Hippocampus and entorhinal cortex in mild cognitive impairment and early AD

ARTICLE *in* NEUROBIOLOGY OF AGING · APRIL 2004

Impact Factor: 5.01 · DOI: 10.1016/S0197-4580(03)00084-8 · Source: PubMed

ELSEVIER

Neurobiology of Aging 25 (2004) 303–310

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Hippocampus and entorhinal cortex in mild cognitive impairment and early AD

Corina Pennanen [a], Miia Kivipelto [a], Susanna Tuomainen [a], Päivi Hartikainen [b], Tuomo Hänninen [a,b], Mikko P. Laakso [a], Merja Hallikainen [a], Matti Vanhanen [a], Aulikki Nissinen [c], Eeva-Liisa Helkala [d], Pauli Vainio [e], Ritva Vanninen [e], Kaarina Partanen [e], Hilkka Soininen [a,b,*]

[a] *Department of Neuroscience and Neurology, University of Kuopio and Brain Research Unit, Clinical Research Center, Mediteknia, P.O. Box 1627, FIN-70 211 Kuopio, Finland*
[b] *Department of Neurology, Kuopio University Hospital, 70211 Kuopio, Finland*
[c] *National Institute of Public Health, Mannerheimintie 116, 00300 Helsinki, Finland*
[d] *Department of Public Health, University of Kuopio, 70211 Kuopio, Finland*
[e] *Department of Clinical Radiology, Kuopio University Hospital, 70211 Kuopio, Finland*

Received 28 October 2002; received in revised form 14 March 2003; accepted 27 March 2003

Abstract

Magnetic resonance imaging (MRI) has been suggested as a useful tool in early diagnosis of Alzheimer's disease (AD). Based on MRI-derived volumes, we studied the hippocampus and entorhinal cortex (ERC) in 59 controls, 65 individuals with mild cognitive impairment (MCI) and 48 patients with AD. The controls and individuals with MCI were derived from population-based cohorts. Volumes of the hippocampus and ERC were significantly reduced in the following order: control > MCI > AD. Stepwise discriminant function analysis showed that the most efficient overall classification between controls and individuals with MCI subjects was achieved with ERC measurements (65.9%). However, the best overall classification between controls and AD patients (90.7%), and between individuals with MCI and AD patients (82.3%) was achieved with hippocampal volumes. Our results suggest that the ERC atrophy precedes hippocampal atrophy in AD. The ERC volume loss is dominant over the hippocampal volume loss in MCI, whereas more pronounced hippocampal volume loss appears in mild AD.

Keywords: Aging; Mild cognitive impairment; Alzheimer's disease; Dementia; Hippocampus; Entorhinal cortex; Magnetic resonance imaging

1. Introduction

Magnetic resonance imaging (MRI)-based volumetric measurements have attracted great interest as representing one aid to the early diagnosis of Alzheimer's disease (AD). There is evidence that the earliest neuropathological changes in AD appear in the entorhinal cortex (ERC) and then progress to the hippocampus [2,6,21,45,46]. Accordingly, the MRI-based volumetric measurements of the ERC and hippocampus have been proposed as valuable tools in detecting early AD [9–14,16,23–26,28,29,33–35]. Mild cognitive impairment (MCI) [39,40] is considered as a transition state between normal aging and dementia. Longitudinal studies have shown that the subjects fulfilling the criteria of MCI have a 10-fold risk for developing dementia and most often AD [39,40]. The consensus statement states that individuals with MCI should be clinically followed using neuropsychological tests or cognitive screening instruments [41]. A few studies [9,10,12–14,23,24,28,29,47,48,51] have focused on MRI-based volumetric measurements of different brain regions in individuals classified for MCI in line or not with the criteria proposed by Mayo Clinic Alzheimer's Disease Research Center (MCADRC) [39,40]. In our study we pursued MRI volumetric measurements of the hippocampus and ERC in subjects with MCI and in patients with AD and compared the results with those obtained in cognitively normal elderly subjects. The main aim of our study was to determine: (1) whether the ERC and hippocampal volumes are significantly reduced in subjects with MCI compared with control subjects and in patients with AD compared with MCI subjects and with controls;

* Corresponding author. Tel.: +358-17-173-012; fax: +358-17-173-019.
E-mail address: hilkka.soininen@kuh.fi (H. Soininen).

