Memory impairments associated with hippocampal versus parahippocampal-gyrus atrophy: an MR volumetry study in Alzheimer’s disease

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Abstract — Delayed memory impairments and medial temporal-lobe atrophy are considered to be cardinal features of Alzheimer’s disease (AD). The goal of the present magnetic resonance (MR) volumetry study was to investigate the relationship between both features. We determined MR-derived estimates of hippocampal and parahippocampal volume in a sample of 27 AD patients and in a group of 26 healthy control subjects (NCs) of comparable age and education. We examined the performance of the two groups on immediate and delayed recall trials of an auditory-verbal list-learning task (CVLT), a visual non-verbal memory task (Visual Reproduction of the WMS-R), and a screening procedure that provides an estimate of overall cognitive functioning (DRS). Volumes of the hippocampus and the parahippocampal gyrus were significantly smaller in AD patients than in NCs. AD patients were impaired in their overall level of cognitive functioning and showed memory deficits under immediate and delayed recall conditions. The association between medial temporal-lobe atrophy and cognitive impairments in AD was found to be highly specific: Hippocampal volume correlated positively with delayed but not immediate recall of the verbal-auditory list learning task. In contrast, parahippocampal gyrus volume, specifically in the right hemisphere, was positively related to delayed but not immediate recall of the non-verbal visual memory task. In NCs, there was a trend towards a negative association between hippocampal volumes and delayed verbal recall. Our results suggest that hippocampal and parahippocampal gyrus atrophy in AD are related to distinct aspects of the patients’ memory impairments. Our findings have implications for current discussions regarding contributions of the hippocampus and the parahippocampal gyrus to memory in the intact human brain.

Key Words: anterograde amnesia; hippocampus; medial temporal lobes; human; episodic memory; magnetic resonance.

Impairments in episodic memory, i.e. memory for events distinct in time and space, and medial temporal-lobe atrophy are considered to be core features of Alzheimer’s disease (AD) and play a crucial role in its diagnosis [10, 28, 76, 83]. A large body of neuropsychological and neurological research has focused on either one or the other but few studies have investigated both abnormalities together in the same participants. Recently, however, research has started to address the relationship between memory impairments and medial temporal-lobe atrophy in AD [13, 16, 62, 84]. In the present study, we aim to show how an investigation of this relationship may help not only to determine the neural basis of specific cognitive impairments in AD but also to contribute to understanding of the organization of memory in the intact human brain.

Similar to research on the amnesic syndrome, much neuropsychological research on AD has been conducted in order to specify the nature of anterograde episodic memory impairments. It has generally become accepted that the memory impairment in AD is multifaceted and can affect processes that pertain to encoding, storage and
retrieval [24, 50]. It remains to be determined, however, whether all facets are caused by pathological changes in a single structure, conceivably the hippocampus, or whether pathological changes in multiple structures inside and possibly outside the medial temporal-lobe region contribute to these deficits.

Findings from lesion studies in rats, non-human primates and humans indicate that medial temporal-lobe structures are crucially involved in episodic memory [15, 70]. Whereas research has traditionally focused on contributions of the hippocampus, results from recent studies in non-human primates with isolated medial temporal-lobe lesions outside the hippocampus suggest that the parahippocampal gyrus, including perirhinal and entorhinal cortex, may also play an important role in memory [53, 72]. This notion is supported by functional brain imaging research in healthy humans in which involvement of the hippocampus and the parahippocampal gyrus in episodic memory has been found [19, 54, 55, 61, 73, 78]. Although there is evidence to suggest memory contributions of both structures, it remains to be determined whether the hippocampus and the parahippocampal gyrus play a similar role in memory or whether they support distinct processes [77].

Both the hippocampus and the parahippocampal gyrus are among the primary regions targeted by AD [4, 10, 25, 31, 32]. Recent advances in quantitative analysis of magnetic resonance (MR) imaging have made it possible to quantify atrophy (i.e. remaining tissue volume) of the hippocampus and of the parahippocampal gyrus independently with a high degree of reliability [26, 58, 81, 87]. MR-volumetric estimates of atrophy can be used as indices of hippocampal and parahippocampal functioning in AD and can be correlated with behavioral data to obtain insight into the functional role of these structures in memory. Previous MR volumetry studies in AD have demonstrated that atrophy in the hippocampus but not in structures outside the medial temporal-lobe region, namely lateral temporal cortex and the cingulate nuclei, correlates with anterograde memory impairments [13, 16, 84]. Within the medial temporal lobes, hippocampal but not parahippocampal atrophy has been found to be associated with verbal anterograde memory impairments [84]. In line with findings in other organic memory disorders and with lesion research in non-human species [42, 57, 67, 85, 86, 89, 90], this association has been shown to be delay-dependent in AD in that it was observed for recall after a delay but not immediately after learning [84].

For the most part, previous research using MR volumetry in AD has concentrated on verbal memory [13, 84]. It has yet to be examined whether the reported pattern of relationships between memory deficits and atrophy in distinct medial temporal-lobe structures generalizes to recall of non-verbal information. Furthermore, previous studies have failed to compare the brain-behavior relationships observed in AD with those observed in healthy control participants. Thus, it is unclear whether the reported association between MR volumes and memory performance is specific to AD or whether it can also be observed when brain pathology and behavioral deficits are absent, as the results of some [21, 22] but not all studies [59] that have focused on healthy individuals alone, suggest.

