and malignancy in the subject group did not allow for statistical analysis.

Neurofibrillary Tangles

Neurofibrillary tangles were present in variable numbers in 51 out of 59 (86%) subjects. Ten subjects did not have significant entorhinal NFT and were equivalent to Braak stage 0; 11 subjects did not have hippocampal NFT. Neurofibrillary tangles were most abundant in the hippocampal CA1, entorhinal cortex, and basolateral amygdala in our subjects (Fig. 1). There was a statistically significant increase in mean NFT density in the CA1 region and the amygdala in the tenth decade compared with the eighth and/or ninth decade ($p < 0.01$) but not between the eighth and ninth decades (Fig. 1). The mean NFT density was highest in the eighth decade in the entorhinal cortex and did not exhibit a significant change in the next 2 decades. Twenty-six subjects (44.1%) displayed no neocortical NFT. Of the 33 (55.9%) subjects with neocortical NFT, mean densities were highest in the temporal neocortex and lowest in occipital association areas. No significant age group differences were found in the 3 decades studied. Mini-Mental State Examination performance correlated with NFT formation in only the hippocampal CA1 region ($r = 0.43$).

Diffuse Plaques

Diffuse senile plaques occurred in the neocortex and/or allocortex in 46 of 59 (78%) subjects. In the neocortex, mean DP densities were most abundant in middle frontal gyrus and inferior parietal lobule and least common in the occipital association areas (Fig. 2). In the allocortex,

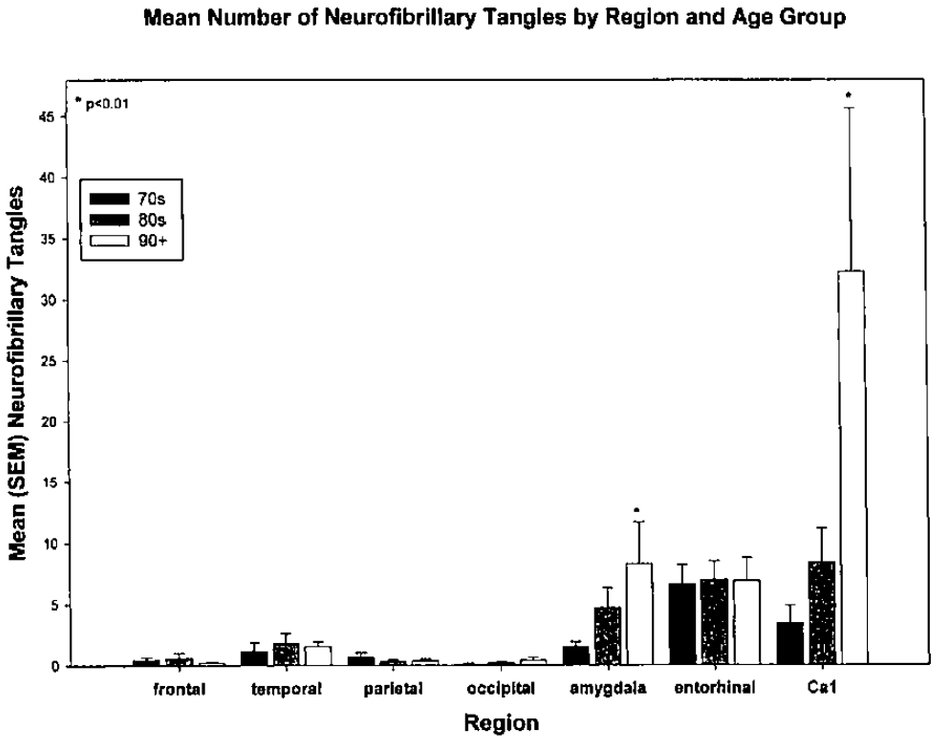

Fig. 1. Mean count of neurofibrillary tangles (NFT) averaged in neocortical regions and medial temporal lobe structures. NFT counts performed using 20× objective (field size 0.74 mm²). Subjects were divided into 3 age groups: <79 years, 80–89 years, and >90 years. Error bars represent standard error of the mean (SEM).

