Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment

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ABSTRACT Objective: To evaluate the total number of synapses in the stratum radiatum (str rad) of the human hippocampal CA1 subfield in individuals with mild Alzheimer disease (mAD), mild cognitive impairment (MCI), or no cognitive impairment (NCI) and determine if synapse loss is an early event in the progression of the disease. **Methods:** Short postmortem autopsy tissue was obtained, and an unbiased stereologic sampling scheme coupled with transmission electron microscopy was used to directly visualize synaptic contacts. **Results:** Individuals with mAD had fewer synapses (55%) than the other two diagnostic groups. Individuals with MCI had a mean synaptic value that was 18% lower than the NCI group mean. The total number of synapses showed a correlation with several cognitive tests including those involving both immediate and delayed recall. Total synaptic numbers showed no relationship to the subject's Braak stage or to *APOE* genotype. The volume of the str rad was reduced in mAD vs the other two diagnostic groups that were not different from each other. **Conclusion:** These results strongly support the concept that synapse loss is a structural correlate involved very early in cognitive decline in mild Alzheimer disease (mAD) and supports mild cognitive impairment as a transitional stage between mAD and no cognitive impairment. **NEUROLOGY 2007;68:1501–1508**

The structural or biochemical changes underlying progressive cognitive decline in Alzheimer disease (AD) remain unknown. Clinicopathologic studies have centered on neuritic amyloid plaques (NP) and neurofibrillary tangles (NFT) as the principal lesions of cognitive decline in AD. Postmortem neuropathologic studies reveal the presence of NP and NFT in nondemented elderly individuals[1-4] as well those classified as having "preclinical" AD,[5-7] who do not display measurable cognitive decline.[2]

Elderly individuals manifesting subtle cognitive deficits atypical of those observed in normal aging[8,9] may be in a transitional stage preceding frank dementia, clinically termed mild cognitive impairment (MCI).[10] Although the structural alterations underlying MCI are unclear, neocortical synaptic loss provides the best correlate of dementia in AD.[11-14]

We reported that a decline in synapse number in the hippocampal dentate gyrus in individuals with mild AD (mAD) correlates with impairment on a variety of cognitive tests,[15] suggesting that hippocampal degeneration is central to memory loss in AD.[16] The current study used these same subjects and evaluated total number of synapses in stratum radiatum (str rad) of the hippocampal CA1, a region linked to learning and memory. The CA1 str rad receives a major input (Schaffer collaterals) from the ipsilateral CA3 pyramidal cells, as part of a trisynaptic pathway originating in entorhinal cortex. The current and our earlier study[16] differ from previous synaptic investigations in that total synaptic numbers were evaluated in individuals with mild rather than more advanced AD.

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Table 1 Subject profile for hippocampal CA1

Group	Gender, M/F	Age, y	PMI, h	Brain wt, g	MMSE	Final diagnosis, y
NCI, n = 10	6/4	81.5 ± 6.7	5.8 ± 4.9	1,262 ± 48	28.1 ± 1.3	6.7 ± 1.8
MCI, n = 9	5/4	87.3 ± 5.5	3.8 ± 1.4	1,189 ± 99	26.8 ± 1.9	2.7 ± 1.3
mAD, n = 9	5/4	87.8 ± 8.1	4.8 ± 1.1	1,241 ± 96	18.6 ± 6.2*	4.2 ± 2.4

Values represent means ± SD.
*$p < 0.01$ compared with NCI and MCI.
PMI = postmortem interval; MMSE = Mini-Mental State Examination; NCI = no cognitive impairment; MCI = mild cognitive impairment; mAD = mild Alzheimer disease.

METHODS

Postmortem human brains. Tissue was examined from 28 individuals (mean age 85.0 ± 7.0 years; range 66 to 103 years; table 1) who were participants in either the Religious Orders Study (ROS), a longitudinal clinical-pathologic studies of aging and Alzheimer disease composed of older Catholic nuns, priests, and brothers,[17-19] or the University of Kentucky's community-dwelling cohort (Biologically Resilient Adults in Neurological Studies [BRAiNS]).[20] The Human Investigations Committee of Rush University Medical Center and the University of Kentucky College of Medicine approved the studies. Individuals included in these studies agreed to an annual clinical evaluation and to brain donation at the time of death. For all subjects, cognitive test scores were available within the last year of life; the average interval from last evaluation to time of death was 8.0 ± 3.1 months, with no differences among the three diagnostic groups ($p > 0.1$). Subjects were categorized as no cognitive impairment (NCI; n = 10; mean age = 81.1 ± 6.7 years, mean Mini-Mental State Examination [MMSE] = 28.1 ± 1.3), MCI (n = 9; mean age = 87.3 ± 5.5 years; mean MMSE = 26.8 ± 1.9), or mAD (n = 9; mean age = 87.8 ± 8.1 years; mean MMSE = 18.6 ± 6.2) based on cognitive testing prior to death.