The goal of the present MR volumetry study was to investigate the relationship between atrophy in distinct medial temporal-lobe structures and anterograde episodic memory deficits in a sample of AD patients and to compare it with the relationship between volumes of these structures and memory performance in a sample of healthy normal control participants (NCs). We obtained MR-derived estimates of hippocampal and parahippocampal gyrus volumes in both groups and examined them in relation to behavioral performance on immediate and delayed recall trials of a verbal list-learning task and a non-verbal visual memory task. To address the behavioral specificity of possible associations between MR volumes and memory performance, we also obtained an estimate of the overall level of cognitive functioning in each participant as a control measure.

Methods

Participants

Twenty-seven community-dwelling patients who met the NINCDS-ADRDA criteria [45] for probable AD participated in the study. Their demographic characteristics are described in Table 1. The severity of their disease as indicated by their Mini-Mental State Scores (MMS; [17]) ranged from mild to severe. Thirteen patients were mildly demented (MMS 21–27), 12 were moderately demented (MMS 11–20) and two were severely demented (MMS ≤10). The control group was comprised of 26 elderly community-dwelling individuals of comparable age and educational background (Table 1) with no presence or history of neurological and psychiatric impairments (as determined by a detailed health questionnaire). Inclusion in this control group was contingent on the absence of signs of dementia and age-associated memory impairment (AAMI; [9]) on the Mattis Dementia Rating Scale (inclusion with Total Score >123) and the California Verbal Learning Test (inclusion with Immediate Recall age- and sex-adjusted T-Score > 40), respectively. The average time that elapsed between neuropsychological testing and MR imaging was 3.0 months in the AD sample and 3.9 months in NCs. All par-

<p>| Table 1. Demographic and clinical description of AD patients and NCs |
|-------------------------------|-------------------|----------------|---|</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>AD patients</th>
<th>NCs</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>27</td>
<td>26</td>
<td>—</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>13/14</td>
<td>12/14</td>
<td>—</td>
</tr>
<tr>
<td>Age</td>
<td>70.1 (8.5)</td>
<td>70.8 (6.3)</td>
<td>ns</td>
</tr>
<tr>
<td>Years of education</td>
<td>12.7 (2.7)</td>
<td>13.2 (2.8)</td>
<td>ns</td>
</tr>
<tr>
<td>Mini mental state score</td>
<td>19.5 (4.7)</td>
<td>28.5 (1.3)</td>
<td>***</td>
</tr>
</tbody>
</table>

Note: $*** P < 0.001; n.s. $P > 0.10. Standard deviations are shown in parentheses.
Participants (or their caregivers) gave written informed consent. Ethical approval was obtained from the Research Ethics Board at Sunnybrook Health Sciences Centre.

Neuropsychological testing

Participants underwent individual testing on a large battery of neuropsychological tests as part of their clinical work-up. Performance data for the Mattis Dementia Rating Scale (DRS; [40], the California Verbal Learning Test (CVLT; [12]) and the Visual Reproduction Test of the WMS-R (VRT; [82]) were analysed for the purpose of the present study.* The DRS is a cognitive screening procedure that provides a score of overall cognitive functioning. The CVLT is a neuropsychological test that measures immediate and delayed recall for auditorily-presented verbal material. By contrast, the VRT measures immediate and delayed recall for visually-presented non-verbal material. The three test procedures were administered in the standardized manner. Administration of the CVLT, however, did not include all subsections of the test.

Participants were asked to learn and recall List A of the CVLT in five learning and free recall trials (immediate recall). The list is comprised of 16 words from hippocampal and parietal categories. Subsequently, an interference list of 16 words (List B) was presented for learning and free recall in a single trial. Upon completion of this trial, memory for the words of the first list (List A) was tested again in a free-recall trial (delayed recall). The delay between the last learning trial and the delayed recall trial was approximately 5 min. In our analyses of the CVLT data, we focused on two measures. To obtain a single score of immediate recall performance, we computed the sum of correctly recalled items from List A on Trials 1–5 (maximum of 80 items). To quantify delayed recall performance, we determined the number of correctly recalled items from List A after the 5 min delay (maximum of 16 items).

Administration of the VRT involved the visual presentation of four abstract figures for 10 s each. Participants were asked to memorize the first figure. Then, immediately after learning, they were asked to draw this figure from memory. The subsequence figures were presented in the same manner. Memory for the designs was tested again in a delayed recall trial after approximately 30 min filled with neuropsychological testing in unrelated domains. Scores for the immediate and delayed recall trials (each with a maximum of 41 points) were determined using the detailed scoring criteria of the WMS-R manual. The VRT was administered to only 18 of the 26 AD patients. Due to time constraints, the clinical protocol did not include a non-verbal memory test initially. Through the course of completion of the clinical investigation, however, the protocol was extended to include the VRT.

MR scanning

Brain images were acquired on a 1.5-T Signa system with a standard head coil (General Electric Medical Systems, Milwaukee, WI, U.S.A.). A sagittally-acquired 3D T1-weighted SPOG sequence was used for determination of hippocampal and parahippocampal gyrus volumes (TR/TE of 35/5 ms; 1 NEX; flip angle of 35°; field of view 22 cm; matrix size 256 × 256; imaging time 14.4 min). This sequence produced 124 contiguous slices of 1.3 mm thickness, which covered the whole brain. Images from a second sequence were used to allow for correction of variations in overall brain size. These additional images were obtained from an axial two-spin-echo sequence (i.e. proton density and T2-weighted images; half-Fourier sampling; 192 phase-encoding steps; TR/TE of 3000/30, 50 ms; 0.5 NEX; field of view 22 cm; matrix size 256 × 256; imaging time 11.6 min) that produced 58 contiguous and interleaved slices of 3 mm thickness, which also covered the whole brain.