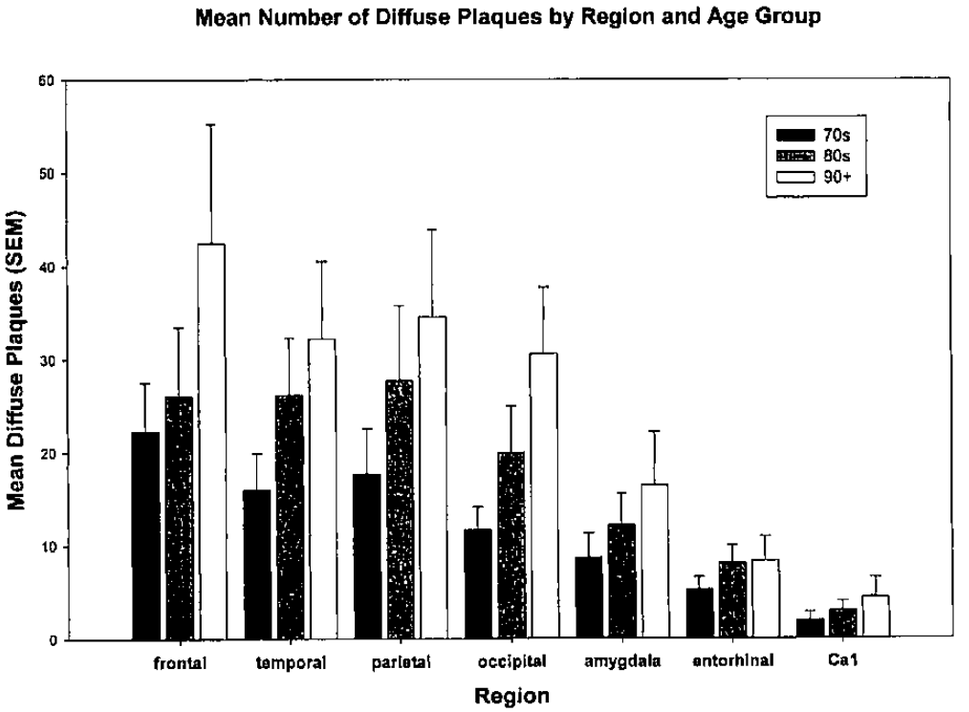

Fig. 2. Mean counts of diffuse plaques (DP) averaged in neocortical regions and medial temporal structures. DP counts performed using 10× objective (field size 3.08 mm²). Subjects were divided into 3 age groups: <79 years, 80–89 years, and >90 years. Error bars = SEM.

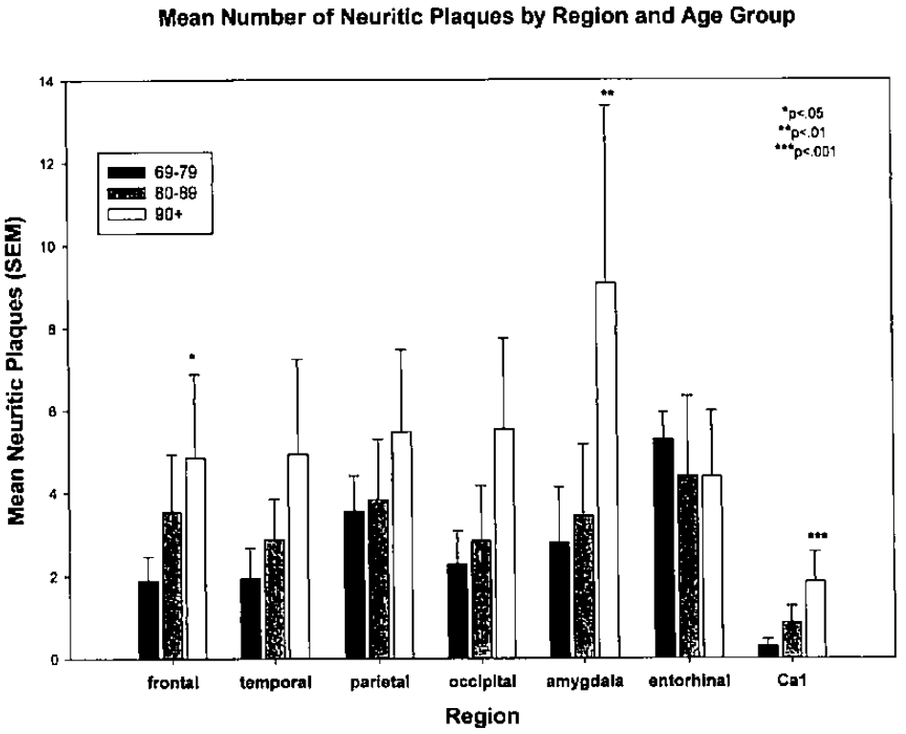

Fig. 3. Mean counts of neuritic plaques (NP) averaged in neocortical regions and medial temporal structures. NP counts performed using 10× objective (field size 3.08 mm^2). Subjects were divided into 3 age groups: <79 years, 80–89 years, and >90 years. Error bars = SEM.

DP densities were most abundant in the entorhinal cortex. Thirty subjects (51%) lacked DP in CA1, whereas only 13 (22%) did not have neocortical DP. There was a gradual increase in DP density over the 3 decades studied, which was statistically significant only when comparing the occipital lobe in the tenth decade with the eighth decade.

Neuritic Plaques

Forty-one of 59 (69%) subjects had at least 1 NP in at least 1 neocortical or allocortical section. Mean NP densities were highest in the basolateral amygdala and entorhinal cortex (Fig. 3). Mean NP densities were significantly increased in the tenth decade compared with the eighth and ninth decades in the amygdala ($p < 0.01$), hippocampal CA1 ($p < 0.001$), and frontal region ($p < 0.05$) (Fig. 3).

Braak Staging

Using the Braak and Braak staging method (20, 21), our subjects were classified as follows: Stage 0 (n = 10), Stage I (n = 15), Stage II (n = 12), Stage III (n = 7), Stage IV (n = 9), Stage V (n = 5), and Stage VI (n = 1). As expected, mean NFT densities increased in the entorhinal cortex, hippocampal, CA1 and amygdala with increasing Braak stages (Fig. 4). Although the neocortex contained only small numbers of NFT, mean densities increased with higher Braak stages in frontal, temporal, and parietal regions and reached statistical significance in comparing stage V with 0, I, II, and III in frontal lobe ($p < 0.01$). Similarly, NP counts increased in the entorhinal cortex, CA1, amygdala, and all 4 neocortical regions with increasing Braak stages and reached statistical significance in all regions ($p < 0.01$) except for CA1 and the amygdala (Fig. 5). In general, mean DP densities increased in all neocortical and allocortical regions with increasing Braak stages, but none of these increases were statistically significant. The mean MMSE score for subjects with Braak stages were as follows: stage 0 was 28.2 ± 1.2, stages I–II was 28.6 ± 1.3, stages III–IV was 26.5 ± 2.4, and stage V/VI was 26.8 ± 3.9. There was a significant difference in the MMSE scores between Braak stages I–II and III–IV ($p < 0.01$). Post hoc tests for group differences disclosed significant differences between stage 0 and V–VI as well as between stage I–II and V–VI ($p < 0.05$).

