

Neurofibrillary Tangles, Amyloid, and Memory in Aging and Mild Cognitive Impairment

Angela L. Guillozet, PhD; Sandra Weintraub, PhD; Deborah C. Mash, PhD; M. Marsel Mesulam, MD

Background: Large numbers of neurofibrillary tangles (NFTs) and amyloid plaques are diagnostic markers for Alzheimer disease (AD), but lesser numbers of these lesions are also seen in nondemented elderly individuals. Much of the existing literature suggests that the NFTs of AD have a closer correlation with cognitive function than do amyloid plaques. Whether a similar relationship exists in normal aging and mild cognitive impairment (MCI), a condition that frequently reflects a pre-clinical stage of AD, remains unknown.

Objective: To determine the distribution patterns of β -amyloid plaques and NFTs and the association of these lesions with memory performance in nondemented individuals.

Methods: We investigated regional distributions and neuropsychological correlates of NFTs and amyloid plaques in cognitively normal elderly persons and subjects with MCI who received neuropsychological testing before death.

Subjects: Eight nondemented subjects who volunteered to receive annual neuropsychological testing and agreed to brain donation were studied. Five subjects showed no cognitive impairment, and 3 were diagnosed with MCI.

Results: Distribution of NFTs followed a rigorous and hierarchical pattern, but distribution of amyloid plaques varied among individuals. Subjects with MCI displayed higher NFT densities than did nonimpaired subjects. In addition, NFT density in the temporal lobe correlated with memory scores, whereas density of amyloid plaques did not.

Conclusions: Neurofibrillary tangles are more numerous in medial temporal lobe regions associated with memory function and show a relationship to performance on memory tests in nondemented individuals. These results suggest that NFTs may constitute a pathological substrate for memory loss not only in AD but also in normal aging and MCI.

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From the Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago, Ill (Drs Guillozet, Weintraub, and Mesulam); and the Department of Neurology, University of Miami School of Medicine, Miami, Fla (Dr Mash).

ALZHEIMER DISEASE (AD) is characterized by severe and progressive memory loss that interferes with daily living activities. Mild impairments of memory are typical of "normal" aging.¹⁻³ Some individuals display an isolated loss of memory that is more severe than what would be expected on the basis of age alone but without the additional impairments of other cognitive domains or disruption of daily living activities characteristic of AD. This condition has been labeled mild cognitive impairment (MCI).⁴⁻⁶ Although focal memory loss may have heterogeneous origins,⁷ individuals with MCI convert to AD at a higher rate than do individuals without cognitive impairment,^{5,8} suggesting that, in many cases, MCI may represent an intermediate stage between aging and AD.

The best-characterized neuropathological lesions associated with AD are neurofibrillary tangles (NFTs) and amyloid

plaques. However, both amyloid plaques⁹⁻¹⁴ and neurofibrillary tangles^{6,9-11,13-15} have been found in nondemented individuals. The relevance of these lesions to memory performance is poorly understood, although NFTs show a strong relationship to dementia severity in AD^{16,17} and all mutations resulting in AD appear to alter amyloid processing.

In the course of events that eventually lead to AD, NFTs are initially limited to the hippocampus, the entorhinal cortex, and adjacent limbic structures.^{18,19} As the numbers of tangles in these areas increase, adjacent temporal neocortex, including the fusiform and inferior temporal gyri and temporal polar cortex, begin to show clusters of tangles. As pathological changes worsen further, NFTs become more numerous in these regions and begin to appear in the lateral temporal cortex. A small number of lesions then emerge in the frontal or parietal neocortex. Finally, tangles in the frontal or parietal neocortex become plentiful, con-

Table 1. Neuropsychological Test Scores in Nonimpaired (Control) Subjects and Subjects With Mild Cognitive Impairment (MCI)

Neuropsychological Test	Maximum Raw Score*	Cutoff Score, † M/F	Score, Mean ± SD		P Value
			Control	MCI	
MMSE ⁴⁰	30	28/27	28.4 ± 2.1	26.9 ± 0.2	.26
Memory tests					
CERAD word list					
Acquisition ⁴¹	30	15/15	22.2 ± 3.3	12.3 ± 2.1	.004
Delayed recall ⁴¹	10	4/5	7.0 ± 1.6	1.7 ± 0.6	.002
Forgotten	NA	NA	1.4 ± 0.5	3.3 ± 0.6	.003
Logical memory, WMS-R					
Acquisition ⁴²	50	6/6	20.2 ± 13.6	18.0 ± 3.6	.80
Delayed recall ⁴²	50	3/3	15.2 ± 14.2	11.0 ± 11.5	.68
Forgotten	NA	NA	5.0 ± 3.4	7.0 ± 9.2	.66
CERAD constructions, ⁴¹ delayed recall	11	NA	8.6 ± 2.1	2.7 ± 1.2	.004
Language tests					
Category fluency ^{43,44} (animals, 1 min)	NA	10/10	15.0 ± 4.6	9.0 ± 2.7	.09
Boston Naming Test ^{41,45}	15	12/12	14.8 ± 0.5	13.5 ± 2.1	.19
Letter fluency ⁴⁶ (C-F-L test, 3 min)	NA	8/16	40.6 ± 13.3	22.0 ± 10.2	.09
Visuospatial tests					
CERAD constructions (copy) ⁴¹	11	9/8	9.8 ± 1.8	6.67 ± 2.9	.10
Benton Visual Retention Test ⁴⁷	14	2/2	5.8 ± 2.2	1.00 ± 0.0	.11

Abbreviations: CERAD, Consortium to Establish a Registry for Alzheimer's Disease; MMSE, Mini-Mental State Examination; NA, no upper limit to the score or no published mean available for that test; WMS-R, Wechsler Memory Scale-Revised.