Volumes of interest were determined using ANALYZE software (Mayo Foundation, Rochester, MN, U.S.A.) on a SUN workstation (SUN Microsystems, Mountain View, CA, U.S.A.) for display, reformatting of images, demarcation of structures, and volume calculations. Hippocampal measurements were obtained by adapting the protocol described by Jack et al. [26, 27]. According to this protocol, the hippocampus includes Ammon’s horn (CA1–CA4), dentate gyrus, alveus, fimbria and parts of the subiculum. We modified this protocol with the goal of excluding the subiculum, which is transitional cortex that connects Ammon’s horn with the parahippocampal gyrus, from the hippocampal measurements. A protocol comparable to the one described by Yoneda et al. [87] was used to measure the parahippocampal gyrus and to distinguish it from the hippocampus. This measurement included the parahippocampal gyrus proper (excluding portions inferior to the collateral sulcus), caudal aspects of entorhinal cortex, underlying white matter and medial aspects of the subiculum. In addition, it included caudal aspects of perirhinal cortex, which lines the superior bank of the collateral sulcus before it becomes the parahippocampal gyrus proper [3]. Care was taken not to include parts of the amygdala in either of the medial temporal-lobe measurements. Reference was made to a detailed atlas of the human hippocampus throughout the course of the measurements [14].

The measurements of the hippocampus and of intracranial capacity were made by either of two raters. The parahippocampal measurements were obtained by a single rater. Raters were blind to the cognitive performance data. To establish that the volumetric measurements were sufficiently reliable inter-rater agreement (indexed by the intraclass coefficient; ICC [65]) was determined for the measurements of the hippocampus and the parahippocampal gyrus. In addition, inter-rater agreement (also indexed by the ICC) was determined for the hippocampal measurement. All reliability coefficients were found to be above 0.80. The intra-rater reliability for the hippocampal volumes was estimated based on 36 hemispheres and was 0.91. The corresponding inter-rater reliability based on 20 hemispheres was 0.82. The intra-rater reliability for the parahippocampal volumes based on 20 hemispheres was 0.89.

The protocol for the volumetric measurements included the following steps. The T1-weighted images were first displayed in the sagittal plane to determine the longitudinal axis of the hippocampus and its anterior and posterior boundaries. Subsequently, they were reformatted into a series of approximately 55–60 contiguous slices of 0.859 mm thickness that were oriented perpendicular to this axis (oblique coronal orientation, see Fig. 1) and covered the hippocampus in its complete rostral-caudal extent. The anterior endpoint of the hippocampus was determined on the oblique coronal slices as previously described by Jack et al. [27]. Specifically, the most rostral slice was chosen on which the alveus (a white-matter marker with high signal intensity) forms the border between the hippocampus and the overlying amygdala. The posterior endpoint was determined by locating the most rostral oblique coronal slice on which the temporal horns separated from the main body of the lateral ventricles [5]. The location of this bifurcation relative to other anatomical structures is presumably unaffected by ventricular enlargement or cortical atrophy. The parahippocampal gyrus was also measured along the rostral-caudal extent of the hippocampal axis, starting anteriorly on the first slice on which the

* The CVLT and the VRT were the only neuropsychological tests of memory that were included in the larger battery of tests administered for the clinical work-up.
The hippocampus was visible. It was followed posteriorly up to the point where the anterior calcarine sulcus, which separates the parahippocampal sulcus from the isthmus of the posterior cingulate gyrus, becomes visible. In most cases, this endpoint was found anterior to the most posterior slice on which the hippocampus was measured. The hippocampal and parahippocampal regions were delineated manually between posterior and anterior endpoints in each hemisphere on every third slice of the reformatted images (Fig. 2). Manual tracing (planimetry) was used to delineate the hippocampus. To delineate the parahippocampal gyrus, we used stereology (point counting with grid-size = 3 × 3 × 3, shape coefficient = 3.91) because it provides a more efficient method to determine volumes when structures are sufficiently large [43, 64]. The demarcated areas were multiplied by slice thickness, interpolated across slices on which no measurements were taken and summed to provide the hippocampal and parahippocampal gyrus volume in each hemisphere. Because left and right volumes were found to be highly correlated (r = 0.94 for the hippocampus; r = 0.70 for the parahippocampal gyrus across participants of both groups) they were summed for the group comparison and most of the analyses of brain-behavior correlations to provide single measures of total hippocampal and parahippocampal volumes. Due to a technical problem with one of the scans, measurements for the parahippocampal gyrus were available in 15 of the 16 AD patients only.

To correct the hippocampal and parahippocampal measurements for variations in total intracranial capacity, whole brain volume was determined, using the T2-weighted images [32]. Images were first “edited” using a semi-automated procedure with intensity thresholds to separate brain tissue, including CSF, from non-brain tissue. Subsequently, each slice was visually inspected for the presence of remaining non-brain tissue. If present, this tissue was removed using manually-placed tracing limit lines. Finally, the whole brain volume, including cortex, cerebellum, brainstem and surrounding CSF was calculated from the edited images as an index of intracranial capacity. Following Jack et al. [27], this measure was included as a covariate in the multiple regression analyses on brain-behavior relationships.