Alzheimer Disease Diagnostic Classifications

Khachaturian (22), CERAD (1), and NIA-RI (2) AD neuropathologic diagnostic guidelines were applied to all cases. The following number of cases were diagnosed as having AD with each: Khachaturian (n = 29), CERAD

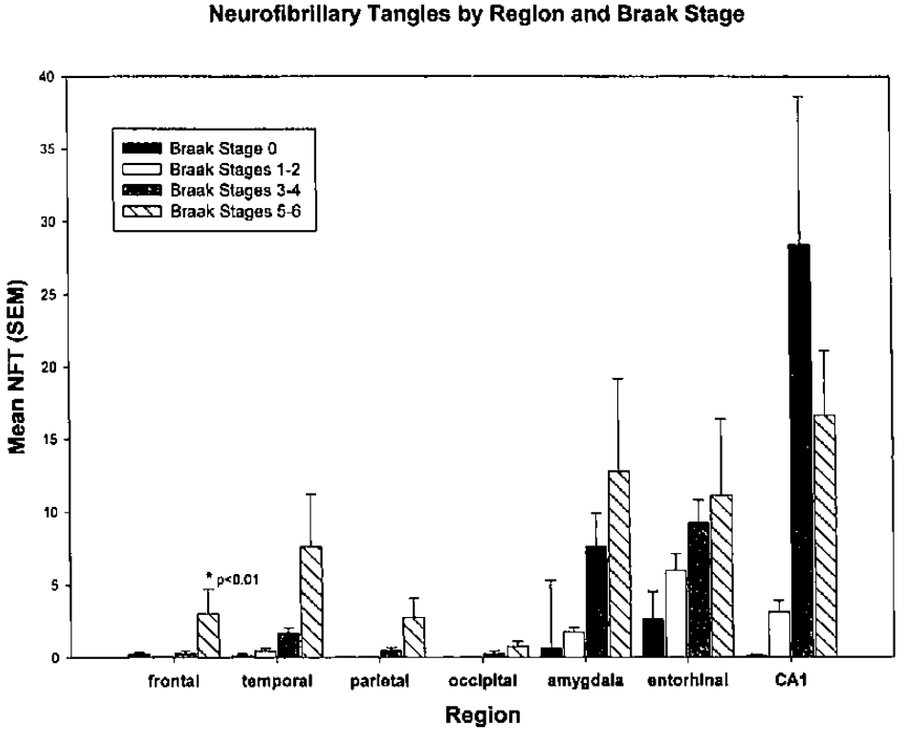

Fig. 4. Mean neurofibrillary tangle (NFT) counts in neocortical regions and medial temporal lobe structures were compared between Braak stage groups. Subjects were separated into 4 groups using Braak staging. NFT counts performed using 20× objective (field size 0.74 mm²). Error bars = SEM.

(n = 15), and NIA-RI (n = 7) (Table 4). By definition, all subjects meeting NIA-RI guidelines had abundant hippocampal NFT and some neocortical NFT. Of the 7 subjects who met NIA-RI guidelines, 5 had MMSE scores of 28 or 29, 1 had a score of 27, and 1 100-year-old subject scored 25. All subjects who met NIA-RI guidelines also met CERAD and Khachaturian guidelines. Of the 7 subjects who met CERAD, but not NIA-RI guidelines, 5 had MMSE scores of 28 to 30, 1 had a score of 26, and a 96-year-old had a score of 21.

Lewy Bodies

Three subjects had brainstem and neocortical LB. In 2 of these, the neocortical density of LB was greater than 5 inclusions per neocortical region, compatible with the neocortical category outlined by the consensus guidelines for the pathologic diagnosis of dementia with LB (23).

Argyrophilic Grains

Fourteen subjects (23%) demonstrated argyrophilic grain formation in entorhinal cortex, hippocampal CA-1, or amygdala. Mini-Mental State Exam scores in 12 of these patients were at 28 or above and the remaining 2 scored 27 and 25. No statistical differences could be attributed to the presence of argyrophilic grains in our subjects.

Vascular Amyloid

Vascular amyloid was present in the subarachnoid blood vessels in 75% of the subjects, intraparenchymal blood vessels in 62%, both locations in 62%, and absent in either site in 23%. The frequency and severity of VA deposition was most pronounced in the occipital lobe, and least in hippocampal CA1. It was similar in frequency and severity in the frontal, parietal, and temporal lobes. In the occipital lobe, meningeal VA deposits occurred in 40 (68%) subjects with 24 (41%) subjects having 6 or more blood vessels involved. Parenchymal VA deposits occurred in 32 (54%) subjects in the occipital lobe with 11 (19%) subjects having 6 or more blood vessels involved. There were significant positive relationships between parenchymal VA deposition and DP ($p < 0.05$), NP ($p < 0.01$), and meningeal VA deposits and DP ($p < 0.01$) in the occipital lobe (Fig. 6A, B). Similar significant positive relationships were present between parenchymal VA deposition and DP in the temporal lobe ($p < 0.05$) and frontal lobe ($p < 0.01$), and parenchymal deposits and NP in