0197-4580/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0197-4580(03)00084-8

(2) which of the MRI volumetric measures are the best discriminants between the diagnostic groups; (3) what are the results of discriminant analyses when mild cases of the AD group are considered. To this end, we studied a large number of individuals and used strict criteria for MCI, and a validated, histology-based protocol to accurately measure the ERC and the hippocampus.

2. Methods

2.1. Subjects

The present study included 59 control subjects, 65 subjects with MCI and 48 subjects with AD. The study subjects derived from population-based cohorts in which cognitive functions of the elderly have been evaluated [20,30], or from hospital series of well characterized AD patients investigated in the Department of Neurology, Kuopio University Hospital, Kuopio, Finland. The study was approved by the local ethics committee, and all the participants gave informed consent for their participation in the study. Some of the participants, with all the MCI cases excluded, have been used in our previous studies, but their regions of interest have been measured again by a single rater to avoid two-rater bias.

2.1.1. Diagnosis of AD

Diagnostic evaluations for AD patients included medical history, physical and neurological examinations performed by a physician, and a detailed neuropsychological evaluation administered by a neuropsychologist. The severity of cognitive decline was graded according to the Clinical Dementia Rating (CDR) Scale [3]. Furthermore, brain MRI scan, CSF analysis, EKG, chest radiography and blood tests were performed. These were not used in the diagnostic phase except for excluding other possible pathologies underlying the symptoms. The diagnosis of dementia was based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [1] and the diagnosis of AD on the National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [37].

2.1.2. Diagnosis of MCI

The subjects with MCI were identified from two different population cohorts. In both cohorts the evaluation consisted of a structured interview including CDR Scale and a large neuropsychological assessment. The scoring of the CDR was independent of the scores obtained from neuropsychological tests.

MCI was diagnosed using the criteria proposed by Mayo Clinic Alzheimer's Disease Research Center. Later, these criteria have been modified, but at the time this study was conducted the criteria required: (1) memory complaint by patient, family, or physician; (2) normal activities of daily living; (3) normal global cognitive function; (4) objective impairment in memory or in one other area of cognitive function as evident by scores >1.5 S.D. below the age-appropriate mean; (5) CDR score of 0.5; and (6) absence of dementia [39,44].

In the first cohort, the following test battery was used for a comprehensive neuropsychological evaluation of different cognitive domains: *Memory*: Visual Reproduction Test (immediate and delayed recall) from Wechsler Memory Scale [43], Logical Memory Test (immediate and delayed recall) from Wechsler Memory Scale-Revised [50], Word List Recall (immediate and delayed recall) from the CERAD Neuropsychological Assessment Battery [38], delayed recall of the Constructional Praxis from CERAD [38], New York University Paragraph Recall (immediate and delayed recall) [32]; *Language*: Abbreviated (15 items) Boston Naming Test [27], vocabulary subtest from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [49]; *Attention and executive function*: Verbal Fluency Test [5,8], Trail Making Test [42] parts A and B; *Visuospatial skills*: Constructional Praxis from CERAD [morris3], Block Design from the WAIS-R [49]; *Global functioning*: Mini-Mental State Examination [15] (MMSE), Clock Drawing Test (the CERAD version) [38]. In this cohort, however, two memory test scores only were used as the objective psychometric criteria of memory impairment in MCI diagnosis: according to the normative data [20] in delayed recall in the Logical Memory Test from the WMS-R or in the Visual Reproduction Test from the WMS, he was defined as impaired. The diagnostic procedure used in the second cohort has been described in detail previously [30]. All the MCI subjects included in the present study had memory impairment.