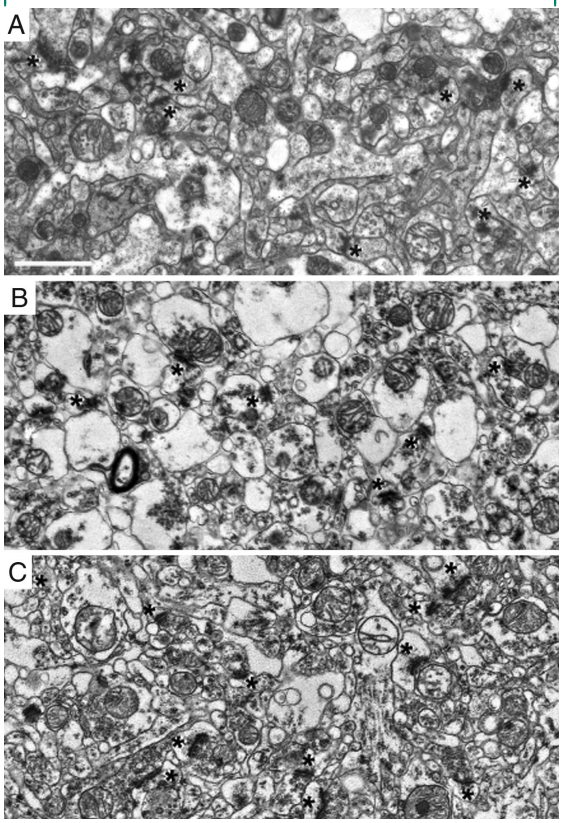
Clinical evaluation: ROS and BRAiNS subjects. Details of the ROS and the BRAiNS clinical evaluations have been published elsewhere.[17,19-21] In brief, trained neuropsychology technicians administered a battery of tests measuring performance in five cognitive domains: orientation, attention, memory, language, and perception.[22] A board-certified clinical neuropsychologist used these results to summarize impairment in each of the five cognitive domains. Following review of all clinical data for that year and examination of the participant, a board-certified neurologist with expertise in the evaluation of the elderly made a clinical diagnosis. The diagnosis of dementia and mAD followed the recommendations of the Joint Working Group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.[23] There are no consensus criteria for the clinical classification of MCI. Our MCI population was defined as those individuals rated as impaired on neuropsychological testing by the neuropsychologist but who were not found to have dementia by the examining neurologist.[17,19] These criteria are similar to, or compatible with, those used by others in the field to describe persons who are not cognitively normal but do not meet established criteria for dementia.[10,24] A postmortem interview similar to that used previously[17,25] was conducted at the time of death to identify medical conditions that occurred during the interval between the last clinical evaluation and death. Finally, a consensus conference of neurologists and neuropsychologists reviewed all available data and made a summary clinical diagnosis. Only subjects that were diagnosed clinically as having amnestic MCI were used in these studies.

Pathologic evaluation and tissue preparation for electron microscopy. At autopsy, brains were processed as previously published.[19,26] The postmortem interval (PMI) did not differ across groups ($p = 0.315$; table 1). A neuropathologist conducted a gross examination of brain neuropathology and cases were excluded if they exhibited non-AD type of pathologic conditions (e.g., brain tumors, encephalitis, large stokes, multiple lacunar infarctions).

The procedure used for ultrastructural assessment of synapses was identical to that described previously.[16] In brief, at the time of autopsy, the entire hippocampal formation from the left side was removed in toto, measured for its anterior–posterior length, and within the first 0.5 cm, a random starting point was chosen. The hippocampus was subsequently hand cut into 0.5-cm coronal slabs beginning at the hippocampal pes and ending at the hippocampal tail. Every other 0.5-cm hippocampal tissue slab (five to seven tissue slabs per subject) was immediately immersion fixed for 24 hours in 4% paraformaldehyde with 1% glutaraldehyde. Fixed tissue slabs were exhaustively sectioned at 100 μm in the coronal plane with a vibratome (Vibratome Co., St. Louis, MO). A die was thrown to choose which sections from each of the hippocampal slabs would be investigated ultrastructurally. The designated sections were postfixed in 1% osmium tetroxide (OsO_4), stained en bloc with 0.5% uranyl acetate, dehydrated in a graded series of ethanol, treated with propylene oxide, infiltrated with epoxy embedding resin, and flat embedded in circular molds (Ted Pella, Redding, CA). All hippocampal sections were coded so that all analyses could be done blind with respect to subject group designation. Sections immediately adjacent to those used for ultrastructure were designated for determination of the reference volume (V_{ref}) and were treated as previously described.[16] The operational definition of the str rad for the current measurements used the fused hippocampal fissure as one boundary and the CA1 pyramidal cell layer as the other with a change in staining pattern to differentiate str rad from stratum lacunosum moleculare regions. On each section used for ultrastructural analysis, the length of the CA1 str rad region was measured with a microscope and this length portioned into six equal segments. A die was used to determine which portion of CA1 length to analyze.