*Maximum raw score indicates the total number of possible points on each test.

†Cutoff score indicates 2 SDs before the published mean for individuals older than 80 years with 12 or more years of education.

sistent with Braak stages 5 and 6.¹⁸ Individuals suffering from the memory loss symptomatic of AD most often fall into these 2 stages of neurofibrillary degeneration.²⁰

All AD-causing genetic mutations also affect the processing and/or deposition of β -amyloid ($A\beta$), either by increasing the overall amount of $A\beta$ or by selectively increasing the ratio of longer, more toxic forms to the shorter forms of the molecule.²¹⁻²⁸ Presence of the $\epsilon 4$ allele of the *ApoE* gene, a known risk factor for the development of AD,^{29,30} has also been shown to affect the deposition of $A\beta$ in the cortex.³¹ However, unlike NFTs, no specific regional pattern of amyloid distribution has emerged. Few studies have found relationships between the amount of amyloid and dementia severity in AD,³²⁻³⁶ and many nondemented individuals display amyloid plaques throughout the cortex.^{9,37-39}

In this study, we examine the correlation of NFTs and amyloid plaques in reference to memory function in cognitively normal elderly individuals and subjects with MCI. We hypothesize that having lesions within the medial-temporal regions will be correlated with memory function, whereas subjects with lesions in nontemporal regions will lack such a relationship.

METHODS

Subjects participated in a longitudinal study conducted at the University of Miami, Miami, Fla, in which they agreed to neuropsychological testing and brain donation (University of Miami Brain Endowment Bank). Brain specimens from subjects who enter the study without dementia and who remain nondemented throughout the study are sent to our laboratory at Northwestern University, Chicago, Ill, for further analysis. This study was limited to consecutively received specimens from subjects who underwent multiple neuropsychological evaluations, were not demented at the time of the last neuropsychological evaluation, and whose brains did not show non-AD-

type abnormalities upon autopsy, such as Lewy bodies, Pick bodies, or vascular changes. Eight brains met these criteria within the time periods spanned by this study.

Subjects received neuropsychological testing between 2 and 6 times before death; the last test occurred between 15 days and 12.5 months before death. Neuropsychological evaluation included tests to examine overall dementia severity, memory function, language function, and visuospatial function (see **Table 1** for a list of tests).⁴⁰⁻⁴⁷ Additionally, we calculated the number of items forgotten on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word lists⁴¹ (maximum number of words recalled in the acquisition phase minus the number of words recalled after a delay) and on the logical memory subtest of the Wechsler Memory Scale-Revised (WMS-R)⁴² (total number of items immediately recalled minus the number of items recalled after a delay). This was done to control for interindividual differences in encoding the information. A reviewer blinded to the neuropathological status of each subject (S.W.) retrospectively analyzed medical records and neuropsychological test scores and determined whether subjects were nondemented or showed signs of memory impairment consistent with MCI. Mild cognitive impairment was defined as a selective loss of memory function reflected in abnormal neuropsychological test scores for age while other cognitive test scores remained normal and activities of daily living, as corroborated by clinical information, were relatively unaffected by the memory loss. This definition is consistent with the criteria defined by Petersen et al.⁴ Test scores were considered abnormal if they fell 2 or more SDs below the published mean for individuals older than 80 years. Activities of daily living were assessed by questionnaires and with unstructured interviews with the patients and family members. Nonimpaired control subjects showed no measurable cognitive deficits or alterations in daily living activities and performed at or above the level expected for age, sex, and years of education. Although only the final neuropsychological evaluation was used in data analysis, the results from previous evaluations were used to establish whether an actual decline in function had occurred.

Upon autopsy, one hemisphere from each brain was cut coronally into slabs approximately 2 to 3 cm thick and immersed in

Table 2. Maximum Focal Density of Neurofibrillary Tangles (NFT_{max}) and β -Amyloid (A β) Burden in Nonimpaired (Control) Subjects and Subjects With Mild Cognitive Impairment (MCI)