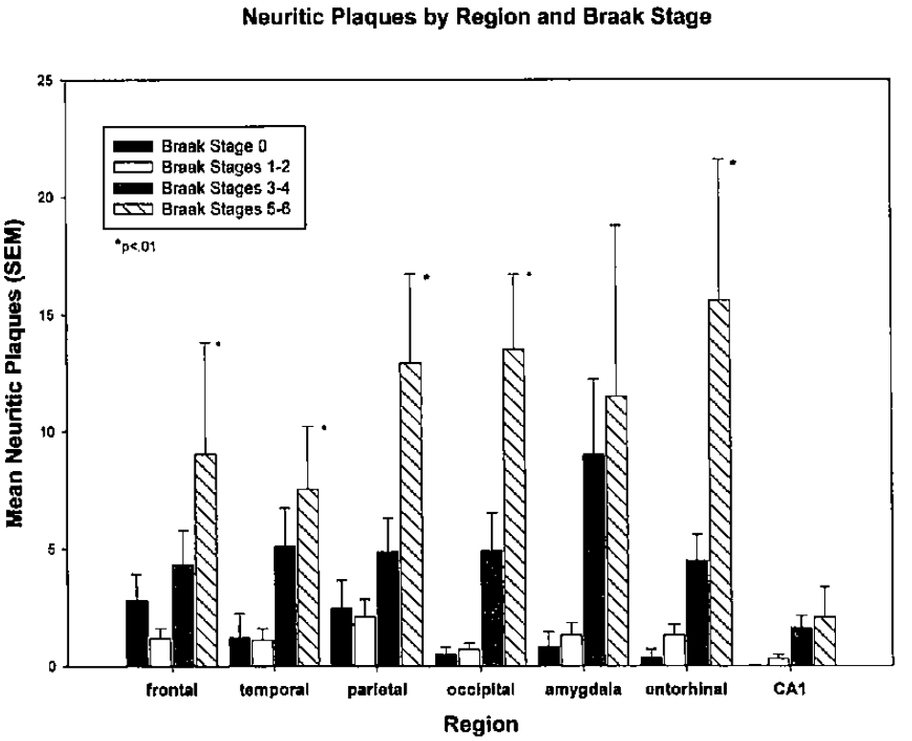

Fig. 5. Mean neuritic plaque (NP) counts in neocortical regions and medial temporal lobe structures were compared between Braak stage groups. Subjects were separated into 4 groups using Braak staging. NP counts performed using 10× objective (field size 3.08 mm²). Error bars = SEM.

TABLE 3
Neuropathologic Summary of non-AD Changes in Subjects

	Number of cases	Percent of cases
Lewy bodies	5	8%
Argyrophilic grains	14	23%
Gross infarcts		
Frontal	3	5%
**Temporal	3	5%
**Parietal	4	6%
**Occipital	4	6%
Neostriatum or thalamus	10	17%
Cerebellum	2	3%
Hippocampal sclerosis	2	3%

Neuropathologic Summary of non-AD Changes in Subjects. ** Because some of the infarcts overlapped contiguous lobes, the number of cases in the temporal lobe includes 3 cases listed in parietal lobe tally. The number of cases in the occipital lobe includes 2 cases listed in the parietal lobe tally. The number of cases in the occipital lobe includes 1 case listed in the temporal lobe tally. Infarcts in neostriatum and thalamus were lacunar. Microinfarct data are summarized in Figures 7a and b.

TABLE 4
Frequency of Alzheimer Disease Using Different Neuropathologic Diagnostic Guidelines

Guidelines met?	Freq	%
Khachaturian guidelines		
No	30	50.8
Yes	29	49.2
Total	59	100.0
CERAD guidelines		
No	44	74.6
Yes	15	25.4
Total	59	100.0
NIA-REAGAN Institute guidelines		
No	52	88.1
Yes	7	11.9
Total	59	100.0

Classification of 59 cognitively "normal" subjects using Alzheimer disease histopathologic classification guidelines of Khachaturian, CERAD and NIA-RI.

the temporal lobe ($p < 0.05$) Of cases with meningovascular amyloid, 42% demonstrated microinfarcts, whereas 57% of cases lacking VA had microinfarcts.

Infarcts, Gross and Microscopic

Grossly detectable infarcts in these subjects were limited (Table 3) and not of statistical significance in this study. Microinfarcts were present in a preponderance of subjects (61%) and were most often chronologically remote. Microinfarcts were most common in occipital lobe,