Statistical analysis

Two-sided t-tests were performed to compare AD patients with NCs on the demographic, neuropsychological, and MR-volumetric measures. The relationship between medial temporal-lobe volumes and neuropsychological test measures was determined in each group separately using a three-step procedure. First, we computed the matrix of Pearson product-moment correlations between MR volumes and neuropsychological test scores in each group and performed an omnibus test on each matrix to determine whether any of the correlation coefficients significantly departed from zero. Performing an omnibus test before examining individual coefficients is important in situations in which large numbers of coefficients are tested because it guards against misinterpreting spuriously significant individual coefficients [8]. If the omnibus test showed a positive result we tested the significance of each individual coefficient in the matrix in a second step using t-tests. In a third step, we further investigated those associations that emerged as significant in step two by applying a multiple regression approach. For each neuropsychological measure that correlated with MR volumes significantly, we tested a single one-step regression model in which the test score was the dependent variable and the parahippocampal and hippocampal volumes were the predictors (i.e. independent variables). By including the volumes of both structures as predictors in the same regression model, we could directly determine their independent contributions to performance on each neuropsychological measure examined (see [16], for rationale). Moreover, we also controlled for possible confounding effects of intracranial capacity, age, education and sex by including them as covariates (i.e. additional independent variables) in these regression models.

Results

A comparison of the group means for the MR-volumetric measures and the neuropsychological measures is
Fig. 2. Samples of anterior (top) and posterior (bottom) oblique coronal slices perpendicular to the longitudinal hippocampal axis in an AD patient (left) and a NC (right). The top slices were taken at the level of the anterior commissure. The bottom slices were taken approximately 5 mm anterior to the bifurcation of the temporal horns of the lateral ventricles. For illustrative purposes, the hippocampus is delineated in the left hemisphere (right-hand side) and the parahippocampal gyrus is delineated in the right hemisphere. Note the atrophy present in both medial temporal-lobe structures in the AD patient as compared to the NC.
Table 1. Means and Ranges for MR volumetric and Behavioral Measures of AD Patients and NCs

<table>
<thead>
<tr>
<th>Variable</th>
<th>AD patients</th>
<th>NCs</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MR volumetric measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal volume (in mm(^3))</td>
<td>2065.2(^a)</td>
<td>2613.7(^b)</td>
<td>**</td>
</tr>
<tr>
<td>Parahippocampal volume (in mm(^3))</td>
<td>4342.1(^b)</td>
<td>5210.0(^b)</td>
<td>***</td>
</tr>
<tr>
<td>Intracranial capacity (in cm(^3))</td>
<td>1410.4(^c)</td>
<td>1434.3(^c)</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Dementia Rating Scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall cognitive functioning score</td>
<td>108.2(^a)</td>
<td>139.9(^b)</td>
<td>***</td>
</tr>
<tr>
<td><strong>California Verbal Learning Test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall</td>
<td>17.8(^a)</td>
<td>53.3(^b)</td>
<td>***</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>0.9(^a)</td>
<td>11.1(^b)</td>
<td>***</td>
</tr>
<tr>
<td><strong>WMS-R Visual Reproduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall</td>
<td>11.9(^c)</td>
<td>32.4(^b)</td>
<td>***</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>2.5(^c)</td>
<td>26.5(^b)</td>
<td>***</td>
</tr>
</tbody>
</table>

*Note: \( P \) values are for corresponding \( t \)-tests; \( * P < 0.05; ** P < 0.01; *** P < 0.001; n.s. P > 0.10; ^a n = 27; ^b n = 26; ^c n = 18."

shown in Table 2. The table also presents the results of the \( t \)-tests performed on these data. Figure 3 demonstrates that the total (i.e. sum of left- and right-sided) hippocampal volume (\( P < 0.01 \)) and the total parahippocampal volume (\( P < 0.001 \)) were smaller in AD patients than in NCs. This was also true when interindividual differences in total intracranial capacity were partialled out by means of analyses of covariance (\( F(1,50) = 9.3, P < 0.01 \) for hippocampus; \( F(1,49) = 15.4, P < 0.001 \) for parahippocampal gyrus). Hippocampal and parahippocampal volume were positively correlated in AD patients (\( r = 0.49, P < 0.05 \)) but not in NCs (\( r = 0.24; P > 0.10 \)).

Behaviorally, AD patients exhibited a significantly lower level of overall cognitive functioning than NCs as indicated by their DRS scores (Table 2). When episodic memory functioning was examined with the CVLT and the VRT, AD patients were found to be impaired on the immediate and the delayed recall measure of both tests (Table 2). The difference between groups in delayed recall performance persisted on both tests even when interindividual differences in initial learning were partialled out by means of covariance analyses (\( F(1,50) = 14.6, P < 0.001 \) for the CVLT; \( F(1,41) = 7.6, P < 0.01 \) for the VRT). A subgroup of AD patients was unable to recall any items on the CVLT (16 patients) and the VRT (9 patients) after the delay.