Area	NFT _{max} , Mean \pm SD, NFTs/mm ²		P Value	A β , Mean \pm SD, %		P Value
	Control	MCI		Control	MCI	
Medial-temporal region	71.52 \pm 73.03	197.88 \pm 44.56	.04	2.3 \pm 1.0	2.6 \pm 1.5	.97
Entorhinal layer 2	148.24 \pm 159.05	400.67 \pm 119.07	.06			
Entorhinal layer 5	81.64 \pm 92.74	201.33 \pm 37.112	.08	2.9 \pm 2.0	2.1 \pm 1.5	.66
Fusiform gyrus	31.96 \pm 29.26	138.33 \pm 40.08	.005	4.3 \pm 3.3	4.2 \pm 3.6	.98
Temporal pole	24.24 \pm 22.08	51.20 \pm 0.53	.09	3.1 \pm 2.2	3.5 \pm 2.8	.83
All other regions	8.01 \pm 11.69	13.93 \pm 9.51	.49	3.9 \pm 2.5	3.2 \pm 2.3	.67
Inferotemporal gyrus	16.32 \pm 20.22	42.67 \pm 21.61	.13	4.8 \pm 3.0	3.2 \pm 2.3	.47
Superotemporal gyrus	17.67 \pm 30.60	21.33 \pm 13.61	.86	6.8 \pm 2.8	3.2 \pm 3.0	.21
Cingulate gyrus	7.08 \pm 9.44	13.33 \pm 10.28	.41	5.0 \pm 3.8	3.5 \pm 2.9	.59
Orbital-frontal cortex	11.55 \pm 10.83	0.80 \pm 1.13	.26	3.1 \pm 1.2	2.5 \pm 0.7	.44
Lateral-parietal cortex	12.20 \pm 27.28	13.33 \pm 15.54	.95	4.5 \pm 3.3	4.3 \pm 4.3	.93
Intraparietal sulcus	11.20 \pm 25.04	3.20 \pm 5.54	.62	4.4 \pm 3.3	4.2 \pm 3.6	.92
Dorsolateral-prefrontal cortex	0.96 \pm 2.15	9.07 \pm 8.21	.07	6.2 \pm 4.0	4.4 \pm 4.1	.56
Primary motor cortex	2.80 \pm 5.60	5.33 \pm 5.62	.58	1.8 \pm 1.6	1.4 \pm 0.9	.72
Primary visual cortex	0.32 \pm 0.72	0.00 \pm 0.00	.58	1.6 \pm 2.0	1.8 \pm 2.3	.90

4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4, at 4°C for 30 hours. The slabs were then rinsed and cryoprotected in ascending sucrose solutions. Slabs were frozen on dry ice and cut at 40 μ m using a sliding microtome. Sections that were immediately adjacent before sectioning were stained with cresyl violet, an antibody directed against the A β protein, and thioflavine-S for tangles as described below. The postmortem interval ranged from 2.5 to 9.5 hours.

Twelve areas were examined for the presence of NFTs and A β plaques (Table 2). The analysis focused on the paralimbic belt and temporal regions where neurofibrillary abnormalities are known to begin. Additional areas in the prefrontal, parietal, and occipital regions were analyzed to ensure that a large portion of the brain was examined and to determine the extent of pathological lesions. For NFT counts, the entorhinal cortex was divided into layer 2 and layers 3 and 5. For measurements of amyloid burden, the area from pia to white matter was analyzed. Areas were identified grossly using neuroanatomical boundaries and confirmed by cytoarchitecture as visualized on cresyl violet-stained sections.

We chose to analyze the entorhinal region rather than the hippocampal region because tangles initially appear in the entorhinal cortex and appear later in the hippocampus. Because some of our cases contained very minimal abnormalities, we did not wish to introduce a floor effect in tangle counts in those cases in which tangle formation had not yet reached the hippocampus. Additionally, it is well established that the entorhinal cortex and hippocampus are intimately connected and work as a unit. The primary output of the entorhinal cortex is to the hippocampus, and it receives many reciprocal connections from the hippocampus. Damage to the entorhinal cortex has been shown to result in memory loss, even when the hippocampus itself was not affected.

Sections were mounted, dried, and stained with 0.1% thioflavine-S as previously described.⁴⁸ Slides were viewed under fluorescence using a violet filter to eliminate residual autofluorescence. Sections adjacent to those stained with thioflavine-S were stained for A β immunohistochemical analysis using the well-characterized rabbit antibody R1280,⁴⁹ as previously described.⁵⁰

For NFT measurement, thioflavine-S-stained sections were viewed under the microscope and traced, with the outline and placement of each NFT recorded on an XY plotter (Hewlett-Packard, Palo Alto, Calif). A reviewer blind to the clinical diagnosis of each subject counted the most densely affected fields

in each of the 13 areas examined. The NFTs in a minimum of 30 of the most densely affected visual fields of each were counted, and the 10 densest were used to compute the maximum focal density of NFT (NFT_{max}). Counts were then extrapolated to compute the number of tangles per square millimeters.

For amyloid densitometric measurement, cresyl violet-stained sections immediately adjacent to A β -immunostained sections were used to delineate areas of interest. Within these areas, 4-mm fields were marked off, and digital images were acquired of each of the fields for use as reference images. Matching fields within the A β -stained sections were aligned to the cresyl violet-stained images, and digital images were acquired. The area of interest was outlined on the reference image and transferred to images of tissue stained for amyloid immunohistochemistry. The percentage of cortical area covered by immunoreactivity (amyloid burden) was then calculated using image analysis software (NIH Image; National Institutes of Health, Bethesda, Md).