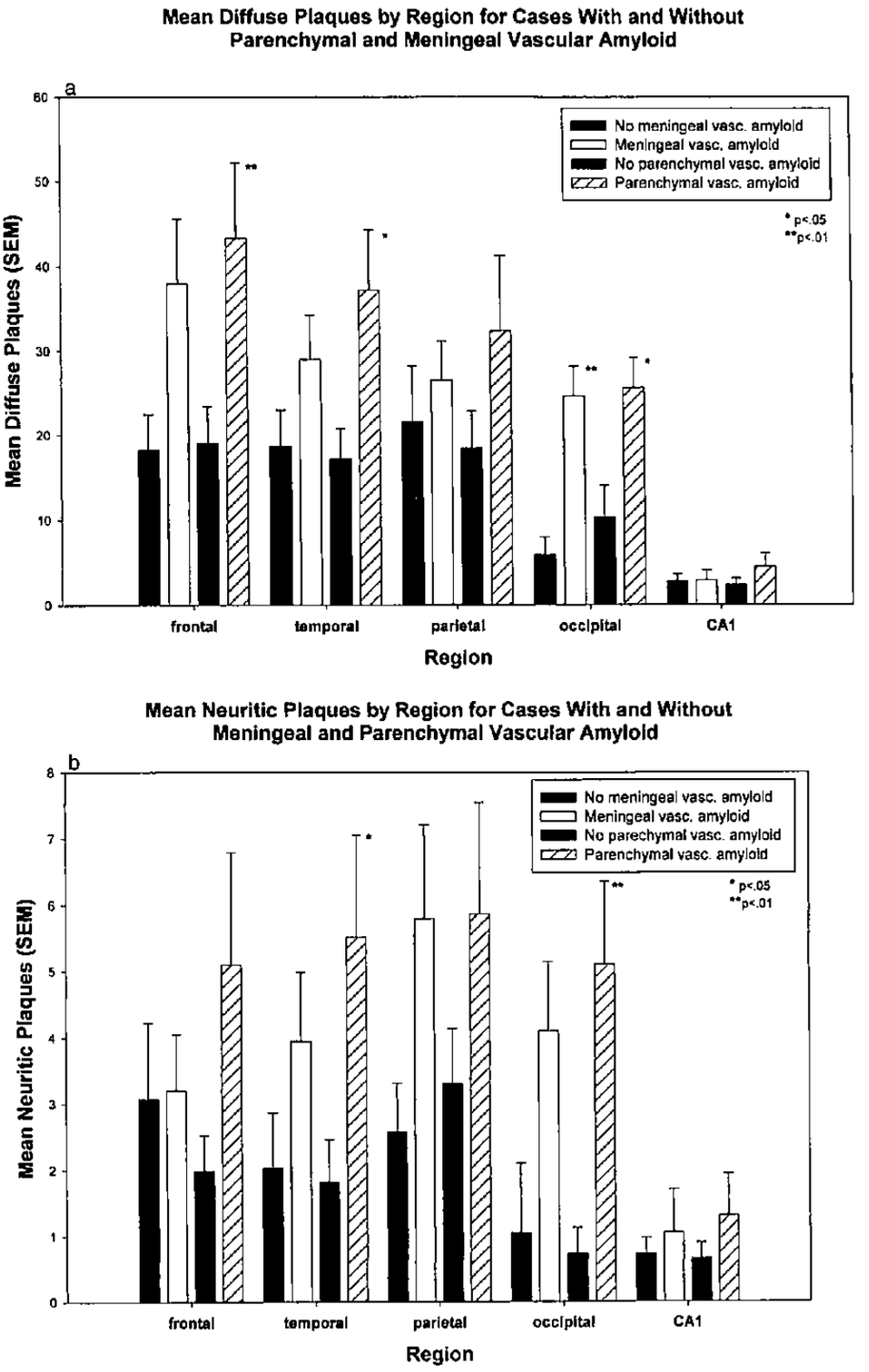

Fig. 6. (a) Mean number of diffuse plaques by region in subjects with and without either meningeal or parenchymal vascular amyloid. Diffuse plaque counts performed using 10× objective (field size 3.08 mm²). Error bars = SEM. (b) Mean number of neuritic plaques found regionally in subjects with and without either meningeal or parenchymal vascular amyloid. Neuritic plaque counts performed using 10× objective (field size 3.08 mm²). Error bars = SEM.