The omnibus test for the correlation matrix between neuropsychological test scores and volumetric measures in AD patients revealed that some elements of the matrix were significantly different from zero (\( \chi^2(10) = 29.0, P < 0.005 \)). When individual coefficients were examined, we found that only delayed memory performance correlated significantly with hippocampal or parahippocampal volume in AD. Delayed recall on the CVLT correlated positively with hippocampal volume whereas delayed recall on the VRT correlated positively with parahippocampal gyrus volume (Table 3). Importantly, the distribution of behavioral scores at floor also followed this relationship, i.e. their occurrence was related to the degree of hippocampal and parahippocampal gyrus atrophy. Those AD patients who obtained a delayed recall score of zero on the VRT showed smaller parahippocampal gyrus volumes than those who obtained a score above zero (\( P < 0.01 \)). Similarly, those AD patients...
Table 3. Pearson correlations between MR volumes and behavioral measures in AD patients

<table>
<thead>
<tr>
<th>Behavioral variable</th>
<th>Parahippocampal gyrus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dementia Rating Scale</td>
<td>−0.04^b</td>
<td>−0.04^a</td>
</tr>
<tr>
<td>Overall cognitive functioning score</td>
<td>0.15^b</td>
<td>0.21^a</td>
</tr>
<tr>
<td>California Verbal Learning Test</td>
<td>0.37^b</td>
<td>0.61***</td>
</tr>
<tr>
<td>Immediate recall</td>
<td>0.32^d</td>
<td>−0.02^e</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>0.61***</td>
<td>0.08^e</td>
</tr>
<tr>
<td>WMS-R Visual Reproduction</td>
<td>0.76***</td>
<td>0.01^c</td>
</tr>
</tbody>
</table>

Note: An omnibus test of the matrix showed an association between MR-volumetric measures and behavioral variables ($P < 0.005$). Significance levels are displayed for $t$-tests of individual coefficients; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. $^a$ $n = 27$; $^b$ $n = 26$; $^c$ $n = 18$; $^d$ $n = 17$.

who obtained a delayed-recall score of zero on the CVLT showed a trend towards smaller hippocampal volumes than those who obtained a score above zero ($P < 0.07$). To ascertain that recall scores of zero did not inflate the observed correlations we also examined the brain-behavior correlations in only those individuals who obtained recall scores above zero. Both correlations, i.e. the one between parahippocampal gyrus volume and delayed VRT recall, as well as the one between hippocampal and delayed CVLT recall, were numerically larger in the subsamples of AD patients with recall scores above zero ($VRT r = 0.72$; $P < 0.05$; $CVLT r = 0.64$; $P < 0.05$) than in the entire group. Thus, including AD patients with behavioral performance at floor in the total sample led, if at all, to an under-estimation but certainly not on over-estimation of the size of these associations.

In two subsequent multiple regression analyses, in which CVLT delayed recall or VRT delayed recall served as the dependent measure, we determined the independent (i.e. residualized for shared variance) contributions of hippocampal and parahippocampal volumes to behavioral performance while controlling for effects of other confounding variables. When the volumes of both structures together with intracranial capacity, sex, age and education were used as predictors, the regression analyses showed that the association between hippocampal volume and delayed CVLT performance and the association between parahippocampal gyrus volume and delayed VRT performance were robust and dissociable. Both regression models reached statistical significance ($CVLT$ delayed recall $R^2 = 0.49$, $P < 0.05$; $VRT$ delayed recall $R^2 = 0.76$, $P = 0.01$). In the model for the CVLT delayed recall score, hippocampal volume emerged as a significant predictor but parahippocampal gyrus volume did not (Table 4). By contrast, in the model for the VRT delayed recall score, parahippocampal gyrus volume emerged as a significant predictor but hippocampal volume did not (Table 4). To ascertain that these results were not due to differences in sample size, we repeated the regression analysis for the delayed CVLT score, including only the subsample on which the delayed VRT regression analysis was based. The pattern of results observed with this subsample was identical to the pattern observed with the larger sample.

Because atrophy in medial temporal-lobe regions was found to be highly symmetrical in AD, affecting left- and right-hemisphere structures to the same degree, we concentrated our analyses on total volumes of the hippocampus and the parahippocampal gyrus summed across both hemispheres (as reported previously). Nevertheless, to address laterality effects in brain-behavior correlations, we did perform additional regression analyses for those behavioral measures which were found to be related to hippocampal and parahippocampal volumes (CVLT delayed recall score, VRT delayed recall score). As in our previous analyses, we included the behavioral measures as the dependent variable and the MR-volumetric measures as the predictors while controlling for differences in intracranial capacity, age, sex and education. For each behavioral variable, we examined one model in which left-sided volumes served as predictors and another model in which right-sided volumes served as predictors. For the CVLT delayed recall score, both the left-sided and right-sided models were significant ($R^2 = 0.48$ and $0.49$, respectively; $P < 0.05$). In both models, hippocampal (left $\beta = 0.77$, $P < 0.05$; right $\beta = 0.77$, $P < 0.05$) but not parahippocampal gyrus volume (left $\beta = -0.07$, $P > 0.10$; right $\beta = -0.06$, $P > 0.10$) emerged as a predictor for delayed CVLT recall. For the VRT delayed recall score, the model in which right-sided volumes were included ($R^2 = 0.78$, $P < 0.01$) showed that the volume of the parahippocampal gyrus ($\beta = 0.60$, $P < 0.05$) but not of the hippocampus ($\beta = 0.38$, $P > 0.05$) was a significant pre-
dictor of performance. By contrast, in the model with
left-sided volumes \( (R^2 = 0.65, P = 0.05) \), neither the vol-
ume of the parahippocampal gyrus \( (\beta = 0.47, P > 0.10) \)
nor of the hippocampus \( (\beta = -0.16, P > 0.10) \) emerged
as a significant predictor of delayed VRT recall. In sum,
these analyses revealed laterality effects for delayed VRT
recall, with the right but not the left parahippocampal
gyrus emerging as a significant predictor with a positive
relationship to behavioral performance. By contrast, no
laterality effects were found with respect to delayed
CVLT performance, presumably due to the higher cor-
relation between left- and right-sided hippocampal vol-
umes \( (r = 0.97) \) as compared to parahippocampal gyrus
volumes \( (r = 0.71) \) in our sample of AD patients.