Two types of analyses were conducted. In the first, MCI and control groups were compared for measures of regional tangle density and amyloid burden using a 1-way analysis of variance. In the second, Pearson correlation coefficients were used to examine the relationship between various factors such as age and memory test scores to measures of tangle density and amyloid burden. Correlation coefficients tend to be overestimated, especially when the sample size is small.⁵¹ In this study, the squared correlation coefficients were minimally overestimated by amounts ranging from 2% to 12%. Adjusted values for the squared correlations are presented.

RESULTS

There was no difference in age between nondemented and MCI subjects (mean \pm SD: control group, 88.6 \pm 5.0 years vs MCI group, 93.7 \pm 4.7 years; $P = .21$), education (14.8 \pm 2.7 years vs 13.0 \pm 1.0 years; $P = .32$), postmortem interval (6.7 \pm 3.5 hours vs 3.8 \pm 1.0 hours; $P = .23$), or brain weight (1160.8 \pm 69.9 g vs 1174.7 \pm 178.0 g; $P = .88$). Although *ApoE* genotype was determined, almost all subjects displayed a similar genotype, which did not allow analysis of the effect of genotype on neuropsychological measures or lesion distribution. The geno-

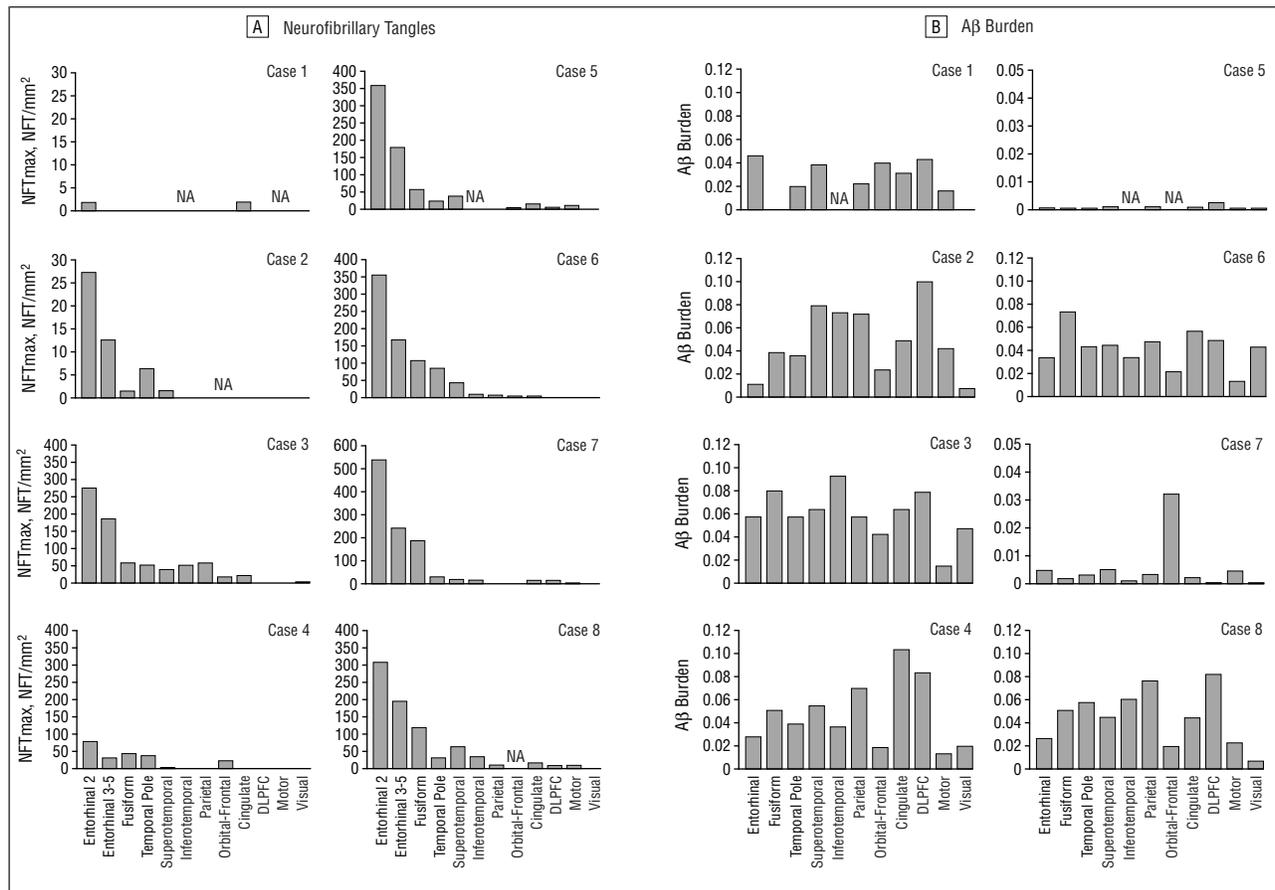


Figure 1. Distribution of neurofibrillary tangles (NFTs) (A) and β -amyloid plaques ($A\beta$) (B) in the 8 brains analyzed. Cases 1 to 5 are control subjects, and cases 6 to 8 are subjects with mild cognitive impairment. The y-axis scale varies from case to case to illustrate the regional variations in maximum NFT density (NFTmax) and amyloid burden. These graphs show that regional variation in the relative magnitude of NFTs remains stable from case to case, whereas this is not the case for amyloid burden. Because severity of neurofibrillary abnormalities varies among cases, the y-axis bars differ among some subjects to show the relative distribution patterns within the individual rather than the comparison between individuals. DLPFC indicates dorsolateral prefrontal cortex; NA, not available.

type of one of the control subjects (case 3) was 2/3, whereas the genotype of all other subjects was 3/3.