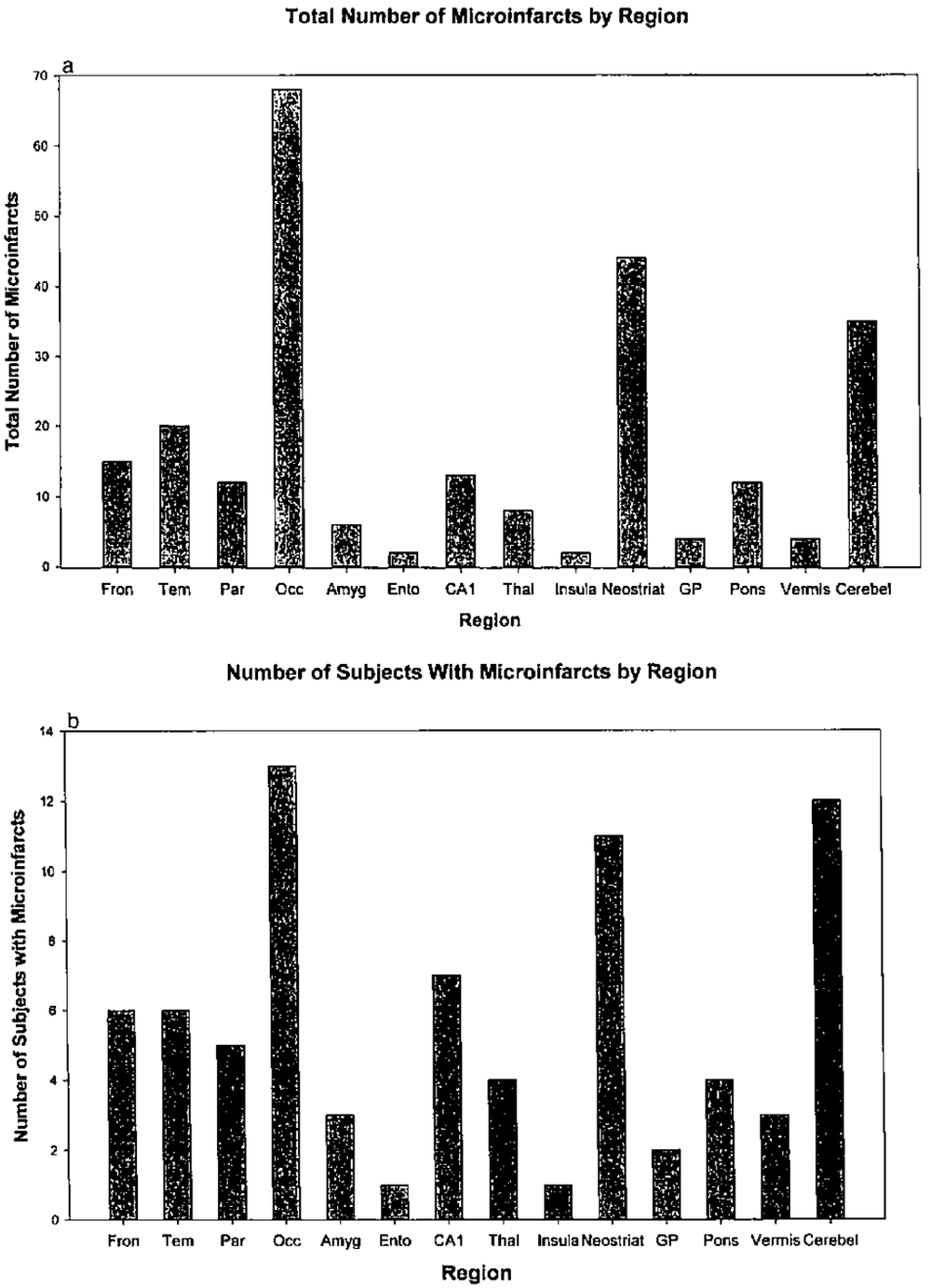

Fig. 7. (a) Total number of microinfarcts identified in each brain region, totaled across all subjects. Fron = frontal, Tem = temporal, Par = parietal, Occ = occipital, Amyg = amygdala, Ento = entorhinal, Thal = thalamus, Neostriat = neostriatum, GP = globus pallidus, Cerebel = cerebellum. (b) Total number of subjects with a microinfarct in a specific brain region. Abbreviations same as in Figure 7a.

neostriatum, cerebellum and temporal lobe (Fig. 7A) in descending order frequency when site affected was totaled across all cases. Examining the number of subjects manifesting infarcts in a specific location resulted in a reordering of the descending frequency to occipital, cerebellum, neostriatum, and hippocampal CA1 (Fig. 7B). Although there was a tendency for the presence of microinfarcts to be associated with a lower MMSE score

(no microinfarcts: MMSE = 28.3 ± 2.1; microinfarcts present: MMSE = 27.8 ± 1.7), it was not statistically reliable ($p = 0.33$).

Apolipoprotein E Genotyping

Apolipoprotein genotype testing in 57 subjects demonstrated the following distribution: 1 (2/4), 15 (3/4), 35 (3/3), and 6 (2/3). No genotype 4/4 subjects were found.

DISCUSSION

This study reports the neuropathologic results of 59 cognitively normal subjects who had annual mental status testing over a 1- to 8-year-period. Several features stand out in our study. Ten normal subjects had essentially no neocortical NFT, few or no SP, and/or few hippocampal and entorhinal NFT or SP. These individuals' ages ranged from 70–89 years (mean 76.5 years). They were all Braak stage 0 and their MMSE scores ranged from 27 to 30. The mean brain weight of these cases was 1262 g. Two were heterozygous for ApoE-ϵ4 alleles, 1 was ApoE-ϵ 2/3, and the remainder were 3/3. These individuals were cognitively intact, did not exhibit pathologic aging, or presymptomatic AD, and thus, could serve as controls for comparative study with AD subjects.

Our study also revealed that the brains of many subjects contained abundant SP and/or NFT, yet they did not show cognitive impairment on mental status testing. Twenty-nine (49%) of the subjects met the Khachaturian guidelines for the histopathologic diagnosis of AD (22), mainly because of the presence of abundant DP in the neocortex. Although many of these subjects had a large neocortical amyloid burden, they did not show significant cognitive decline. This would indicate that the Khachaturian guidelines, which do not discriminate between DP and NP and do not place emphasis on the presence of NFT, are not useful criteria to discriminate between AD and normal subjects. This view has been underscored by the recent NIA-RI report which places diagnostic priority on NP and NFT (2). The finding also suggests that deposits of amyloid in the form of DP are not always an indication of the presence of AD, or incipient or presymptomatic AD. This view differs from that of Morris et al (10) who interpreted the presence of abundant DP to be evidence of AD rather than normal aging. They found very mild cognitive impairment in 9 subjects with high DP densities in the neocortex, and suggested these subjects had early AD. It is possible that AD is clinically underdiagnosed in a few of our subjects who had large numbers of neocortical DP, but it is unlikely that they all had early AD. Morris et al (10) used the Clinical Dementia Rating Scale in their study which may have allowed them to detect a mild degree of functional decline that we might not detect. However, the issue of whether abundant DP are the forerunners of AD will remain unsolved until more sophisticated methods are available to address this problem.