2.1.3. Control subjects

Control subjects were volunteers from the population-based cohorts matched with age, and gender for the demented subjects. The methods used for the identification of control subjects have been published earlier in detail [19,30]. The controls showed no impairment in the cognitive tests, and had no history of neurological or psychiatric diseases.

2.2. MRI acquisition and volumetric assessment

The subjects were scanned with a 1.5 T Vision (Siemens, Erlangen, Germany) using a three-dimensional magnetization prepared rapid acquisition gradient echo sequence (TR 10 ms, TE 4 ms, matrix 256 × 192, 1 acquisition). The images were then aligned to correct the undesirable effects of head tilt and rotation. Standard neuroanatomical landmarks (such as the orbits, sulci and the commissures) were used to correct for possible deviations in any of the orthogonal planes and the scans were reconstructed into 2.0 mm thick contiguous coronal slices, oriented perpendicular to the intercommissural line.

The hippocampi and ERC were manually traced by a single tracer (C.P.), blinded to the clinical data, using custom made software for a standard Siemens work console. Tracing of the hippocampus started rostrally where the hippocampus first appears below the amygdala and ended posteriorly in the section where the crura of the fornices depart from the lateral wall of the lateral ventricles [34]. The ERC volumes were traced according to the histology-based criteria designed for MRI volumetric measurements [22]. In brief, the most anterior slice measured was the one after the appearance of the temporal stem, and the last slice was the one where the uncus and gyrus intralimbicus were no longer separable. Once the ROI has been traced, the software calculates the volume for every structure by computing the number of voxels for each traced image. The intraclass correlation coefficients for intrarater reliability were 0.96 for the hippocampus and 0.95 for the ERC measured from 10 subjects. The coronal intracranial area at the level of the anterior commissure was measured and used for normalization of the volumetric data [16,34]. For the purpose of data presentations, the volumes were normalized to the intracranial area according to the formula: (volume/intracranial area) × 100.

2.3. Statistical analyses

The statistical software SPSS for Windows V10.0 (SPSS Inc., Chicago, IL) was used to analyze the data. In all statistical analyses of the volumetric data, we used volumes normalized for the intracranial area. One-way ANOVA with Bonferroni post hoc analysis was used to compare the means of age, education, and MMSE scores between the groups. The relationship of volumes with gender, diagnostic groups and age was assessed with the ANCOVA test, which had hippocampal and ERC volumes as dependent variables, gender and diagnostic groups as factors, and age as covariate. Pearson's correlation coefficients were used to analyze the correlation between the hippocampal and ERC volumes within each study group. We tested the value of the hippocampal and ERC volumes in discriminating between the groups using discriminant analyses with enter and stepwise methods, respectively. In the analyses, the normalized volumes to the intracranial area and adjusted for age were used. First, discriminant analyses with an enter method were used to analyze the value of normalized total volumes of the hippocampus and ERC to distinguish AD patients or MCI subjects from controls and MCI subjects from patients with AD, using volumes as independent variables. Then, the value of the hippocampal and ERC total volumes for group classification were tested using stepwise discriminant function analyses (Wilks' method). Finally, stepwise discriminant analysis was used to test unilateral volumes: the right and left hippocampal volume, the right and left ERC volume in discrimination between pairwise combinations of clinical groups. The results are expressed as mean ± S.D. The level of statistical significance of differences is $P < 0.05$.

Table 1
Descriptive characteristics

	Controls	MCI	AD
Number	59	65	48
Female/male	37/22	43/22	25/23
Age	72.7 ± 4.3	72.8 ± 4.5	71.1 ± 8.1
Education	8.1 ± 3.2	6.7 ± 1.6	6.8 ± 3.4
MMSE	27.3 ± 1.8	24.0 ± 2.5	21.4 ± 3.5

MCI: mild cognitive impairment; AD: Alzheimer's disease; MMSE: Mini-Mental State Examination; age, education and MMSE expressed as mean ± S.D. Education differs significantly across the study groups one-way ANOVA ($F[2, 164] = 4.3$, $P < 0.05$), Bonferroni post hoc analysis Controls vs. MCI $P < 0.05$.