NEUROPSYCHOLOGICAL TESTS

Three subjects had neuropsychological and behavioral profiles consistent with MCI. Five subjects performed at or above the level expected for their age (nonimpaired controls). As expected, the MCI subjects had abnormal scores on memory tests (≥ 2 SDs below the mean) compared with age-matched controls but no differences in scores on tests of language and visuospatial functioning (Table 1). The decline in memory function of these individuals was apparent across multiple neuropsychological testing sessions but was not sufficient for a diagnosis of AD. Additionally, there was no significant difference in scores on the "Mini-Mental State" Examination,⁴⁰ which has been shown to be valuable in differentiating stages of dementia but not in distinguishing the early stages of memory loss from normal aging.⁵

There was a trend toward a decrease in test scores associated with increasing age, but this did not reach significance for any of the tests. The limited age range of our subjects (81-99 years) may have prevented age from becoming a contributing factor in test performance.

NEUROFIBRILLARY TANGLES

Distribution

The distribution of NFTs was consistent with previous findings.^{18,19} The entorhinal cortex always contained the most tangles, whereas primary sensory motor regions almost always contained the least. The fusiform gyrus, temporal pole, and inferior temporal gyrus contained the next-highest NFT density, followed by the superotemporal and parietal cortices. The orbital frontal cortex, cingulate gyrus, and dorsolateral prefrontal cortex almost always contained more tangles than did primary sensory motor regions (**Figure 1A**).

Clinical Diagnosis

There was a significant difference between MCI and control subjects in NFTmax in the fusiform gyrus ($F_{6,1} = 19.18$; $P = .005$) and the medial-temporal region overall ($F_{6,1} = 7.10$; $P = .04$). Although the difference between control and MCI subjects did not reach significance in other areas of the medial-temporal region, the NFTmax was greater in MCI subjects than in control subjects for all temporal regions (Table 2).