In our group of cognitively normal subjects, 15 (25%) met CERAD guidelines for the diagnosis of AD. Of these, 10 had MMSE scores of 30 to 28, 2 had scores of 27 to 26, a 100-year-old had a score of 25, and a 96-year-old had a score of 21. It is possible that the latter subject was in the early stages of AD, but his advanced age, systemic illness, and medication use made it difficult to determine the course of his cognitive decline. Seven (11%) subjects met the NIA-RI guidelines for the diagnosis of AD. Six had MMSE scores of 29 to 27 and 1 had a score of 25. Our study suggests that neither CERAD nor NIA-RI guidelines discriminate AD from pathological aging changes in the absence of clinical findings.

In 69% of subjects, this study showed variable numbers of NP in neocortex, amygdala, and entorhinal cortex. These were most prominent in the amygdala, inferior parietal lobule, and entorhinal cortex. The increasing NP density appeared to be related to higher Braak stages and to increasing age. Katzman et al (8) described 10 prospectively evaluated normal subjects with few or no neocortical NFT, but with considerable numbers of neocortical NP. Kazee et al (18) found a subgroup of cognitively normal control subjects who had neocortical NP. Morris et al (10) described that NP made up less than 14% of SP in cognitively intact subjects and 15% in very early AD subjects.

The entorhinal cortex processes information from the neocortex into the hippocampus and plays a major role in memory (24). Subjects with a minor number of degenerative changes in the entorhinal cortex and the hippocampus would not be expected to show a decline in memory. However, subjects with abundant entorhinal and hippocampal NFT and other degenerative changes would be expected to show a decline in memory. In our study, 22 (37%) subjects had abundant entorhinal cortex and hippocampal NFT, yet did not show recognizable cognitive decline. It suggests that partial disruption of a critical cortical circuitry is tolerated by some elderly individuals and that different degrees of degeneration are required to cause clinical symptomatology. In this regard, our study suggests that Braak staging does not correlate well with the psychometric findings in this group of control subjects. Nine Braak stage IV, 5 stage V, and 1 stage VI subjects were present in our group, but none of these individuals had clinical evidence of dementia. All of the Braak stage V and VI subjects met CERAD and NIA-RI guidelines for the diagnosis of AD. These individuals had a mean age of 84.5 years. These results are in agreement with Geddes et al (25, 26) who found that Braak staging was not sensitive in subjects with a low prevalence of dementia.

There was a hierarchical pattern of progression of NFT from entorhinal cortex to hippocampus and amygdala as a function of age. There was a significant increase in NFT in the hippocampal CA1 region and amygdala in those over 90 years of age compared with the 80+ and 70+ age ranges. Of interest, this pattern did not exist in the entorhinal cortex, where the level of NFT remained essentially the same throughout all 3 decades. This would suggest that the entorhinal cortex has the earliest formation of NFT and reaches a threshold level, at least in cognitively intact subjects. Subsequent increases occurred in the hippocampus and amygdala with increasing age. This is in keeping with the hierarchical pattern of NFT formation as described by Braak and Braak in AD (20, 21). The mild number of NFT in neocortex was not altered by age in our subjects. It should be underscored that a few neocortical NFT were found in over half of our subjects without detectable cognitive decline.

Two of the subjects had abundant NFT pathology limited to the entorhinal cortex, the hippocampus, and to a lesser degree in the amygdala, without significant SP formation. Neuropil threads were also prominent in these areas. These 2 subjects were not demented and their cognitive tests did not indicate a decline over the last testing periods. The MMSE scores in the 2 subjects were 30 and 25. These 2 cases are comparable to the "neurofibrillary tangle prominent dementia" previously described by several authors (27–30). In the study by Ulrich et al (30), neuropsychologic tests demonstrated the subjects to be demented. Bancher and Jellinger (29), in a series of 265 AD subjects, found 10 subjects with abundant limbic NFT, but no or few neocortical NFT or SP. Four of these subjects were severely demented, 5 were moderately demented, and 1 was mildly demented. Our study suggests that a limbic NFT burden is well tolerated by some elderly subjects.

Two subjects with intact intellectual function had sufficient numbers of neocortical, limbic, and brainstem LB to meet the guidelines for a neuropathologic diagnosis of dementia with LB (23). A previous study demonstrated that the density of LB in different cortical areas correlates with dementia (31). Others have indicated that, in some demented individuals, LB found in the absence of other pathological lesions may be sufficient to produce the clinical disorder (32). Of our 2 subjects, 1 was 69-years-old and scored 30 on the MMSE and the other was 84-years-old and scored 26. Both had moderately abundant DP in the neocortex. To our knowledge, this is the first report of the presence of abundant LB in brainstem, limbic areas, and neocortex in cognitively intact individuals.