Blocks containing the randomly selected portion of str rad were trimmed to the appropriate region and semithin (2-μm-thick) sections containing the full width of the CA1 pyramidal cell dendritic field were taken to accurately determine the boundaries of the str rad and to aid in further trimming of the blocks. These sections were stained with toluidine blue. On stained semithin sections, the str rad region was divided into three equal zones (proximal, middle, distal) with reference to the pyramidal cell layer, and a die was thrown to determine from which subzone ultrathin sections would be taken. After determining the subzone of str rad to assess, blocks were further trimmed and ribbons of six to eight ultrathin sections (silver–gold interface range) were obtained and collected on Formvar-coated, carbon-stabilized slot grids (1 × 2–mm slot size). The ultrathin sections were stained with uranyl acetate (3%) and Reynold lead citrate. A die was thrown to identify the first of a pair of consecutive sections in the series. The identical area on both sections was identified. Two electron micrographs were taken on each section at an initial magnification of ×4,400 and photographically enlarged to approximately ×20,000. At this final magnification, synaptic profiles could be easily recog-

Figure 1 Representative transmission electron microscopic views of tissue stained with osmium tetroxide



Many normal-appearing synaptic complexes (*) can be observed in the hippocampal CA1 regio superior from individuals clinically defined as having mild Alzheimer disease (A), mild cognitive impairment (B), or no cognitive impairment (C). Bar = 1 μm.

nized. A grating replica was photographed and printed with each series of micrographs to ensure magnification. For each tissue block used, an additional set of micrographs was acquired by sectioning deeper into the block (approximately 100 μm) and repeating the above steps. Thus, a total of 10 to 12 different regions of the str rad were assessed.

The stereologic physical dissector technique[27] was used to count and estimate the numerical density of synapses per unit volume (N_v). Every synaptic profile on each micrograph was labeled. Synaptic profiles were defined by the presence of the postsynaptic density (PSD) in association with the postsynaptic element and synaptic vesicles in a presynaptic terminal (figure 1). After all micrographs had been assessed and all synapses labeled, adjacent dissector pairs of micrographs were identified with one micrograph assigned as a reference section and the other as a look-up section.[28] An unbiased counting frame was randomly superimposed over the reference section with the stipulation that the edge of the micrograph and the counting frame were separated by a distance that was greater than the dimension of a synaptic profile. Those synapses located either entirely or partially within the counting frame and not intersecting the forbidden edges or extension lines were counted. Discontinuous or perforated synapses were treated as a single synapse. Only those synaptic profiles that were observed on the reference micrograph within the counting frame and not on the look-up micrograph were counted. To increase efficiency, the look-up and reference sections were reversed, and the counting frame was again applied in a random fashion. The thickness of the ultrathin sections was estimated with the Small method of minimal folds.[29]

The numerical density of synapses per unit volume, N_v, was calculated using the following formula: $N_v = Q^-/V_{dis}$, where Q^- is the mean number of synapses counted in each dissector and V_{dis} is the mean dissector volume. The total number of synapses, N_{syn}, was calculated for each case using the following formula: $N_{syn} = N_v \cdot V_{ref}$.

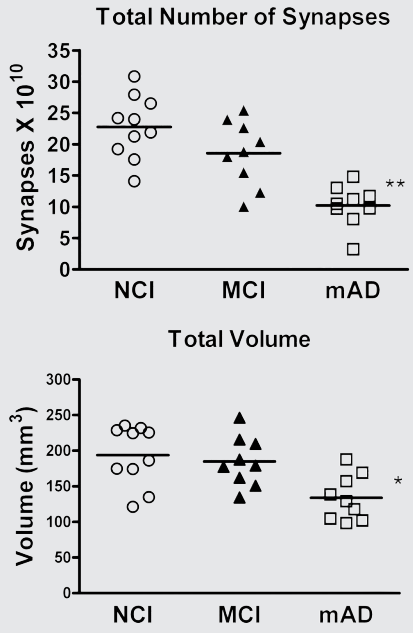
Statistical analyses. The relationship between dependent variables (e.g., total synaptic counts) and clinical group was examined with an analysis of variance (ANOVA). If a significant ANOVA was found, post-hoc tests (Student–Newman–Keuls) controlling for multiple comparisons were used to identify pairs of diagnostic groups that differed significantly. The diagnostic groups also were compared for possible differences in demographic characteristics (i.e., age, sex, PMI, and education). Because the diagnostic groups were somewhat heterogeneous in their clinical features, the relationship between total synaptic counts and performance on neuropsychological tests at last clinical evaluation was examined using linear regression models. Level of significance was set at $p < 0.05$.

RESULTS

Demographics. Table 1 shows characteristics of the sample population by diagnostic group. The NCI, MCI, and AD groups were found to be similar in gender, PMI, and brain weight. An ANOVA showed an expected difference in MMSE between groups ($F_{2,24} = 17.365$; $p < 0.0001$). Post-hoc analysis revealed no difference between NCI and MCI groups ($p > 0.05$) but did reveal a difference between AD and the other two diagnostic groups ($p < 0.05$). An ANOVA ($F_{2,25} = 2.25$; $p > 0.09$) revealed no difference in group mean age at time of death, eliminating age as a major contributor to lower cognitive scores.

