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Frequency of Hippocampal Formation Atrophy in Normal Aging and Alzheimer's Disease

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DE LEON, M. J., A. E. GEORGE, J. GOLOMB, C. TARSHISH, A. CONVIT, A. KLUGER, S. DE SANTI, T. MCRAE, S. H. FERRIS, B. REISBERG, C. INCE, H. RUSINEK, M. BOBINSKI, B. QUINN, D. C. MILLER, H. M. WISNIEWSKI. *Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease.* NEUROBIOL AGING **18**(1) 1–11, 1997.—We used CT and MR to examine the frequency of occurrence of hippocampal formation atrophy (HA) in a research clinic population of 130 normal elderly, 72 nondemented patients with very mild memory and cognitive impairments (MCI), 73 mild Alzheimer's disease (AD) patients, and 130 patients with moderate to severe AD. HA was found in 29% of the normal elderly group and its frequency of occurrence was strongly related to increasing age. For normal elderly 60–75 years of age, 15% had HA; the proportion rose to 48% in subjects 76–90 years of age. Among the three groups of impaired patients, the frequencies of HA ranged from 78% in the MCI patients to 96% in the advanced AD group. Unlike the normal elderly group, the percentages were not related to age. In both the normal elderly group and MCI group disproportionately more males than females had HA. After controlling for learning and the effects of generalized brain changes as reflected in ventricular size, only in the normal group was HA associated with reduced delayed verbal recall performance. Follow-up examinations for 15 individuals with baseline HA, 4 who at entry were MCI and 11 probable AD, yielded clinical and neuropathologic diagnoses of AD in all cases. The results of the present study indicate that hippocampal formation atrophy is associated with memory and cognitive impairments. Further longitudinal and neuropathologic work is required to validate the relationship between hippocampal formation atrophy and AD. *Copyright © 1996 Elsevier Science Inc.*

Hippocampal formation MRI Neuroimaging Aging Alzheimer's disease Neuropathology Memory

A growing body of literature utilizing either clinical neuroimaging or postmortem neuropathology has observed hippocampal formation atrophy in both normal aging and in patients with mild cognitive impairments possibly representing an early stage of Alzheimer's disease (AD). Human hippocampal pyramidal neurons are vulnerable to age-related degenerative changes (8), and pathologic evidence suggests that the boundary between clinically defined (so-called) normal aging and the dementia associated with Alzheimer's disease (AD) can be anatomically appreciated in both the pattern and extent of involvement of the hippocampal formation and the neocortex. West et al. (49) reported substantial age-associated neuronal losses in the hilus of the dentate gyrus and the subiculum. These same regions showed larger neuronal losses in AD, but only the CA1 pyramidal region showed changes that were specific to AD. Other recent studies of nondemented elderly (including individuals with very mild cognitive impairments) have shown that with increasing age, medial temporal lobe regions were more vulnerable than neocortical regions to the deposition of neurofibrillary tangles (2,40,46). However, in demented patients, neurofibrillary tangles and senile plaques were consistently found postmortem in both the neocortex and the hippocampal formation (36). Together, these results suggest a medial temporal lobe locus for the early expression of AD-related pathologic changes and an association between dementia and the involvement of the neocortex.

In vivo human imaging studies have shown that the volume of the hippocampus is decreased in AD (10,29,31,33,43,44) and there is increased CSF in the surrounding complex of hippocampal fissures (14,16). However, there is less information describing the normal aging of the medial temporal lobe and the relationship of such brain changes to mild cognitive impairments. The available data do, however, support observations derived across species, namely, hippocampal damage is related to memory dysfunctions (45). Several recent neuroimaging studies reported that hippocampal atrophy is associated with the very mild memory and other cognitive changes often found in community residing elderly. It was recently reported that variability in memory performance among carefully selected high functioning and well-educated normal elderly was significantly related to both the subjective rating of hippocampal formation atrophy (HA) (25) and the volume of the hippocampal formation (26). Evidence for the anatomic specificity for these changes comes from recent MRI studies of gyral subvolumes of the medial and lateral temporal lobe. Nondemented elderly subjects with mild memory impairments showed volume

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losses that were restricted to the hippocampus (9,10). Moreover, the hippocampal atrophy in such patients appears to have prognostic value in the prediction of further cognitive decline. A 4-year longitudinal follow-up of elderly subjects with mild memory impairments revealed that baseline HA ratings were sensitive and specific predictors of progressive cognitive and functional deterioration and the diagnosis of AD (14,16).

Given the large numbers of elderly in our society and our increasing awareness of the high percentage of individuals with complaints of memory impairments, the determination of the frequency of hippocampal formation atrophy in selected patient samples and the estimated prevalence in society is of great interest. Unfortunately, up until now this has not been possible because the small size of data sets in the literature, in part due to the difficulty of conducting large numbers of quantitative studies. In the present cross-sectional study we examined 405 carefully evaluated and medically screened elderly subjects for the presence of atrophy of the hippocampal formation using a rapidly determined qualitative measure. The groups studied included normal elderly, elderly individuals with mild cognitive impairment, and patients with probable Alzheimer's disease whose severity of dementia ranged between mild and severe. In addition, HA was evaluated for its relationships to memory performance, age, ventricular size, and gender.

METHOD

Subjects

Between 1983 and 1991, 616 subjects were evaluated at the New York University School of Medicine—Aging and Dementia Research Center. Selecting subjects based on their most recent clinic visit, a total of 405 carefully screened subjects met criteria for inclusion in the study. Approximately 68% of the study group were individuals receiving their first evaluation at the clinic. The study sample, like the community surrounding our medical center, was largely middle class, Caucasian and well educated (see Table 1). Subjects were considered for entry if they were between 60 and 90 years of age; and met both NINDS-ADRDA (35) and DSM III-R (1) diagnostic criteria for probable AD; or showed evidence of very mild cognitive impairments in the absence of a recognized or likely etiologic factor; or were considered cognitively normal. A comprehensive screening evaluation was used to identify pure groups of study patients who were free of potentially confounding conditions that could effect brain functioning or brain structure. The screening procedures, administered to all participants by the Clinical Core of our Alzheimer Disease Center, included medical, neurological, psychiatric, neuropsychological, and neuroradiological examinations. For both patients and normal elderly, all screening and clinical examinations were completed within 3 months of their CT or MRI scan date.