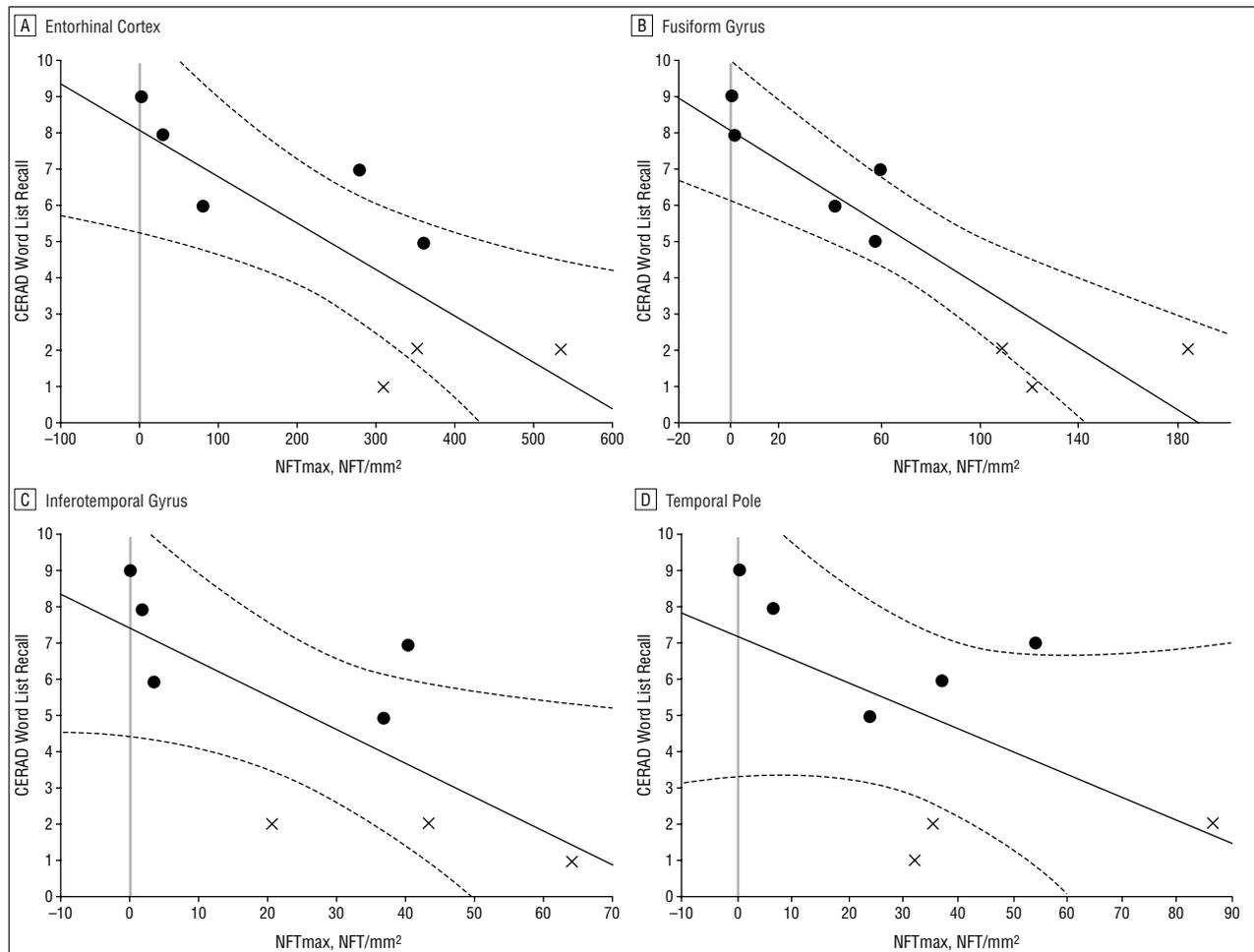


Figure 2. Maximum neurofibrillary tangle (NFT) density (NFTmax) is related to memory performance. Regression plots for scores on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word list recall test vs NFTmax in entorhinal cortex (A), fusiform gyrus (B), inferotemporal gyrus (C), and temporal pole (D). Closed circles indicate control subjects; x's, subjects with mild cognitive impairment; solid lines, regression lines; and dotted lines, 95% confidence intervals around the mean.

Neuropsychological Tests

The NFTmax in the medial temporal regions was related to scores on neuropsychological tests of acquisition. Scores on the CERAD word list acquisition measure correlated significantly with NFTmax in the layer 2 island cells of the entorhinal cortex ($r^2=0.81$; $P=.001$), the layer 3 and layer 5 pyramidal cells of the entorhinal cortex ($r^2=0.54$; $P=.02$), and the fusiform gyrus ($r^2=0.84$; $P<.001$). There was also a significant correlation between scores on the delayed-recall portion of the CERAD word list test and the NFTmax of layer 2 entorhinal neurons ($r^2=0.58$; $P=.02$), layer 3 and layer 5 pyramidal cells of the entorhinal cortex ($r^2=0.54$; $P=.04$), and the fusiform gyrus ($r^2=0.78$; $P=.01$) (**Figure 2**). When the numbers of items forgotten on the CERAD word list tests were calculated, the number of words forgotten was correlated with NFTmax of the fusiform gyrus ($r^2=0.61$; $P=.02$). Although there was no correlation between NFTmax and scores on either the immediate or delayed-recall portions of the logical memory subtest, the number of items forgotten was significantly correlated to the NFTmax of the temporal pole ($r^2=0.65$; $P=.02$). Scores on tests of language and visuospatial function did not correlate with NFTmax in any area.

β -AMYLOID

Distribution

There was considerable interindividual variance in the deposition pattern of A β plaques. Unlike the pattern found with NFTs, no area consistently displayed the highest amyloid burden (Figure 1B). For example, in one case, the entorhinal cortex showed the highest burden, whereas, in another case, the amyloid burden of the entorhinal cortex was the next to lowest. Similarly, the dorsolateral prefrontal cortex displayed the highest burden in one case, but one of the lowest burdens in another. In general, the lowest amyloid burdens were found in primary sensory motor regions, except in cases 3 and 6, in which the A β burden of the visual cortex was comparable to that found in most other areas of the cortex.

Age and Clinical Diagnosis

The amyloid burden showed no relationship to age or clinical diagnosis. The A β burden for control subjects was essentially equivalent to that for subjects with MCI (Table 2), and in almost all areas, the highest amyloid burden