Synaptic number and neuropil volume. The total synaptic counts for the str rad of the CA1 region for each subject in each of the three diagnostic groups were calculated (figure 2). Approximately 300 synapses were counted in approximately 20 to 24 dissectors for each subject. An ANOVA showed a difference between group means ($F_{2,25} = 17.953$; $p \leq 0.0001$). Post-hoc analyses showed that the mAD group was 55 and 45% lower than the NCI and MCI groups ($p < 0.001$). Post-hoc analysis showed no difference in total synaptic number between the NCI and MCI groups ($p > 0.1$) (table 2), although the overall group mean was 18% lower in the MCI group. An ANOVA showed a difference between group means ($F_{2,25} = 7.166$; $p < 0.005$) in volume of str rad of CA1 regio superior. Post-hoc analysis revealed a lower volume in the mAD group ($p < 0.01$), with the mAD cases 31% lower than both the NCI and the MCI groups. There were no differences between NCI and MCI diagnostic groups in terms of neuropil volume. Changes in synaptic size were also evaluated as previously described.[28,30] There was a change in synaptic size ($F_{2,25} = 4.561$; $p < 0.03$) with the mAD group showing a ($p < 0.05$) size in-

Figure 2 Estimates of total number of synapses in stratum radiatum subregion of the hippocampal CA1 in the three different diagnostic groups examined



The total volume of CA1 stratum radiatum was estimated with the Cavlieri method directly from tissue sections. Individual points represent individual subjects' scores for each group. Horizontal line indicates group mean. *$p < 0.05$; **$p < 0.01$.

crease (12%) compared with the NCI group. The MCI group's synaptic size was 5% larger than the NCI group. There was an association between synaptic size and total synaptic numbers ($r = -0.414$; $p < 0.03$), indicating that as the number of synapses decreased, the size of the residual contacts increased.

Table 2 CA1 stratum radiatum synapses, postsynaptic density length, and volume measurements

	NCI	MCI	mAD
Synapses, $\times 10^{10}$			
Sample size	10	9	9
Mean ± SD	23 ± 5.0	19 ± 5.2	10 ± 3.3†
Change, %		−18	−55
PSD length, μm			
Sample size	10	9	9
Mean ± SD	0.32 ± 0.03	0.34 ± 0.02	0.36 ± 0.04*
Change, %		+5	+12
Str rad volume, mm^3			
Sample size	10	9	9
Mean ± SD	194 ± 42	185 ± 35	134 ± 32†
Change, %		−5	−31

*mAD < MCI; AD < NCI ($p < 0.05$).
†mAD < MCI; AD < NCI ($p < 0.01$).
NCI = no cognitive impairment; MCI = mild cognitive impairment; mAD = mild Alzheimer disease; PSD = postsynaptic density.

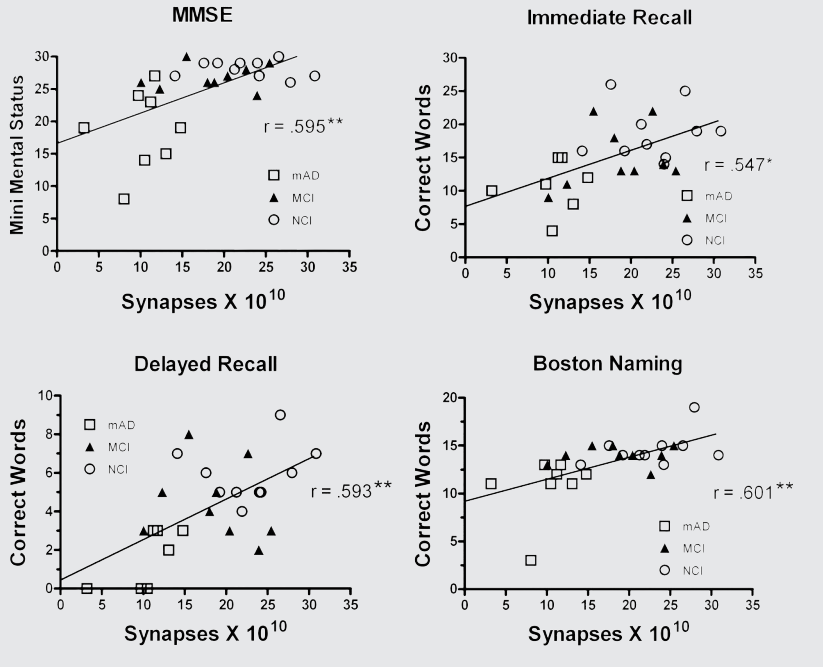
Total synaptic number, neuropil volume, and neuropsychological test scores. We evaluated the association of the total number of synapses in str rad of CA1 regio superior and scores achieved on neuropsychological tests obtained during the subject's last clinical evaluation. As the total synaptic counts in str rad declined, we observed a decline in the MMSE ($r = 0.595$; $p = 0.001$), the immediate word list recall ($r = 0.547$; $p = 0.005$), the delayed word list recall ($r = 0.593$; $p = 0.005$), and the Boston Naming ($r = 0.601$; $p = 0.001$) (figure 3). Total synaptic counts did not correlate with delayed word list recognition ($r = 0.371$; $p > 0.05$). Volume of str rad also correlated with psychometric testing. Loss of synaptic input to str rad reflects a loss of afferent input most notably from the ipsilateral CA3 pyramidal cells (Schaffer collaterals) that could account for a decline in neuropil volume. The decline in str rad of CA1 regio superior neuropil volume mirrored the decline in both the MMSE ($r = 0.445$; $p = 0.02$) and the delayed word list recall ($r = 0.485$; $p = 0.012$).