Individuals with identifiable conditions potentially associated with cognitive impairments, other than those thought to be AD related, were excluded (e.g., modified Hachinski Ischemia Scale scores (42) greater than 3, vitamin deficiency states, insulin dependent diabetes, history of significant head trauma, or substance abuse). Individuals with evidence for structural brain alterations that included masses, stroke or lacunar infarcts or evidence for hydrocephalus were excluded. In addition, subjects were excluded if they had a prior psychiatric history, received a Hamilton Depression Scale Score (28) of 18 or greater, or if they failed to discontinue any psychotropic or cognitively acting medication at least 2 weeks prior to the evaluation period. Of the 211 subjects (34%) that did not meet study criteria, 113 received other diagnoses, typically stroke or depression, 59 subjects were out of the age range (typically younger), and 39 did not have a technically correct CT or MR scan.

Staging for the general level of functional ability was conducted using the 7-point Global Deterioration Scale (GDS) (41). These ratings were administered by clinicians blind to the neuropsychological and neuroradiological test results. Based on the global staging, the selected study population was divided into four groups: 130 normal (NL) elderly controls (GDS 1–2) comprised of volunteers, often spouses or friends of patients; 72 elderly patients with mild cognitive impairment (MCI) that was not sufficient to meet criteria for dementia (GDS = 3); and 203 AD patients further subdivided into a mild AD group (GDS = 4, n = 73) and a moderate to severe AD group (GDS = 5–6, n = 130). Very severely impaired GDS = 7 patients are rarely evaluated at our outpatient research laboratory and were not included in this study. Table 1 shows that across the four groups there were no significant age differences. There were, however, differences in the years of education with the more cognitively impaired showing less educational achievement. Of the four groups studied, the NL group was included in a previously published work examining the relationship between hippocampal formation atrophy and memory (25).

Psychiatric and Psychometric Evaluations

In addition to the above screening instruments, the Mini Mental Status Examination (MMSE) (21) was included to further characterize the overall level of functioning and to facilitate comparisons with other published studies (see Table 1). Each subject was ad-

TABLE 1

SUBJECT CHARACTERISTICS FOR FOUR CLINICAL GROUPS (n = 405)

GDS Group	n	% Female	Age	MMSE	% MR	Educ	% HS
1–2 Normal	130	48.5	71.8 (7.1)	29.1 (1.1)	58.5	15.5 (2.8)	95.5
3 MCI	72	51.4	74.3 (7.4)	25.8 (3.1)*	45.8	14.2 (2.9)*	91.4
4 AD-mild	73	61.6	73.5 (7.4)	21.6 (4.4)*†	47.9	13.8 (2.8)*	95.5
5–6 AD mod/sev	130	62.3	73.9 (7.4)	12.0 (6.0)*†‡	40.0	12.7 (3.4)*†	80.7

% MR = % of group with MR scan.
% HS = % of group graduated from high school.
(sd) = standard deviation
* Different from NL Group ($p < 0.05$).
† Different from MCI Group ($p < 0.05$).
‡ Different from AD-mild Group ($p < 0.05$).

ministered a psychometric test battery derived from the Guild Memory Test (23,24) and the Wechsler Adult Intelligence Scale (WAIS) (47) (see Table 2). The Guild Memory Test battery included the immediate and 5-min delayed recall of 2 paragraphs and 10 verbal paired associates, the immediate recall of 10 numbers paired with 10 abstract designs. The WAIS subtests included digit symbol substitution, digits span forward and backward and vocabulary.

CT/MR Studies

Of the 405 individuals included in the study, 209 had a CT scan and 196 an MR scan (see Table 1 for the scan modality by clinical group breakdown). The CT scans were performed either on a GE 8800 or GE 9800 scanner and the MR scans were performed on a 1.5 tesla Phillips Gyroscan imager. In order to assess the comparability of the two imaging modalities, 56 subjects received both examinations. For both CT and MR, subjects were imaged using a clinical screening protocol and a research protocol.

The screening protocol. This examination was designed to cover the whole brain in order to identify specific lesions and permit a characterization of the extent of brain atrophy. For CT, this consisted of 10 mm contiguous axial slices from the base to vertex parallel to the cantho-meatal (CM) plane. For MR, we first obtained 12 T_1-weighted scout sagittal images (TR = 630 ms, TE = 20 ms, with 6 mm thick with 20% gaps). Based on visual inspection of the sagittal scout images, two additional scan orientations were defined for the acquisition of the additional MRI images. An axial plane and was defined as parallel to the long axis of the hippocampal body and a coronal plane was defined as perpendicular to the axial plane. For the screening MR study, axial spin echo image data were obtained consisting of 18 proton density weighted and 18 T_2-weighted slices (TR = 2516 ms, TE = 29 ms and 80 ms, with 6 mm slice thickness and 10% gaps). The coronal imaging resulted in a set of 18 T_1-weighted (TR = 630 ms, TE = 20 ms, with 4 mm slice thickness and 10% gaps). All CT and MR images were read by a neuroradiologist and formally assessed for the presence of intracranial abnormalities.

The research protocol. Both the CT and the MR research protocols yielded sets of axial images approximately parallel to the long axis of the hippocampus. These images were used in the hippocampal formation evaluations (see below). For CT this consisted of six contiguous 5 mm axial slices with a negative gantry angulation that permitted scanning at approximately 20 degrees negative to the CM plane. For the axial MR study, 18 T_1-weighted images were obtained (TR = 630 ms, TE = 20 ms, with 4 mm slices with 10% gap). The consequence of relying on internal (MR) versus external (CT) landmarks for the set up of the scan angulation resulted in MR angulations that were on the average 20 degrees steeper (closer to a coronal plane) than the CT. The CT/MR comparability studies showed that these protocol differences did not affect the hippocampal formation atrophy evaluations (Section C., below).