Sparks et al (14, 15) and Soneira and Scott (16) linked ischemic cardiovascular disease with SP formation. These studies demonstrated higher plaque counts in neocortex of subjects with ischemic cardiovascular disease compared with "nondemented" subjects without coronary artery disease; neither study used subjects with prospective clinical and mental status evaluation. Forty-one percent (n = 25) of subjects in the present study had a clinically documented myocardial infarction and were on medication for angina (n = 11) or were on medication for angina without clinical or electrocardiographic evidence of a myocardial infarction (n =14). No statistical differences were identified between subjects with or without ischemic heart disease and the presence or absence of SP. Other studies have examined hypertension (13) and found a link to the development of NFT. Fifteen subjects (25%) in this study had clinically documented hypertension and were on pharmacologic therapy for this disease. No statistically significant relationship between the presence or absence of NFT and hypertension was present in our study. Subjects who had hypertension and ischemic cardiac disease were combined and compared with those without these diseases. There was no statistically significant difference in either group in their relationship to the presence or absence of SP or NFT.

Infarcts in our subjects were common although predominately consisted of remote microinfarcts. We found no statistically significant effect on MMSE related to the presence of gross or microinfarcts. Two previous studies (33, 34) demonstrated a relationship between the additive effects of infarcts on cognition in AD. One of the studies (33) also found that cerebral infarctions were weakly correlated with dementia in subjects not meeting CERAD neuropathologic criteria for AD. Their study examined the effects of gross or lacunar infarcts and did not examine the effect of microinfarcts. The number of their subjects not meeting AD criteria (n = 41) and having a gross or lacunar infarct (n = 15) was comparable to our study group where gross or lacunar infarcts were present; we identified no statistically significant difference in MMSE scores in this group. Nagy et al (34) found the density of neocortical SP or NP was lower in AD with vascular lesions and other neurodegenerative pathology than in AD without other CNS pathology. Statistical correction for multiple comparisons was applied in this study. These 2 studies (33, 34) indicate that vascular lesions coexisting with AD pathology may summate the 2 pathological processes to produce a greater cognitive deficit than if AD degenerative pathology were present in isolation. However, in the absence of AD pathology, cognitive deficits are not measurably evident with the presently available testing tools as the functional reserve of an otherwise normal subject may be able to compensate for small gross infarcts and/or the presence of many microscopic infarcts. Our study and theirs emphasize the point that vascular lesions in aged subjects, whether associated with a degenerative disease or not, are a common finding.

Twenty-three percent of our cases contained argyrophilic grains in entorhinal cortex, hippocampal CA1, or amygdala. Argyrophilic grain dementia was initially described by Braak and Braak (35, 36) as a non-AD dementing disorder. These cytoskeletal abnormalities have been identified in asymptomatic elderly individuals (37, 38, 39) and in association with a variety of diseases including: AD, Pick disease, corticobasal degeneration, progressive supranuclear palsy, multiple system atrophy, motor neuron disease, and dementia with LB. Two previous studies (37, 38) demonstrated a relationship between increasing age and the presence of argyrophilic grains; our study did not statistically substantiate this finding. The biologic significance of the argyrophilic grain cytoskeletal abnormality is unclear. What is clear is that their presence is not always associated with a degenerative dementing disorder.

Cerebral amyloid angiopathy (CAA) increases in frequency with age. The incidence of CAA was higher in our series than in other reported autopsy series (40–43). In our subjects, a 75% incidence of leptomeningeal CAA was present, whereas 62% of subjects had amyloid in parenchymal vessels. Previously published autopsy series of clinically normal individuals have not exceeded a CAA frequency of 50% (40–43). Our findings parallel these autopsy series in that CAA was present in much greater frequency in the leptomeningeal than parenchymal vessels.

A recent study by Hulette et al (17) examined neuropathological and neuropsychological change in normal aging and concluded there was evidence for preclinical AD in some subjects. The study included 31 subjects from 2 separate institutions, only 12 of which had neuropsychological testing. Of the 12, 8 were normal and 4 were categorized as "possible AD" by CERAD neuropathology guidelines. One of the "possible AD" patients demonstrated a memory savings of only 16% and could have been eliminated and considered to have definite AD. There was a lack of information about a) the presence of other chronic disease states, b) other neuropathological abnormalities except AD and Parkinson disease, and c) limited information about parenchymal vascular lesions. Their small sample size limited statistical validity and strong conclusion, especially the comment that "primitive neuritic plaques correlate with subtle defects in cognitive functioning would suggest that they are the earliest indicator of the disease process that will ultimately result in AD." Although this statement may eventually prove to be correct, it will require clinicopathological correlation in more than 3 patients. All of the subjects in the current study had longitudinal mental status testing and the sample size of the group is large enough to not limit statistical evaluation.

In summary, this study demonstrates a broad spectrum of histopathologic changes in the brains of cognitively intact individuals. Many of these subjects fulfilled neuropathologic guidelines for the diagnosis of AD and 2 met guidelines for dementia with LB. The changes observed in our subjects raise the question of what criteria can be used for normal controls for comparative studies with AD subjects. Many of the subjects with the brain alterations we found should not be used for comparative studies with AD, challenging the validity of studies in which controls were not evaluated thoroughly. If it is the goal of AD studies to use pure controls without AD changes, our study indicates that these are few in number in a group of community dwelling, well-educated, volunteer subjects.

This study raises an important question of why some patients tolerate the insults of AD pathology, infarcts, and effects of hypertension on the brain without showing cognitive decline while others do not. Perhaps our subjects have greater brain or neurocognitive reserve related to higher educational or occupational attainment (reviewed in44). The actual changes in the brain underlying greater neurocognitive reserve remain to be defined. Perhaps better cortico-cortical connectivity, greater synapse or neuron number and function, enhanced neurotransmitter function, or other morphological or molecular factors lead to increased neurocognitive reserve.

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