Total synaptic number, Braak staging, and Reagan score. Possible associations of NFT pathology and total number of synapses in str rad of CA1 regio superior were evaluated by probing the association of synaptic counts and Braak staging. There was no correlation between the individual's Braak score and total synaptic numbers in str rad ($r = 0.055$; $p > 0.78$). We also evaluated a possible relationship by grouping the data into three different levels of Braak stages (I to II; III to IV; V to VI). An ANOVA showed no difference between group synaptic count means ($F_{2,25} = 0.508$; $p > 0.61$). The results indicate that in the current investigation, Braak staging did not appear to have an influence on total synaptic numbers in str rad of CA1 regio superior. There was no correlation between the total number of synapses and the NIA Reagan score ($r = 0.163$; $p > 0.41$).

Relationship between *APOE* and synaptic numbers. The *APOE* genotype was determined for each of the subjects, and the number of synapses in each allele pairing group was analyzed. An ANOVA showed no difference between group means ($F = 1.702$; $df = 4, 23$; $p > 0.18$). Total synaptic counts were further grouped by whether or not the individual had any *APOE4* allele. An unpaired t test failed to detect a difference between these two groups ($t = 1.482$; $df = 26$; $p > 0.15$). These results indicate that in the current sample, *APOE* genotype did not influence total synaptic numbers in CA1 str rad.

Coefficient of error and coefficient of variance. The procedures for calculating the coefficient of error (CE), representing the sampling variance, and the coefficient of variance (CV), representing the bio-

Figure 3 Relationship of total number of synapses in CA1 stratum radiatum and the subjects score on a Mini-Mental State Examination (MMSE) performed within 12 months of death



A relationship was observed with the score on the MMSE increasing as the total number of synapses increased. The relationship between the subject's total synaptic values and performance on both an immediate and a delayed word list recall and also a recognition tests showed a relationship. As the total number of synapses increased, so did the subjects' performance on this cognitive test, which is dependent on the integrity of the hippocampus. Individual points represent individual subjects' scores for each group. $^{*}p < 0.05$; $^{**}p < 0.01$.

logic variance, for estimating the total synaptic numbers were identical to those used previously.[31] The mean CE for all subjects in the current study was 0.037, representing the intrasubject variance, indicating the precision of the counting scheme. The CV among the three diagnostic groups was 0.39. This observed CV is a combination of the inherent biologic and intersubject variation. The ratio CE^2/CV^2 is 0.009, indicating that the precision of the estimate observed with this sampling scheme meets the criterion for optimal sampling.[31]

DISCUSSION This is the first study to estimate the total number of synapses in the human hippocampal CA1 str rad dendritic field using unbiased stereology. The results support and extend our previous findings of a marked loss of synaptic contacts in the hippocampal dentate gyrus in mAD.[16] The current study used the same subject pool but differs in that CA1 regio superior was studied, assessing primarily associational fiber synaptic input. This study demonstrates a decline (55%) in total synapses in individuals with mAD, which is greater than that observed in the outer molecular layer of the dentate gyrus (44%).[16] In this study, individuals with MCI demonstrated an 18% decline in synaptic contacts, which is also greater than that observed in the dentate gyrus (13%). Total synaptic numbers in the CA1 regio superior associate very strongly with several tests of cognitive ability. These results have important implications for the neuropathologic substrate of both MCI and mAD.