Hippocampal Evaluations

The purpose of this study was to assess the frequency of hippocampal formation atrophy in a large number of subjects. To conduct volumetric measurements, which are very time consuming, in hundreds of subjects is not feasible. Moreover, accurate volume measurements of the hippocampus, which require a coronal orientation, were not possible with the axially derived CT scans. Inadequate Z-axis resolution would have degraded the quality of the reformatted data. Relatively poor gray matter–white matter contrast on the CT scans further limits the anatomic judgments. Consequently, these limitations on quantitative study would have required us to restrict the study to the MR scans, thereby reducing the sample by approximately 50%. We, therefore, used a validated subjective rating of HA that permitted us to combine evaluations from both the CT and the MR scans. Anatomic and pathologic validation studies for the subjective observations were previously conducted (38). The relationship between the subjective rating and MR volumetry have also been reported (9,16).

For all study subjects, the hippocampal formation was examined for CSF accumulation in the regions of the transverse, choroidal, and hippocampal fissures. The anatomical basis for this assessment is described in detail in a previous publication (16). For each hemisphere, using previously published procedures, the extent of HA was rated on a 4-point scale: (0—none, 1—questionable, 2—mild/moderate, and 3—severe) (14,16,17,22,25). A cutoff score of 2 or greater (definite CSF accumulation) on either hemisphere was considered evidence for qualitative HA (see Fig. 1). All scans were rated by an experienced observer blind to the patients clinical status. The interrater reliability for this assessment, conducted blind to other results, was found to be quite high. For 25 CT scans, the kappa measure of agreement between the two observers for the full range of rating scores (0–3), was 0.72 for the right hemisphere and 0.71 for the left hemisphere ($p < 0.001$). Using the cutoff score for HA, the agreement between observers for the right and left ratings was kappa = 0.92 and 0.91, respectively ($p < 0.001$). Dichotomizing the sample for the presence of at least one hemisphere with an HA rating of ≥ 2, there was 100% concordance of classification between observers (16).

TABLE 2

UNCORRECTED GROUP MEANS (SD) FOR THE NEUROPSYCHOLOGICAL EVALUATION

Variable/Group	NL	MCI	Mild AD	Mod AD
Paragraph immed	8.4 (3.4)	4.9 (2.8)	2.8 (2.3)	0.8 (1.1)
Paragraph delay	10.4 (4.0)	4.0 (3.6)	1.0 (2.0)	0.1 (.4)
Pair assoc immed	4.1 (2.3)	1.6 (1.7)	0.6 (1.1)	0.1 (.4)
Pair assoc delay	4.7 (2.7)	1.6 (2.0)	0.5 (1.1)	0.1 (.3)
Designs	5.5 (2.2)	2.9 (2.2)	1.3 (1.3)	0.4 (.8)
Digit symbol	48.0 (12.4)	30.3 (14.8)	23.1 (14.7)	6.4 (8.9)
Digits foward	7.0 (1.3)	6.1 (1.6)	5.7 (1.7)	3.7 (2.4)
Digits back	5.6 (1.3)	4.4 (1.4)	3.7 (1.5)	1.9 (1.6)
Vocabulary	67.0 (11.3)	56.3 (19.2)	46.1 (21.4)	21.0 (20.7)
% Correct-immediate	39.9 (16.1)	19.5 (13.4)	9.3 (8.1)	2.4 (3.5)
% Correct-delay	47.6 (19.9)	17.4 (16.6)	4.6 (8.3)	.6 (2.1)