The omnibus test for the matrix of brain-behavior corre-
relations in NCs (Table 5) also revealed a significant
association between behavioral measures and MR vol-
umes \( (\chi^2(10) = 18.9, P < 0.05) \). Inspection of individual
correlation coefficients showed significant negative
associations between hippocampal volume and immediate
CVLT recall and between hippocampal volume and delayed
CVLT recall. These associations were further investigated in two multiple regression analyses in which we
included the behavioral measures as the dependent
variable and the MR-volumetric measures as the pre-
dictors while controlling for differences in intracranial
capacity, age, sex and education. In the analysis for the
CVLT immediate recall score, neither the overall regression model \( (R^2 = 0.26, P > 0.10) \) nor the stan-
dardized regression coefficients for the volume of the
hippocampus \( (\beta = -0.28, P > 0.10) \) and the par-
ahippocampal gyrus \( (\beta = -0.10, P > 0.10) \) reached sta-
tistical significance. By contrast, the regression model for
the CVLT delayed recall score did reach statistical sig-
nificance \( (R^2 = 0.53, P < 0.05) \). In this model, the hip-
 pocampal volume showed a significant trend as a
predictor \( (\beta = -0.35, P < 0.09) \) whereas the volume of
the parahippocampal gyrus failed to be of any predictive
value \( (\beta = -0.16, P > 0.10) \). The same pattern of results
was obtained when we repeated these analyses with uni-
lateral volumes as predictors, using the strategy pre-
viously employed for the analysis of AD data. Regardless
of whether the regression model included left- or right-
sided structures, the hippocampal volume showed a trend
as a negative predictor for CVLT delayed recall but not
for CVLT immediate recall. By contrast, left- and right-
sided volumes of the parahippocampal gyrus showed no
predictive value in any of these models.

**Discussion**

The results of the present study demonstrate a distinct
pattern of associations between episodic memory impair-
ments and atrophy in medial temporal-lobe structures in
AD. MR-derived volumes of the hippocampus and the
parahippocampal gyrus (with the latter including por-
tions of perirhinal and entorhinal cortex) were found
to correlate with delayed memory recall but not with
immediate memory recall nor with an estimate of overall
cognitive functioning. Hippocampal volume correlated
positively with delayed recall on a verbal-auditory list
learning task (CVLT) but not on a non-verbal visual
memory task (VRT). In contrast, parahippocampal gyrus
volume, specifically in the right hemisphere, was posi-
tively associated with delayed recall on the non-verbal
visual memory task but not the verbal-auditory list learn-
ing task. The pattern of brain-behavior correlations
observed in AD contrasted with the pattern of cor-
relations observed in NCs. In the latter group, a sig-
nificant trend towards a negative association between
hippocampal volume and CVLT delayed recall emerged.
Our findings have implications for an understanding of
memory impairments in AD, for theories concerning the
neural organization of memory in the intact human brain,
and for the functional interpretation of volumes of medial
temporal-lobe structures in healthy individuals. These
implications will be discussed in the ensuing paragraphs.

The comparison of the group of AD patients with NCs
showed differences both on the structural and on the
behavioral level. Our MR-volumetric results confirm that
medial temporal-lobe atrophy in AD affects the hip-
 pocampus as well as the surrounding parahippocampal
gyrus \( [26, 31–33, 63] \). Behaviorally, AD patients showed
anterograde episodic memory deficits in verbal and non-
verbal recall immediately after learning and after a
delay. Delayed recall of verbal material from the CVLT

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**Table 5. Pearson correlations between MR volumes and
behavioral measures in NCs**

<table>
<thead>
<tr>
<th>Behavioral variable</th>
<th>Parahippocampal gyrus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dementia Rating Scale</td>
<td>−0.08</td>
<td>−0.32</td>
</tr>
<tr>
<td>Overall cognitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>functioning score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>California Verbal Learning Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall</td>
<td>−0.18</td>
<td>−0.39*</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>−0.20</td>
<td>−0.55**</td>
</tr>
<tr>
<td>WMS-R Visual Reproduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall</td>
<td>0.04</td>
<td>−0.13</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>−0.07</td>
<td>−0.11</td>
</tr>
</tbody>
</table>

*Note: An omnibus test of the matrix showed an association
between MR-volumetric measures and behavioral variables
\( (P < 0.05) \). Significance levels are displayed for \( r \)-tests of individual coefficients; * \( P < 0.05; ** P < 0.01; *** P < 0.001; n = 26 \) for all coefficients.
and non-verbal material from the VRT was found to be impaired even when differences in initial learning were taken into account, suggesting that the patients’ memory deficits involve accelerated forgetting over short delays and, thus, implicate abnormal storage [23, 37]. That memory deficits were present even under immediate recall conditions is consistent with suggestions that the functional deficits also pertain to processes at encoding [36, 39, 47].