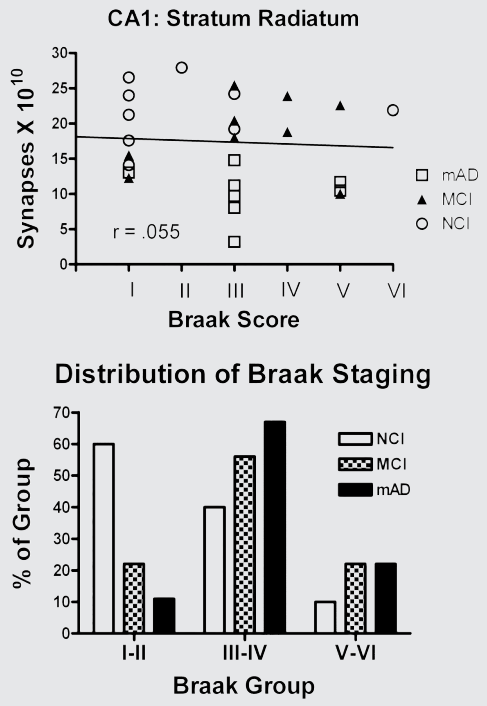
The subjects used in the current investigation were followed for several years (average 6.7 ± 2.4 years) and were initially enrolled without dementia. The groups were age and PMI matched to rule out these as possible variables affecting synapse numbers. The groups were also matched with regard to the number of male and female subjects. For a more complete description of the groups, see Scheff et al.[16] The cognitive testing scores prior to death were used as the sole criterion for differentiating the groups, with morphologic findings used as a dependent variable. The MCI and NCI groups were not different on the MMSE, whereas the mAD was lower from both NCI and MCI. All of the MCI cases used in this study were diagnosed as amnestic MCI.[32]

The mAD subjects, compared with the MCI group, had fewer synapses (45%) in CA1 str rad, suggesting that even in this early stage of AD, this brain structure is severely affected. An earlier biopsy study of the frontal lobe also reported synapse loss in mAD.[11] Although the NCI and MCI groups were not statistically different, the MCI group had a mean that was lower than the NCI cohort. The variance within the MCI group was quite large, with several subjects within the range of the mAD subjects and also within the range of the NCI subjects. This is not surprising considering the fact that MCI is believed to be a transition stage between NCI and mAD.[32] Individuals within the MCI group were identified using standard, uniform clinical evaluation prior to death,[17] eliminating possible selection bias based on neuropathologic criteria. These standardized cognitive tests show a very strong association with synaptic numbers within the hippocampus. Individuals with high synaptic counts performed the best on these measures. As shown in figure 3, almost all of the individuals with MCI performed within the same range as NCI subjects on the MMSE, Boston Naming, and a test of immediate recall. However, these subjects performed noticeably worse on delayed recall but were still superior to most of those in the mAD group.

The primate CA1 str rad dendritic field receives a variety of afferents, with the most prominent arising from the ipsilateral CA3 region. This projection (Schaffer collaterals) is part of a trisynaptic pathway originating in entorhinal cortex that contacts the dendritic tree of the ipsilateral granule cells, which in turn contact the CA3 pyramidal cells via the mossy fibers. The ipsilateral CA3 pyramidal cells give rise to the Schaffer collaterals that are responsible for the major synaptic input to the CA1

There was no trend in the data indicating the subject's Braak staging had no association with the number of synapses in this subregion of the hippocampus. Individuals with neurofibrillary tangle pathology were grouped into three combined categories of Braak staging (I to II, III to IV, V to VI) and the distribution for each of the three diagnostic groups are shown.

Figure 4 Relationship between total number of synapses in the CA1 stratum radiatum and the subject's Braak score



str rad.[33] There is also evidence of a direct projection from the entorhinal cortex to the CA1 lacunosum moleculare dendritic field.[34] The subiculum receives a major input from the CA1 pyramidal cells and in turn projects to both the ipsilateral and the contralateral entorhinal cortex.[35] As the CA1 region receives afferents from several sources, the synaptic loss seen in this area may arise from intrinsic as well as multiple extra hippocampal sites.

The synaptic decline observed in the MCI group represents a real loss of synapses that is independent of any change in the volume of the str rad. As shown in figure 2, the volume was identical between the NCI and MCI groups but less for mAD. Previous studies have suggested that hippocampal atrophy could be a marker for MCI.[36] The reasons for the loss in neuropil volume in mAD is probably not related to the loss of Schaffer collaterals, considering that several unbiased cell counting studies have failed to detect CA3 pyramidal cell loss in AD.[37-39] Most likely the loss can be attributed to declining pyramidal cell numbers in the CA1 sector that is known to occur in AD.[38,40-42] From the current published literature, it is unclear if CA1 neuron loss is significant in transitional stages.[39,40,43] If the loss is related to CA1 pyramidal cells and not CA3 afferents, it would suggest that postsynaptic contacts lose their ability to maintain adequate synaptic numbers. The maintenance of the volume of the str rad neuropil provides indirect evidence for a maintenance of CA1 pyramidal neurons. Prior studies have noted a change in synaptic size as a compensatory mechanism in AD that strongly associates with synaptic numbers.[44] A similar relationship was also identified in the current study in which total synaptic numbers inversely associated with synaptic size (data not shown).

Currently, there are relatively few studies linking AD-like neuropathology with MCI. The majority of these studies have linked the presence of neuritic plaques and β-amyloid deposition in various cortical regions to cognitive impairment[26,45-48] or the presence of tau-like AD pathology.[5,49,50] It is possible that the absence of any association between synaptic numbers and either the Braak score, as shown in figure 4, or the Reagan criteria is an indication that these classic AD lesions have a limited etiologic role in the onset of dementia of the AD type. One investigation using subjects from the ROS linked cell loss in the entorhinal cortex as an early event in the progression of AD.[51] On the other hand, others have failed to link preclinical AD to cell loss in the hippocampus.[39,40] The current results and those from the hippocampal dentate gyrus[16] generated from the same cohort of cases are the only studies suggesting a reduction in synaptic numbers with MCI.