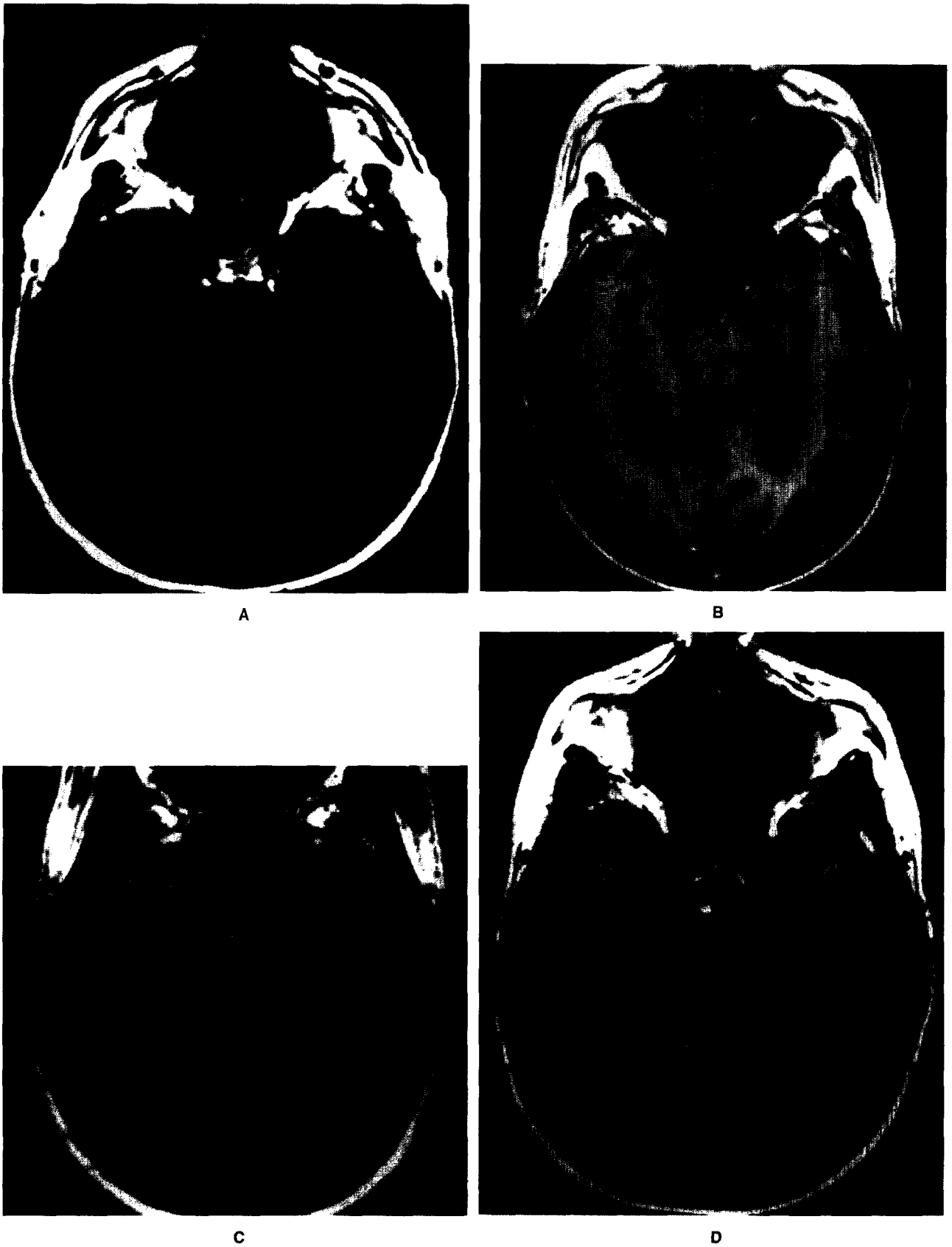

FIG. 1. (A–D) Four MR scans depicting the rating scale evaluations for hippocampal formation atrophy. The arrows in the left hemisphere (on the readers right side) highlight the anterior (level of the pes hippocampus) and posterior extent (level of the pulvinar-thalamus) of the visualized peri-hippocampal CSF. Part A. was rated as normal and B as questionable atrophy, both scans were classified as not atrophic. Part C was rated as mild and D as a moderate level of atrophy, scans C and D were classified as atrophic.

Evaluations of Ventricle Size

For reference purposes, ventricular size was also estimated using a 7-point rating scale. The scale was derived from our previously published 4-point scale, which is based on anchor points for ratings of 1 (normal), 3 (mild), 5 (moderate), 7 (severe) (12). In this earlier study, the scale was shown in elderly cognitively impaired patients to have a good relationship with linear estimates of ventricular size. The additional intermediate rating points we used in this and other studies (13) were scored 2 (very mild), 4 (mild to moderate), and 6 (moderate to severe).

Cross Imaging Modality Methodological Studies

Several studies were conducted to examine the appropriateness of combining the CT with the MR data. During 1986 when the MR scan replaced the CT in our standard AD workup, 56 subjects were intentionally studied on both machines. The average time interval between the CT and the MR scans was 3 months (SD = 3 months). Independent blind evaluation of the hippocampal region for each machine indicated that only 1 out of the 56 cases was not consistently classified for the presence of HA. Specifically, 22 cases were classified HA negative on both machines and of the remaining 34 cases, 33 CT scans and 34 MR scans were classified HA positive (see Fig. 2). The resultant phi-kappa value for cross machine agreement was determined to be 0.96, $p < 0.001$. Using the actual individual hemispheric rating scale values yielded cross machine correlation coefficients that ranged between 0.87 for the right hemisphere and 0.89 for the left. For the ventricle evaluations, an excellent correlation was also determined between the CT and MR ratings ($r = 0.92$, $p < 0.001$, $n = 56$).

Demographic variables were also examined prior to combining the CT and MR patient samples in the current study. One-way analyses of variance (ANOVA) showed no differences in years of education or age between the CT and MR groups. However, the two groups did differ in their average MMSE scores, reflecting the tendency for our physicians to prescribe the briefer, relatively less demanding CT study for the more severe AD patients. We also investigated, but failed to find, a crossmachine bias in the sensitivity for detection of HA by examining cognitive impairment scores. In other words, a machine bias would have produced a difference in the cognitive performances for individuals with CT-HA vs. MRI-HA. For this investigation, a separate ANOVA examined the severity of dementia (MMSE) as the dependent variable and the type of imaging modality, presence or absence of HA, and gender group as the independent variables. We failed to find any significant interactions with HA. Therefore, we concluded that across machines and gender group there was no systematic bias for the detection of HA as reflected in the severity of cognitive impairment. Gender group was examined so as to rule out the possibility that head size was related to the detection of HA.

The excellent agreement between CT and MR ratings as well as the absence of a machine-related bias provided evidence for the equivalence of the CT and MR ratings of HA and ventricle size. Consequently, for the remaining analyses the CT and the MR data sets were combined.

RESULTS

Relationship of HA to Age

Several statistical approaches were used to examine the effects of age. In order to contrast our findings with other published studies the subjects were dichotomized into young-old (60–75) and old-old (76–90) groups. Correlations using the continuous values of age were also examined. Over all 405 subjects, the data showed significantly more HA in the old-old group, $\chi^2(1) = 13.5$, $p < 0.001$. Further breakdown into the four GDS groupings revealed that the age effect was limited to the NL group, $\chi^2(1) = 16.4$, $p < 0.001$). In the NL group, 15% of the young-old and 48% of the old-old showed HA. Similarly, using an ANOVA model with the continuous age values as the dependent variable, HA as the independent variable and years of education as a covariate, only for the NL group was there a significant relationship between HA and age, $F(1, 122) = 21.7$, $p < 0.001$. In the MCI, the mild AD and moderate/severe AD groups the relationships between HA and age were nonsignificant ($ps > 0.05$). Figure 3A and B depicts for each group, the relationship between the frequency of HA and age collapsed into 5-year age groups. The figure shows both the clear age-effect that is limited to the NL group and the disease effect (reported below).

Relationship of HA to Measures of Global Functioning

For the entire sample, the four GDS groups differed in the presence of HA, $\chi^2(3) = 154.2$, $p < 0.001$. HA was common among individuals with probable AD (GDS $\geq$ 4). It was found in 84% of the mild AD and in 96% of the moderate to severe AD patients. For the nondemented patients, 29% of the NL group (GDS $\leq$ 2) showed evidence for HA, as compared with 78% of the patients with MCI (GDS = 3). Comparison of the NL and MCI groups demonstrated a significant association between group and the percentage of subjects with HA, $\chi^2(1) = 45.4$, $p < 0.001$. Across the two AD groups (GDS 4 vs. GDS 5–6), the relationship was not significant.