Atrophy in the hippocampus and the parahippocampal gyrus was found to be related to delayed but not immediate memory impairments in AD patients. Delayed recall of the CVLT word list showed a positive association with remaining hippocampal volume whereas delayed recall of the VRT designs correlated positively with remaining parahippocampal gyrus volume. We confirmed this pattern of associations in multiple regression analyses that allowed us to examine the independent contributions of these structures of interest while controlling for confounding effects of age, sex, years of education and total brain capacity. Hippocampal but not parahippocampal volume emerged as a significant predictor in the regression model for delayed CVLT performance. The reverse pattern was found in the model for delayed VRT performance. In both models, the volume of the structure that did not predict performance had regression coefficients close to zero, suggesting that the observed dissociations are not simply due to statistical thresholds.

Our results indicate that the relationship between atrophy in medial temporal-lobe structures and episodic memory impairments in AD is highly specific, neuroanatomically as well as behaviorally. That atrophy in the two structures of interest correlated with some behavioral measures (i.e. delayed recall on CVLT and VRT) but not others (i.e. immediate recall on CVLT and VRT, overall cognitive functioning on DRS) suggests behavioral specificity; that atrophy in the hippocampus was associated with a different type of delayed recall task (CVLT) than atrophy in the parahippocampal gyrus (VRT) suggests neuroanatomical specificity within the medial temporal lobes. Taken together, these data cannot be accommodated by a hypothesis that assumes a unitary non-specific factor, such as disease severity or global cortical atrophy, as the link between the various behavioral deficits and structural abnormalities examined. An interpretation that considers differences between memory tasks together with the notion of functional specialization within the medial temporal lobes, as discussed later, is clearly better suited to account for the observed pattern of brain-behavior correlations in AD.

Our results confirm those reported by Wilson et al. [84], who also found a positive association between MR-derived volumes of the hippocampus but not the parahippocampal gyrus and delayed verbal recall of auditorily-presented material in AD. Our findings extend these results by showing that the reverse pattern holds for delayed recall on a non-verbal visual memory task. Because no relationship was found between medial-lobar atrophy and immediate memory performance, our findings and those by Wilson et al. hint that changes in structures outside the medial temporal lobes, perhaps in dorsolateral prefrontal cortex [6, 11, 30, 51, 56], underlie the encoding deficits observed in AD. However, given that the volumetric analysis in our study was focused only on the medial temporal-lobe region, this relationship could not be addressed.

Impaired delayed recall over interference-filled delays is not only a core symptom of AD but has more generally been proposed to be the clinical hallmark of anterograde amnesia caused by medial temporal-lobe damage [48, 69]. Several lesion studies in non-human species, including rhesus monkeys and rats, have shown delay-dependent memory impairments produced by hippocampal or combined hippocampal and parahippocampal gyrus damage [85, 86, 89, 90]. Studies in humans who underwent temporal lobectomy for the relief of intractable epilepsy have demonstrated that the amount of medial temporal-lobe tissue removed may crucially determine the degree of delayed-memory deficits observed post-surgery while leaving immediate memory performance unaffected [57, 67]. Together with these findings, the present results support the hypothesis that the medial temporal lobes play a specific role in those memory processes that are involved in storage and counteract forgetting during delays, such as consolidation or cohesion [41, 48, 52, 71]. It should be emphasized, however, that the particular data presented here would not warrant the conclusion that the hippocampus and the parahippocampal gyrus are the only brain structures that support processes related to consolidation and cohesion. Other structures that were not examined here may also contribute to these processes. They may include structures that have strong neuroanatomical connections with the medial-temporal lobe region, such as diencephalic nuclei or retrosplenial cortex [7, 38, 79].

Although a small number of human cases with amnesia due to isolated hippocampal lesions have been documented [60, 80, 88] in most reported cases of medial temporal-lobe amnesia, including those with amnesia due to temporal lobectomy, it is unclear whether the crucial damage is localized in the hippocampus, in surrounding parahippocampal gyrus, or in both. It has been suggested that medial temporal-lobe lesions in humans that include the hippocampus and adjacent regions in parahippocampal gyrus produce more severe memory impairments than lesions restricted to the hippocampus [60]. The present results suggest that this may be the case because damage (or atrophy) to each of these structures affects different aspects of memory functioning. Thus, our findings in AD point to the exciting possibility that the memory contributions of the hippocampus and the parahippocampal gyrus can be double-dissociated [19]. Concerning the nature of these distinct contributions, it is important to note that the two memory tasks of the present study that were related to hippocampal and parahippocampal atrophy differed from each other in
Our results are open to the interpretation that the parahippocampal gyrus plays a role in memory that is restricted to visually-encoded information whereas the hippocampus plays this role for other sensory modalities. Our finding that performance on a visual recall task is highly sensitive to structural damage in the parahippocampal gyrus is in keeping with the evidence that this region has the strongest neuroanatomical connections with ventral occipito-temporal brain regions involved in vision [74] and that lesion studies with non-human primates [20, 46, 53] and functional imaging studies in humans [1, 19, 35, 55] have previously implicated the parahippocampal gyrus, including entorhinal and perirhinal cortex, in visual memory. However, visual recognition memory impairments in non-human primates have also been found after selective hippocampal lesions [2]. Moreover, lesions to the parahippocampal gyrus can produce memory impairments in modalities other than the visual one [75]. An interpretation of the correlational pattern in terms of modality-specificity is further complicated by the fact that both regions receive cortical input from multiple sensory modalities with cortical projections to the hippocampus being relayed in the parahippocampal gyrus [3].