The loss of synapses in str rad could be related to problems associated with pyramidal cells in the CA1 region. These are relatively large projection neurons of the hippocampus, having high energy requirements and the necessity for important trophic support. The uptake and release mechanisms within the synaptic complex are energy dependent requiring ATP. Imaging studies have reported reductions in glucose metabolism as a consequence of AD reflecting true metabolic reductions.[52-54] The decline appears to be related to a reduction in cellular function and not neuronal loss. Decline in cellular function is associated with ATP production involving mitochondrial energy metabolism. There is a loss of mitochondria in AD, and increased mitochondrial DNA damage leads to a greater cellular susceptibility to oxidative damage. Oxidative damage is considered to be one of the earliest events in AD and precedes β-amyloid deposition[55] and is present in MCI.[56] In addition, the accumulation of amyloid precursor protein in neurons also results in mitochondrial dysfunction, further impairing ATP synthesis.[57,58] Finally, β -amyloid has been reported to exacerbate the opening of the mitochondrial permeability transition pore, resulting in mitochondrial swelling and increased oxidative stress. Synapses may be the most vulnerable region of the neuron, and, as such, their loss in conditions such as MCI

and mAD may signal a change in the cellular milieu that most likely influences the fate of the neurons and subsequently cognitive function.

The sampling scheme used in this study was based on general principles for the optimal use of stereology.[59,60] Most of the effort in determining the total number of synapses, aside from the embedding procedure and ultrathin sectioning, was in the estimation of the individual synaptic numerical density. The CE determined for the applied sampling scheme was approximately 4%. The major source of the variance in a study of this type is the biologic variance. In any group of individuals, the number of synapses in individual hippocampi will vary. This biologic variance dictates the upper limit for the precision necessary to estimate the total number of synapses in the str rad. The greatest group CV was found in the MCI subjects (0.28) and the least in the NCI (0.22), most likely reflecting various stages of the disease process in the group with elevated risk for transition to dementia.

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REFERENCES

1. Arriagada PV, Marzloff K, Hyman BT. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 1992;42:1681–1688.
2. Goldman WP, Price JL, Storandt M, et al. Absence of cognitive impairment or decline in preclinical Alzheimer's disease. Neurology 2001;56:361–367.
3. Hubbard BM, Fenton GW, Anderson JM. A quantitative histological study of early clinical and preclinical Alzheimer's disease. Neuropathol Appl Neurobiol 1990;16:111–121.
4. Price JL, Davis PB, Morris JC, White DL. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. Neurobiol Aging 1991;12:295–312.
5. Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. Arch Neurol 2003;60:729–736.
6. Morris JC, Storandt M, McKeel DW Jr, et al. Cerebral amyloid deposition and diffuse plaques in "normal" aging: evidence for presymptomatic and very mild Alzheimer's disease. Neurology 1996;46:707–719.
7. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999;45:358–368.
8. Carlesimo GA, Sabbadini M, Fadda L, Caltagirone C . Word-list forgetting in young and elderly subjects: evidence for age-related decline in transferring information from transitory to permanent memory condition. Cortex 1997;33:155–166.
9. Parkin AJ, Lawrence A. A dissociation in the relation between memory tasks and frontal lobe tests in the normal elderly. Neuropsychologia 1994;32:1523–1532.
10. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999;56:303–308.
11. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 1990;27:457–464.
12. Lipton AM, Cullum CM, Satumtira S, et al. Contribution of asymmetric synapse loss to lateralizing clinical deficits in frontotemporal dementias. Arch Neurol 2001;58:1233–1239.
13. Samuel W, Masliah E, Hill LR, Butters N, Terry R. Hippocampal connectivity and Alzheimer's dementia: effects of synapse loss and tangle frequency in a two-component model. Neurology 1994;44:2081–2088.
14. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30:572–580.
15. Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State." A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
16. Scheff SW, Price D, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 2006;27:1372–1384.
17. Bennett DA, Wilson RS, Schneider JA, et al. Natural history of mild cognitive impairment in older persons. Neurology 2002;59:198–205.
18. DeKosky ST, Ikonomovic MD, Styren SD, et al. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. Ann Neurol 2002;51:145–155.
19. Mufson EJ, Ma SY, Cochran EJ, et al. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. J Comp Neurol 2000;427:19–30.
20. Davis DG, Schmitt FA, Wekstein DR, Markesbery WR. Alzheimer neuropathologic alterations in aged cognitively normal subjects. J Neuropathol Exp Neurol 1999;58:376–388.
21. Aggarwal NT, Wilson RS, Beck TL, Bienias JL, Bennett DA. Mild cognitive impairment in different functional domains and incident Alzheimer's disease. J Neurol Neurosurg Psychiatry 2005;76:1479–1484.
22. Pittman J, Andrews H, Tatemichi T, et al. Diagnosis of dementia in a heterogeneous population. A comparison of paradigm-based diagnosis and physician's diagnosis. Arch Neurol 1992;49:461–467.
23. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
24. Flicker C, Ferris SH, Reisberg B. Mild cognitive impairment in the elderly: predictors of dementia. Neurology 1991;41:1006–1009.

25. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology 2006;66:1837–1844.
26. Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR. Neuropathologic substrate of mild cognitive impairment. Arch Neurol 2006;63:38–46.
27. Gundersen HJ. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson J Microsc 1986;143:3–45.
28. Calverley RK, Jones DG. Determination of the numerical density of perforated synapses in rat neocortex. Cell Tissue Res 1987;248:399–407.
29. Weibel ER. Point counting method. In: Weibel ER, ed. Stereological method. London: Academic Press, 1979: 101–161.
30. Scheff SW, Sparks DL, Price DA. Quantitative assessment of synaptic density in the outer molecular layer of the hippocampal dentate gyrus in Alzheimer's disease. Dementia 1996;7:226–232.
31. West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 1991;231:482–497.
32. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med 2004;256:183–194.
33. Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 1989;31:571–591.
34. Witter MP, Van Hoesen GW, Amaral DG. Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. J Neurosci 1989;9:216–228.
35. Amaral DG, Insausti R, Cowan WM. The commissural connections of the monkey hippocampal formation. J Comp Neurol 1984;224:307–336.
36. Wolf H, Hensel A, Kruggel F, et al. Structural correlates of mild cognitive impairment. Neurobiol Aging 2004;25: 913–924.
37. Simic G, Kostovic I, Winglad B, Bogdanovic N. Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. J Comp Neurol 1997;379:482–494.
38. West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet 1994;344:769–772.
39. West MJ, Kawas CH, Stewart WF, Rudow GL, Troncoso JC. Hippocampal neurons in pre-clinical Alzheimer's disease. Neurobiol Aging 2004;25:1205–1212.
40. Hof PR, Bussiere T, Gold G, et al. Stereologic evidence for persistence of viable neurons in layer II of the entorhinal cortex and the CA1 field in Alzheimer disease. J Neuropathol Exp Neurol 2003;62:55–67.
41. Kril JJ, Patel S, Harding AJ, Halliday GM. Neuron loss from the hippocampus of Alzheimer's disease exceeds extracellular neurofibrillary tangle formation. Acta Neuropathol (Berl) 2002;103:370–376.
42. Price JL, Ko AI, Wade MJ, Tsou SK, McKeel DW, Morris JC. Neuron number in the entorhinal cortex and CA1 in preclinical Alzheimer disease. Arch Neurol 2001;58:1395–1402.
43. von Gunten A, Kovari E, Bussiere T, et al. Cognitive impact of neuronal pathology in the entorhinal cortex and CA1 field in Alzheimer's disease. Neurobiol Aging 2006; 27:270–277.
44. Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. Neurobiol Aging 2003;24:1029–1046.
45. Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. Neurology 2005;64:834–841.
46. Haroutunian V, Perl DP, Purohit DP, et al. Regional distribution of neuritic plaques in the nondemented elderly and subjects with very mild Alzheimer disease. Arch Neurol 1998;55:1185–1191.
47. Morris JC, Storandt M, Miller JP, et al. Mild cognitive impairment represents early-stage Alzheimer disease. Arch Neurol 2001;58:397–405.
48. Mufson EJ, Chen EY, Cochran EJ, Beckett LA, Bennett DA, Kordower JH. Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. Exp Neurol 1999;158:469–490.
49. Mesulam M, Shaw P, Mash D, Weintraub S. Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. Ann Neurol 2004;55:815–828.
50. Mitchell TW, Mufson EJ, Schneider JA, et al. Parahippocampal tau pathology in healthy aging, mild cognitive impairment, and early Alzheimer's disease. Ann Neurol 2002;51:182–189.
51. Kordower JH, Chu Y, Stebbins GT, et al. Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. Ann Neurol 2001;49: 202–213.
52. Drzezga A, Lautenschlager N, Siebner H, et al. Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: a PET follow-up study. Eur J Nucl Med Mol Imaging 2003;30: 1104–1113.
53. Ibanez V, Pietrini P, Alexander GE, et al. Regional glucose metabolic abnormalities are not the result of atrophy in Alzheimer's disease. Neurology 1998;50:1585–1593.
54. Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol 1997;42:85–94.
55. Nunomura A, Perry G, Aliev G, et al. Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001;60:759–767.
56. Keller JN, Schmitt FA, Scheff SW, et al. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 2005;64:1152–1156.
57. Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 2000;1502:139–144.
58. Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. J Neurochem 2002;80:91–100.
59. Coggeshall R, Lekan H. Methods for determining numbers of cells and synapses: A case for more uniform standards of review. J Comp Neurol 1996;364:6–15.
60. Schmitz C, Hof PR. Design-based stereology in neuroscience. Neuroscience 2005;130:813–831.