Relationship of HA to Delayed Recall Measures

The raw score means and standard deviations for the individual subtests of the cognitive test battery for each of the four GDS groups are found in Table 2. Following recent work that has demonstrated the sensitivity of measures of delayed recall to very mild dementia (4,20,34,39,48) and to hippocampal atrophy in elderly normals (25,26), we examined in the NL and MCI groups the relationships between delayed recall performance and HA. Due to "floor" effects on measures of cognitive performance in demented patients, examination of these relationships was restricted to the NL and the MCI groups. First, separate composites for both the immediate and for delayed scores were constructed by averaging the percent correct for both the paired associates and the paragraphs recall subtests of the Guild Memory Test battery (see Table 2). Second, in a hierarchial regression model predicting the composite delayed recall, we controlled for potential confounds by entering as the first step age, education, and the composite immediate recall measure. In the next and final step, HA was entered into the model. The results showed that for the NL group, but not the MCI group, the addition of the HA significantly incremented the explained variance in the delayed recall measure ($R^2_{\text{overall}} = 0.77$, $p < 0.001$; $R^2_{\text{change}} = 0.01$, $F_{\text{change}}(1, 117) = 5.1$, $p < 0.05$). Logarithmic transformation to normalize the distribution of the MCI data did not change the outcome. In order to examine the behavioral specificity of the delayed recall finding, we separately examined the relationships between HA and the other neuropsychological measures including the composite immediate recall measured. The p-value for significance was adjusted using the Bonferroni technique to control for the possibility of spurious findings associated with multiple comparisons. The results revealed that after accounting for age and education in the hierarchical regression, the HA entry into the regression model did not significantly increment the variance accounted for in any other cognitive test score. Consequently, in the NL group only, delayed memory scores alone, after controlling for age, education and immediate memory scores, showed a relationship to HA.

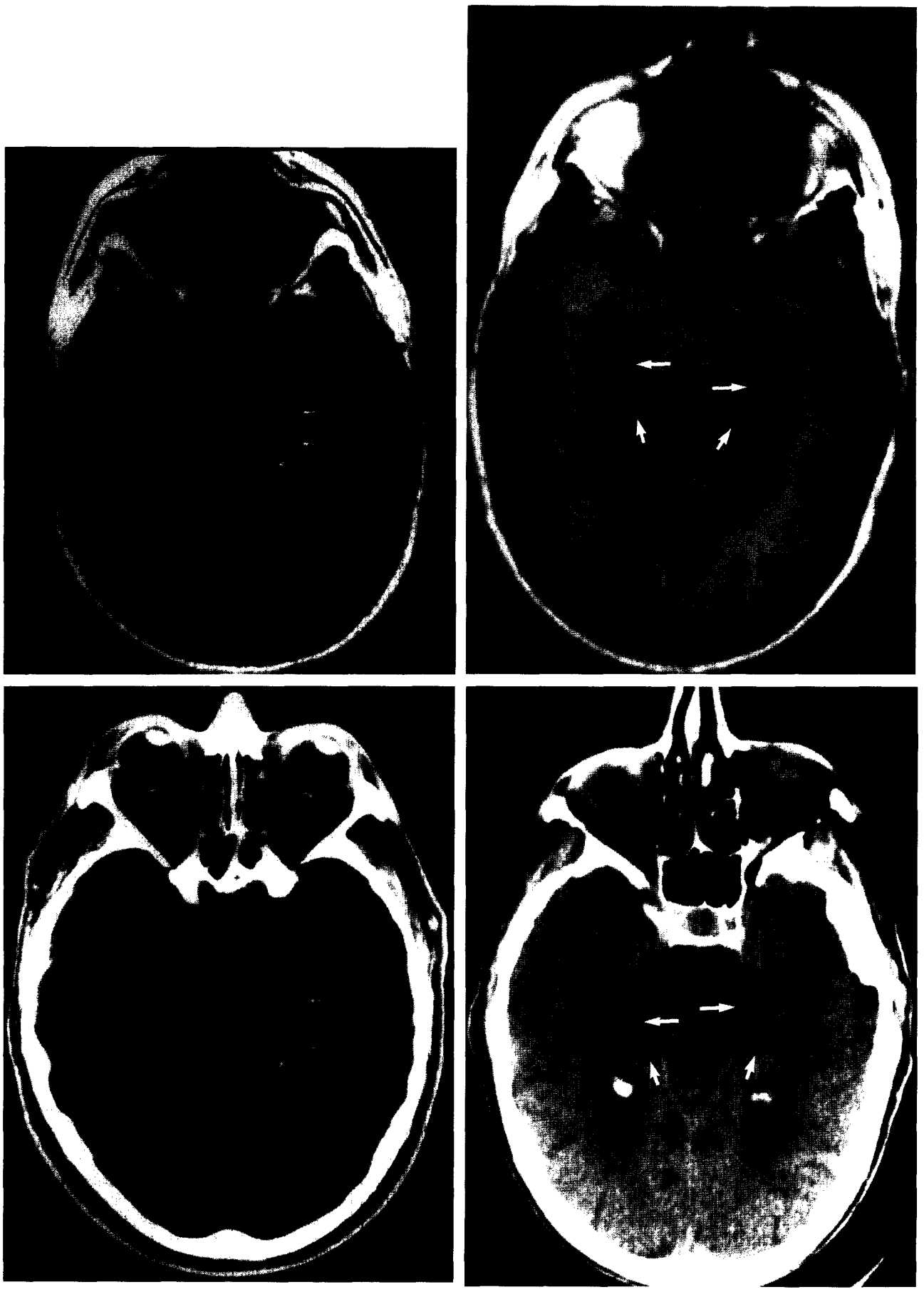

FIG. 2. Paired MR/CT studies derived from the validation study. For each study the MR scan is on the top with the corresponding CT below. The left pair shows a normal right hippocampus (readers left side) and a questionably atrophic left hippocampus on both modalities (small white arrows). This case was classified as normal on both modalities. The second pair, classified as atrophic on both modalities, shows bilateral hippocampal formation atrophy (large white arrows).