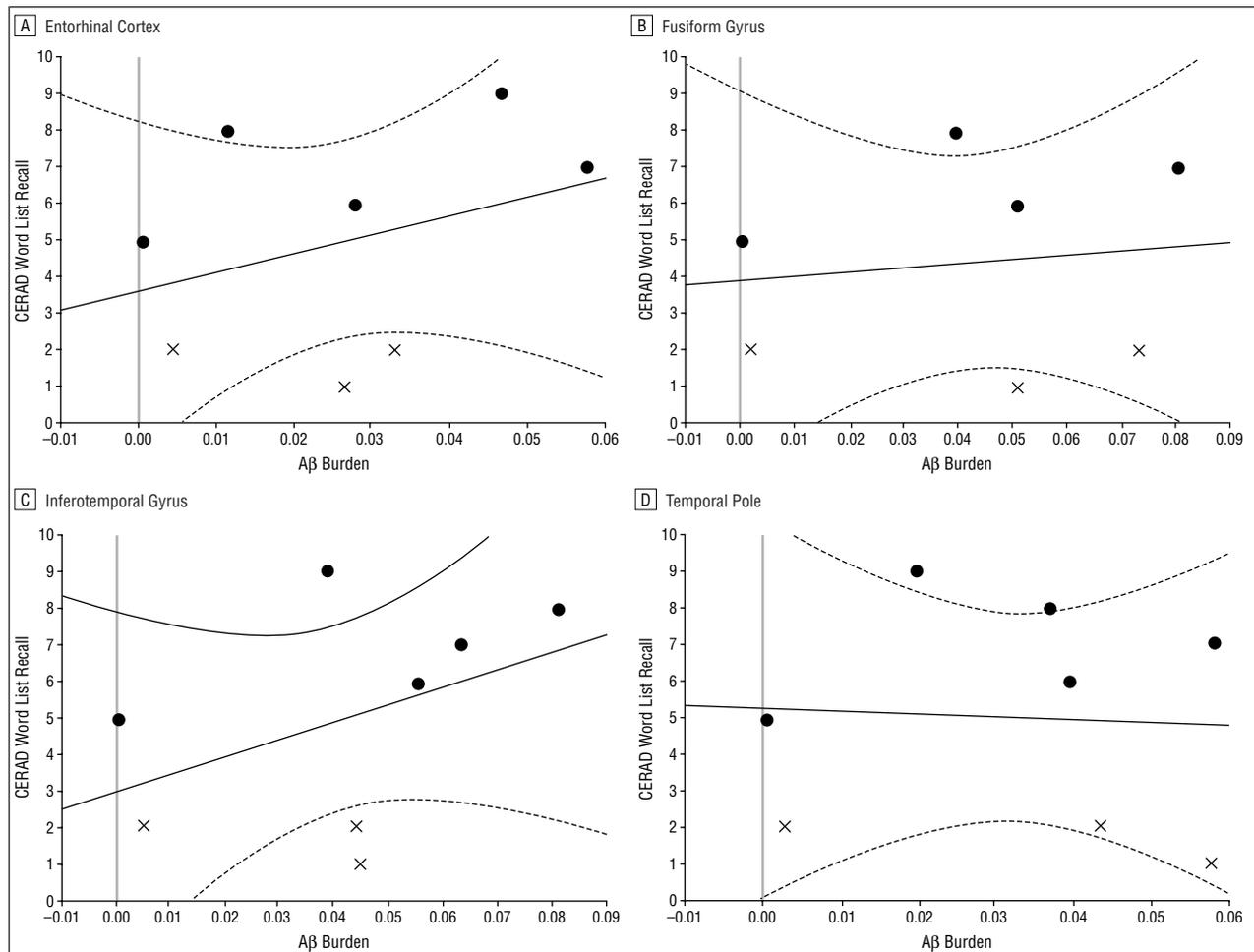


Figure 3. β -Amyloid ($A\beta$) burden is not related to memory performance. Regression plots for scores on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word list recall test vs amyloid burden in entorhinal cortex (A), fusiform gyrus (B), inferotemporal gyrus (C), and temporal pole (D). Closed circles indicate control subjects; x's, subjects with mild cognitive impairment; solid lines, regression lines; and dotted lines, 95% confidence intervals.

observed belonged to a control subject rather than a subject with MCI. The amyloid burdens ranged from almost 0% in 2 cases (1 control and 1 MCI subject) to moderate deposition in which the areas examined contained an amyloid burden of up to 10%.

Neuropsychological Tests

There was no relationship between amyloid burden and scores on any of the neuropsychological tests administered (**Figure 3**). In addition, amyloid burden was not related to NFTmax in any of the areas. This is consistent with the lack of a specific deposition pattern for $A\beta$ coupled with a predictable pattern of tangle development. Areas within the medial-temporal regions associated with memory function did not show higher $A\beta$ burdens than did areas underlying other cognitive functions.

COMMENT

Despite the small number of subjects in this study, we found significant differences in NFT density when subjects with MCI were compared with age-matched controls. Subjects with MCI displayed considerably higher NFT density in

all temporal regions than did nondemented individuals. The NFT density in memory-related cortical regions, such as the entorhinal cortex, fusiform gyrus, and temporal pole, showed strong relationships with scores on tests of memory function in nondemented individuals. These results are consistent with recent findings⁵² and suggest that the accumulation of NFTs may be responsible for the memory loss associated with aging as well as the memory deficits seen in some cases of MCI. Although multiple etiologies undoubtedly exist for memory loss, these results lend further credence to the view that the presence of NFT in aging may represent one of its earliest pathological substrates and that a neuropathological continuum extends from aging to MCI and AD.

The NFT marks a late stage in the process of cellular degeneration. There is circumstantial evidence suggesting that abnormal tau phosphorylation and mitochondrial dysfunction may precede full NFT formation.^{53,54} Neurofibrillary tangles begin appearing in small numbers in the entorhinal and transentorhinal cortices early in aging, around age 60 years.^{15,55} Although only a few tangles may be present in these regions of the brain at this relatively early stage of aging, additional cells that have not yet fully developed NFTs may already be func-