While modality differences warrant further consideration, it is also important to focus on the type of material that has to be recalled in each task. The CVLT requires the recall of related words, which can be grouped in semantic categories, whereas the VRT requires the recall of unrelated meaningless shapes with distinct spatial configurations. Perhaps, the human hippocampus plays a role in consolidation of verbal information encoded in terms of its semantic context [41] whereas the parahippocampal gyrus plays a role in consolidation of non-verbal patterns. Future functional imaging studies in healthy individuals can build upon these results in AD to characterize the distinct memory contributions of the parahippocampal gyrus and the hippocampus using closely matched verbal and non-verbal experimental tasks.

Future functional imaging studies may also address issues regarding the lateralization of hippocampal and parahippocampal gyrus contributions to memory. The analysis of laterality effects in the present study revealed that the volume of the right but not the left parahippocampal gyrus is associated with delayed VRT recall in AD, confirming earlier findings in patients with unilateral temporal lobectomy that showed lateralization of non-verbal memory functions to the right hemisphere [29, 34, 57, 66]. By contrast, our analyses revealed no laterality effects with respect to the association between hippocampal volumes and delayed CVLT performance in AD, presumably due to the high correlation between left- and right-sided hippocampal atrophy that would not allow detection of any differences. If the constraints of symmetrical hippocampal atrophy in AD are taken into account our data are not inconsistent with the notion that hippocampal contributions to delayed verbal recall are lateralized to the left hemisphere [18, 49].

Although not the central focus of this article, the pattern of brain-behavior correlations observed in the elderly NCs also requires comment. While there was no indication for an association between parahippocampal gyrus volume and any of the memory measures in NCs there was a statistical trend towards a negative association between hippocampal volume and delayed CVLT recall that persisted even when possible confounding effects were controlled for. It is important to note that this association replicates the specificity of the relationship between hippocampal volume and delayed memory performance observed in AD. However, the fact that the association is reversed in sign in NCs is intriguing and we do not have a ready explanation for this. It is unlikely that differences in statistical power are responsible for the observed differences in brain-behavior correlations between the two groups because the volumetric measures as well as the behavioral measures showed more variability in the NC sample than the AD sample.

Previous research on the relationship between memory performance and MR-derived medial-temporal lobe volumes in NCs has provided inconsistent results. Work by Golomb et al. [21, 22] suggests a positive association between hippocampal volumes and delayed memory performance in elderly NCs. By contrast, Raz et al. [59] have found no association between hippocampal or parahippocampal gyrus volumes and performance on various delayed memory tasks in a large sample of NCs who ranged in age from 18–77 years. Based on a comparison of subgroups of their larger sample, Raz et al. argued that only samples with a large proportion of participants with poor memory performance may reveal a positive link between episodic memory and medial temporal-lobe volumes. In line with this suggestion, research in nondemented elderly individuals with age-associated memory impairments (AAMI) has demonstrated a positive relationship between memory performance and hippocampal volumes (as indexed by left-right asymmetry, [68]). In the present study, unlike the one by Golomb et al. [21], elderly individuals with AAMI were specifically excluded from the sample of NCs. Eighty-eight percent of the NCs in our sample obtained performance scores at or above the age- and sex-adjusted average [12] on CVLT delayed recall, the measure that showed the nega-
tive association with hippocampal volumes. It is likely that the pathological mechanisms that cause hippocampal atrophy and link it to memory impairments in AD (and possibly AAMI) are absent in these highly-functioning healthy individuals. Other (possibly reversible) neural mechanisms may determine the size of the hippocampus in this group and influence the relationship to cognitive functioning [44]. It remains an important goal for future studies to specify these mechanisms in relation to memory performance in AD and in healthy individuals.

In conclusion, the results of the present study show that hippocampal and parahippocampal gyrus atrophy in AD are related to a specific subset of the patients’ anterograde memory impairments. We found an association between hippocampal tissue loss and delayed verbal–auditory memory deficits and an association between parahippocampal gyrus loss and delayed non-verbal visual memory deficits in AD. Although our use of a correlational design precludes establishing a causal relationship between the structural and the behavioral changes, the present results are at least consistent with the idea that delayed memory impairments in AD result from atrophy in the hippocampus and the parahippocampal gyrus. Our findings support theories which posit that medial temporal-lobe structures play a crucial role in episodic memory processes that occur after encoding such as consolidation or cohesion [41, 48, 52, 71]. Moreover, they suggest that the hippocampus and the parahippocampal gyrus make separate contributions to these processes. On a more general level, our study shows how investigating the relationship between structural abnormalities in distinct brain structures and behavioral performance in AD may not only further our understanding of the cognitive impairments observed in AD but may also advance our knowledge regarding the organization of memory in the intact human brain.

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