FREQUENCY OF SUBJECTS BY DIAGNOSTIC GROUP AND 5-YEAR AGE GROUPS

	60-64	65-69	70-74	75-79	80-84	85-89	TOTALS
NORMAL	24	31	23	35	12	5	130
MCI	9	15	5	24	14	5	72
AD-MILD	14	6	24	11	16	2	73
AD-MOD	17	27	20	30	30	6	130
TOTAL	64	79	72	100	72	18	405

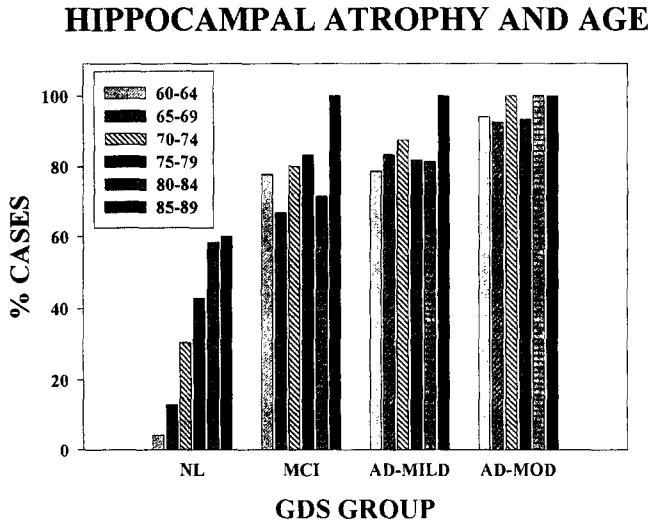

FIG. 3. The top table represents the frequency breakdown of the subjects in 5-year age bins by clinical group. The bottom histogram represents the percent of subjects in each 5-year age bin who have hippocampal atrophy for each of the four clinical groups. The abbreviations refer to: NL = Normal elderly; MCI = Mild cognitive impairment (GDS = 3); ADmild = Alzheimer's disease, mild; and ADmod = Alzheimer's disease, moderate.

Relationships Between HA and Ventricular Enlargement

Ventricular size was evaluated as the dependent variable in a 4 x 2 factorial analysis of covariance (ANCOVA) design with GDS group (four levels) and HA (two levels) as independent variables, and with age and education as covariates. The results indicated that ventricle size increased significantly with both increasing GDS level and the presence of HA, $F_{GDS}(3, 366) = 2.7$, $p < 0.05$; $F_{HA}(1, 366) = 18.6$, $p < 0.001$, and with their interaction, $F_{interaction}(3, 366) = 2.8$, $p < 0.05$. Post hoc Tukey HSD pair-wise comparisons using the same ANCOVA model showed that the NL group had significantly smaller ventricles than the two AD groups ($ps < 0.05$). The NL and the MCI group did not differ in ventricle size nor did the MCI group differ from the two AD groups. Overall, individuals with HA had larger ventricles than individuals without HA. Post hoc analysis of this result for each group indicated that HA was associated with increased ventricular size in the NL group, $F(1, 121) = 22.6$, $p < 0.001$, the MCI group, $F(1, 65) = 4.0$, $p < 0.05$, and the moderate AD group, $F(1, 111) = 8.2$, $p < 0.01$. No relationship was found in the mild AD group.

Evidence for the Anatomic Specificity of Delayed Recall Performance Changes

Following the above observations, we explored the anatomic specificity of the relationship between HA and delayed recall. In a hierarchical multiple regression model we examined separately the NL and the MCI patients. In a three-step model with the composite delayed recall as the dependent variable, we entered the composite immediate recall score in the first step, the ventricle size measure in the second step, and HA in the third and final step. The results indicated that for the NL group but not for the MCI group, HA significantly increased the explained variance in delayed recall performance ($R^2_{overall} = 0.85$), $R^2_{change} = 0.03$, $F_{change}(3, 123) = 12.1$, $p_{change} = 0.001$. With a second multiple regression model, the entry of the ventricle measure in the third step after HA had no incremental effect for either group ($p > 0.05$). We conclude that only in the NL group is there a significant relationship between HA and delayed recall, and that this relationship is independent of ventricle size.

Gender Relationship to HA

For the entire sample of both patients and normal elderly, there was no relationship between gender and HA. However, because our earlier work suggested an increased frequency of HA among elderly normal males, we examined the four study groups separately. Both the NL and the MCI groups showed a statistically significant excess proportion of males with HA. Specifically, in the NL group, 39% of males vs. 18% of females had HA, $\chi^2(1) = 7.3$, $p < 0.01$. In the MCI group, 89% of males vs. 68% of females had HA, $\chi^2(1) = 4.6$, $p < 0.05$. For each of the four study groups, follow-up two-way ANOVAs examined age as a dependent vari-

able with gender and HA as independent variables. The results indicated that males and females were no different in average age nor was there a gender by HA interaction affecting age ($ps > 0.05$). Consequently, in both NL and MCI groups, more males than females showed evidence for HA and this difference was not related to age.

Unilaterality and Bilaterality of HA

An analysis restricted to those subjects with HA revealed that with increasing cognitive impairment (GDS group), there was an increasing percentage of individuals with bilateral HA, $\chi^2(3) = 8.0$, $n = 279$, $p < 0.05$. Over all four groups, the proportion of bilateral HA cases ranged between 60% in the NL group to 80% in the moderate to severe AD group. Among the unilateral HA cases, there was a nonsignificant trend for left hemisphere HA to be more common than right hemisphere HA.

Within each of the four GDS groups, we found no significant relationships ($p > 0.05$) between the dichotomous age grouping and the presence of unilateral or bilateral evidence for HA. That is, for the two levels of age there were similar proportions of bilateral cases within each diagnostic group. Moreover, gender was also not associated with an increased frequency of bilateral HA in any of the GDS groups.

In summary, with increasing cognitive impairment there was an increased frequency of bilateral HA that was unrelated to either age or gender.