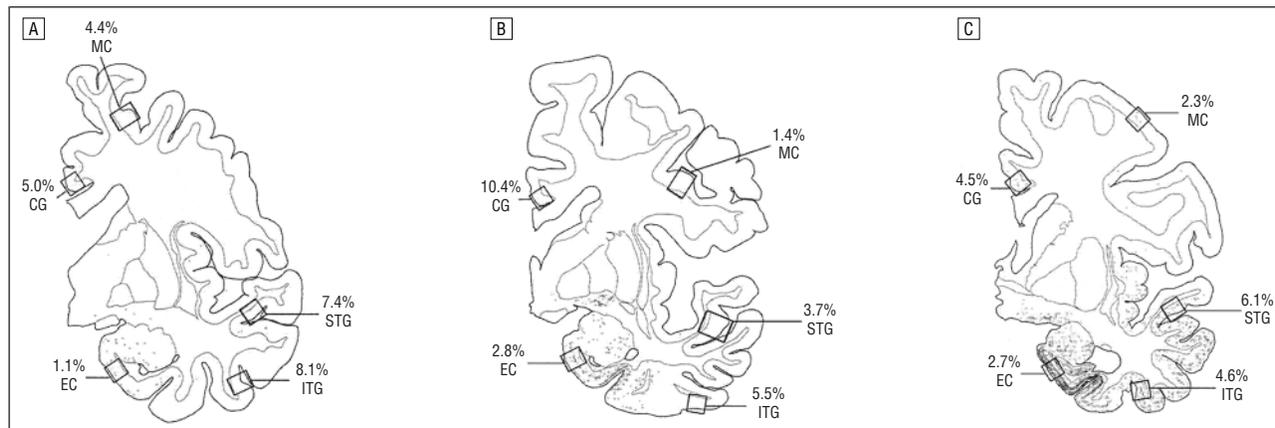


Figure 4. Neurofibrillary tangle density and β -amyloid burdens are not related. Plots of coronal sections from 2 control subjects (A and B) and a subject with mild cognitive impairment (C). Amyloid burdens are given by percentages for the entorhinal cortex (EC), inferotemporal gyrus (ITG), superotemporal gyrus (STG), primary motor cortex (MC), and cingulate gyrus (CG). Gray dots indicate neurofibrillary tangles.

tionally compromised in these same regions and may contribute to the almost universal appearance of “age-associated” memory loss.

Other pathological findings, such as increased cortical atrophy, cell loss, and synaptic loss, have been correlated to memory loss in AD and in aging.⁵⁶⁻⁵⁹ These factors are likely to be interrelated with NFT formation. For instance, cortical atrophy and synapse loss have been correlated to cell loss and NFT density.^{58,60} Neurofibrillary tangles are believed to be capable of inducing neural dysfunction, destruction of synapses, and, eventually, neuronal death,⁶¹ which could account for these multiple findings.

The $A\beta$ burden displayed considerable individual variation. Although a consistent pattern of amyloid plaque deposition has been proposed,⁵⁵ the distribution of amyloid in our subjects did not follow that pattern. In addition, the amyloid burden did not show a relationship with the degree of neurofibrillar abnormalities. Within individual subjects, areas with greater numbers of tangles did not, as a rule, display higher amyloid burdens. Nor did we find a relationship across individuals when single areas were examined (**Figure 4**).

No relationship between cognitive status and amyloid burden was found, consistent with other reports.³⁴ Subjects with MCI did not display a greater amyloid burden than did their nonimpaired counterparts, despite displaying higher densities of tangles. The lack of such a relationship extended to the medial-temporal lobe; there was no relationship between amyloid burden in this part of the brain and scores on tests of memory function. Many subjects displayed numerous amyloid plaques in regions of association cortex underlying cognitive domains that were shown to be intact by testing. The levels of amyloid in these regions often exceeded those found in medial-temporal lobe structures. The lack of correlation with test scores and lack of specificity for memory-associated regions strongly suggest that deposited amyloid does not play a significant role in the initial stages of memory impairment.

These results do not eliminate the possibility that a diffusible species of amyloid may contribute to cognitive decline. New studies suggest that it may not be the deposited amyloid that is linked to declining cognition but a soluble form of the amyloid molecule. Diffusible or soluble

amyloid, present as small oligomers, has been shown to be capable of causing toxic insult to cells.⁶² In contrast to numerous studies that have failed to demonstrate consistent relationships between $A\beta$ plaques and cognitive state, recent studies have reported such a relationship with measures of total (soluble + insoluble) brain amyloid.^{63,64} The levels of these oligomers are higher in the frontal cortex of individuals with dementia compared with nondemented control subjects.⁶³ Whether the levels of soluble amyloid in the medial-temporal regions correlate with the memory decline more closely than does the number of amyloid plaques remains to be determined.

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Author contributions: Study concept and design (Drs Guillozet and Mesulam); acquisition of data (Drs Guillozet and Mash); analysis and interpretation of data (Drs Guillozet, Weintraub, and Mesulam); drafting of the manuscript (Drs Guillozet and Mesulam); critical revision of the manuscript for important intellectual content (Drs Guillozet, Weintraub, Mash, and Mesulam); statistical expertise (Dr Weintraub); obtained funding (Dr Mesulam); administrative, technical, and material support (Drs Mash and Mesulam); study supervision (Drs Weintraub and Mesulam).

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Corresponding author and reprints: Angela L. Guillozet, PhD, Cognitive Neurology and Alzheimer’s Disease Center, Northwestern University, 320 E Superior St, Searle 11-478, Chicago, IL 60611 (e-mail: lgd450@nwu.edu).

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