Postmortem Validation of HA

During the period of observation of this cohort, 15 patients have come to autopsy. Of these patients, 4 were included in the present study as MCI and 11 as probable AD. At the time of death, all 15 patients showed clear features of dementia and all carried a clinical diagnosis of probable AD. Neuropathologic examinations confirmed AD in all 15 patients. Figure 4 shows the in vivo and postmortem hippocampal formation images of a former MCI patient who came to autopsy. Quantitative postmortem study of 13 of the 15 deceased AD patients and 5 controls that were not part of this study, revealed that for the AD group there was extensive hippocampal formation volume loss. There were volume losses, relative to controls, in Ammon's horn (40%), subicular complex (40%), and entorhinal cortex (60%). Only the dentate gyrus failed to show significant volume losses (6). Further examination of this material (three AD and three controls), concentrating on counts of neurons, senile plaques, and neurofibrillary tangles, demonstrated that the hippocampal formation volume losses were associated with both neuronal loss and the number of neurofibrillary tangles. The number of senile plaques and amyloid burden were not associated with hippocampal formation volume (5).

DISCUSSION

Is HA a Marker of Brain AD?

The results of this cross-sectional study showed that the proportion of elderly individuals with detectable CSF accumulations in the region of the hippocampal formation increases sharply with increasing cognitive impairment. The presence of HA was nearly universal in patients with clinically diagnosed AD. For the two groups of probable AD patients, HA was found in more than 90% of 203 patients and its occurrence was independent of age. These results are consistent with the well-established observation that at postmortem examination the hippocampus is nearly always atrophic in AD.

The neuropathologic basis for in vivo determined hippocampal formation atrophy remains preliminary. While both the present study and the results from another group (30) observed excellent relationship between the in vivo determined hippocampal formation atrophy and the pathologic diagnosis of AD, these suggestive associations do not explain the anatomic-pathologic basis of the in vivo determined atrophy. Moreover, owing to the years delay between the clinical observation of HA in our MCI patients and the autopsy, we cannot state with confidence that at the MCI stage (not meeting criteria for the clinical diagnosis of probable AD) these patients had histologic AD-related hippocampal formation changes. While other studies have reported that mildly impaired patients studied at autopsy tend to show AD pathology in the hippocampal formation, these studies did not perform in vivo imaging (2,40,46). Relatedly, some neuropathology reports have documented histologic AD changes in the hippocampal formation of some cognitively normal elderly (7,40,46), and one study reported that normal elderly with histologic AD changes in the hippocampal formation had reduced hippocampal formation volumes (11). However, due to the absence of both cognitive evaluations and neuroimaging in these studies, it must be concluded that the pathologic substrate responsible for in vivo detected hippocampal formation atrophy in cognitively normal elderly is unclear. Finally, because diagnostic specificity studies for HA have not been reported, it remains unknown if other brain diseases also cause HA. We, therefore, conclude that additional work is required to confirm the hypothesized relationship between imaged HA and the neuropathology of AD (15). Until the neuropathologic basis for the imaged atrophy can be determined, HA cannot be considered a marker for brain AD. Until the diagnostic specificity for HA has been longitudinally evaluated, HA cannot be considered a clinical marker for probable AD.

Is HA Related to Memory/Cognitive Impairments

Several lines of evidence contribute to our impression that HA may be related to memory and cognitive changes:

Several studies have shown that HA is associated with memory impairment in normal elderly subjects. Our previous studies showed that both the presence of HA (25) and the reduced volume of the hippocampus in normal elderly (10,26) were associated with relatively poor performance on tests of delayed memory performance. This finding is consistent with other observations that damage to the hippocampus results in memory deficits in both human and nonhuman species (45). The present study, which extends our earlier observations by including the MCI group, indicates that this relationship is only found in the normal elderly, and is independent of ventricular size (a measure of generalized atrophy). The basis for the selectivity of this relationship to the normal group is unknown. It is possible that other sites of brain damage, not reflected in a change in ventricle size and not examined in the present study, contributed to the memory deficits in the MCI cases without HA. Moreover, the lack of an HA-delayed recall relationship in the MCI group may in part be due to the relatively truncated ranges of psychometric performance found in this group. As discussed above, the present study also observed that the frequency of HA was increased with increasing cognitive impairment across the groups.

Longitudinal data from other imaging studies suggest that MCI patients with HA are at increased risk for future AD when compared to MCI without HA (14,16). While the data show that HA has predictive value in patients with mild impairments, there are no long-term prediction studies examining HA in normal elderly.

Cross-sectional MRI volume studies with MCI patients have identified anatomically specific volume reductions in the hippocampus (9,10). Most recently, a PET study reported a selective loss of medial temporal lobe glucose metabolism (18). Overall, these

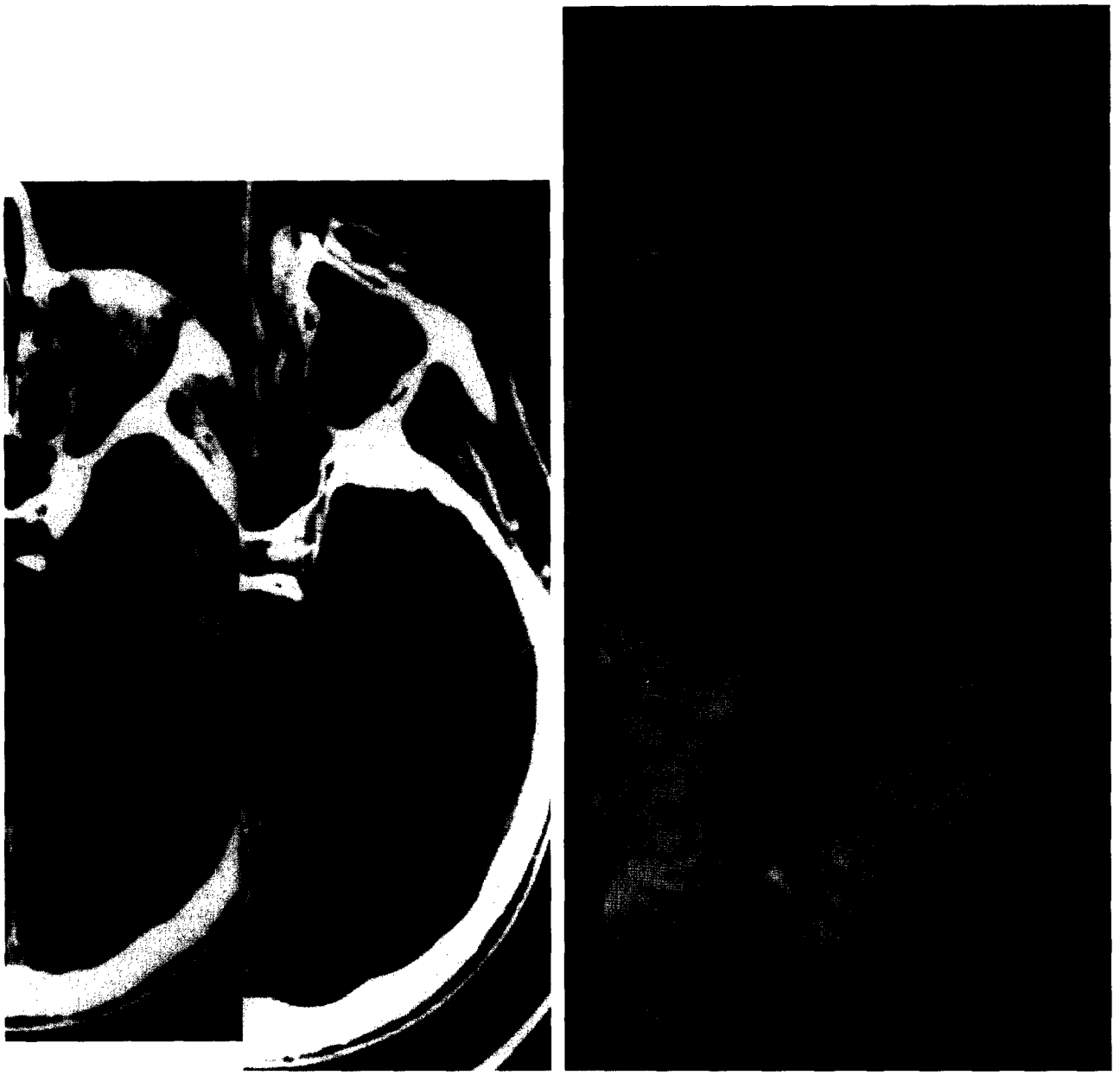

FIG. 4. Two CT and one postmortem MR scan in a male patient studied from 1985 to 1992. At the time of inclusion into the study, the patient was 85 years old and received an MCI diagnosis (GDS = 3). Studied annually, the patient experienced further cognitive decline and received the clinical diagnosis of probable AD (GDS 4) and a second CT scan in 1989. The postmortem examinations, which included MR, confirmed the AD diagnosis in 1992 (GDS 7). The open arrows highlight the CSF that defines the hippocampal formation atrophy.

recent data suggest that relatively localized medial temporal lobe changes occur in patients at increased risk for future AD. Future longitudinal study is required to establish the predictive validity of this pattern.

Population studies indicate an increased risk for dementia with increasing age. While methodologic differences among the population studies have resulted in varying absolute prevalence rates for dementia, there is clear evidence of age-related increases (32). Evans et al. (19) found that the prevalence for dementia rises from 3% in subjects between 65 and 74 years to 19% in those 75 to 84 years and to 47% in those over 85 years. Bachman et al. (3) reported prevalence rates at 0.5% at 65 to 74 years, 4.1% at 74 to 84 years and 13% for those greater than 84 years. In the present study, the frequency of HA showed both a strong relationship to age and memory performance in normal elderly. The frequency of HA was 15% for individuals between 60 and 75 years of age, but it was more than threefold greater (48%) for individuals greater than 76 years. The data from our study are consistent with the possibility that after 80 years of age the majority of elderly develop HA and memory loss. However, our cross-sectional results are derived from a potentially biased clinic-based sample do not permit extrapolation to the general population regarding the prevalence of HA and the related risk for cognitive impairment and dementia.

In summary, in the absence of community-based longitudinal

studies with appropriate neuropathologic validations, it remains unknown if in a normal individual the presence of HA and very mild memory changes is a marker for an increased risk of developing future cognitive impairments and the symptoms of AD. Although more is known regarding the risks for the MCI patient, overall, in the absence of an independent biological marker that is sensitive to early AD, it may prove to be exceedingly difficult to establish the pathologic substrate HA.

The Association of HA with Gender

The results show a disproportionate number of males with HA in the normal and MCI groups. This unexpected result was not due to greater age among males. The present study does not provide an explanation for this finding. Another recent study found that after controlling for age, normal elderly males had more generalized brain atrophy than females (27). The two AD groups in the present study did not show a gender effect for HA.

Sample ascertainment biases could have a strong impact on the gender distributions both across and within the clinical groups. Our sample was comprised of well educated subjects, with the majority of those employed or recently retired at the time of the evaluation being male. It is conceivable that any detected change in competence at work (perhaps in association with HA) would be cause for relatively early self referral, and therefore biased towards males. A similar change in an unemployed or less educated individual might not be as noticeable or disturbing. Relatedly, it was recently reported that higher education was associated with earlier age of onset (due to better detection) and less education was associated with increased severity of dementia at first presentation (longer wait prior to referral) (37). Only longitudinal study, combined with unbiased epidemiologic sampling approaches, will reveal if males are at greater risk for progressive brain deterioration. To date, the longitudinal studies that have examined the prognostic significance of HA have not been large enough to address the gender issue and the present study did not employ unbiased subject sampling strategies.

SUMMARY

The results show that the frequency of cases with in vivo evidence for atrophy of the hippocampal formation increases with increasing levels of cognitive impairment across the range from normal to moderate and several levels of dementia. Nearly all AD patients show hippocampal formation atrophy. For each of the three cognitively impaired patient groups, this brain–behavior relationship was found to be independent of age. We interpret the presence of HA in a dementia patient as consistent with the diagnosis of AD. Only for the normal elderly was the frequency of hippocampal formation atrophy positively related to age, male gender, and to decreased memory performance. Among normals, the preliminary evidence suggests the possibility that the relationship between deficits in delayed recall and HA may be anatomically and neuropsychologically specific. The present results provides strong justification for future studies to examine the diagnostic specificity and the pathologic substrate of HA.

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