3. Results

3.1. Descriptive characteristics

The study groups were well matched for age ($F = 1.5$, $P = 0.24$) and gender ($\chi^2 = 2.4$, $P = 0.30$). The MMSE scores declined in the following order: control > MCI > AD ($P < 0.001$) (Table 1). The level of education differed significantly across the study groups ($F[2, 164] = 4.3$, $P < 0.05$); the subjects with MCI had significantly lower level of education compared to controls (Bonferroni post hoc analysis $P < 0.05$).

3.2. Volumetric measurements

ANCOVA test showed that the diagnostic groups differed significantly in the volumes ($F[2, 164] = 88.2$, $P < 0.001$ for hippocampal volume; $F[2, 164] = 50.1$, $P < 0.001$ for ERC volume), gender had no influence on the volumes ($F[1, 164] = 1.1$ for hippocampal volume, $F[1, 164] = 0.03$ for ERC volume, $P > 0.05$), gender × group had no influence on the volumes ($F[2, 164] = 0.2$ for hippocampus; $F[2, 164] = 2.3$ for ERC volume, $P > 0.05$), and age did not affect the hippocampal volume ($F[1, 164] = 2.6$, $P > 0.05$), but it did affect the ERC volume ($F[1, 164] = 8.2$, $P = 0.01$). While age was not correlated with total ERC volume in the control group ($r = -0.23$, $P = 0.09$) or in the MCI group ($r = -0.15$, $P = 0.22$), in the group of patients with AD there was a significant correlation between age and the total ERC volume ($r = -0.30$, $P = 0.04$). Therefore, all the analyses were adjusted for age. The total hippocampal volume and the total ERC volume, as well as the unilateral volumes of hippocampus and ERC were significantly reduced in the following order: control > MCI > AD (Table 2). The total hippocampal and total ERC volumes were correlated within each group (control: $r = 0.35$, $P = 0.01$; MCI: $r = 0.53$, $P < 0.001$; AD: $r = 0.58$, $P < 0.001$).

The percentual decrease in hippocampal volume was smaller compared with that in ERC volume in the MCI group versus controls, whereas the volume losses in the AD

Table 2
Volumetric measurements and percentual decrease of volume

Region	Controls	MCI		AD	
	Volume	Volume	Decrease (%)	Volume	Decrease (%)
HC					
Right	16.37 ± 2.19	15.20 ± 2.51*	7	10.55 ± 2.86§,‡	36
Left	15.36 ± 2.23	13.90 ± 2.54†	10	9.34 ± 2.48§,‡	39
Total	31.73 ± 4.19	29.10 ± 4.77†	8	19.90 ± 5.10§,‡	37
ERC					
Right	9.02 ± 1.89	7.65 ± 1.59†	15	5.88 ± 1.92§,‡	35
Left	8.47 ± 1.94	7.00 ± 1.50†	17	5.29 ± 1.62§,‡	38
Total	17.49 ± 3.63	14.66 ± 2.96†	16	11.18 ± 3.20§,‡	36

The analyses represent normalized volumes. The results are expressed as mean ± S.D. and the decrease % is compared with controls. HC: hippocampus; ERC: entorhinal cortex; Right: right side; Left: left side; Total: sum of the right plus left side volumes; MCI: mild cognitive impairment; AD: Alzheimer's disease.

* $P < 0.05$ for MCI vs. controls.
§ $P < 0.001$ for AD vs. MCI.
‡ $P < 0.001$ for AD vs. controls.
† $P < 0.001$ for MCI vs. controls.

group versus controls were of the same magnitude for the hippocampus and ERC. Fig. 1 shows the percentual decrease of total ERC and total hippocampal volumes within each study group relative to mean volumes for the control group.

3.3. Discriminant function analysis

In discriminant function analysis we used first enter method to the volumes of the total (right and left) hippocampus and total (right and left) ERC, adjusted for age and thereafter a stepwise method to test the accuracy of first total and thereafter unilateral volumes in classifying the groups.

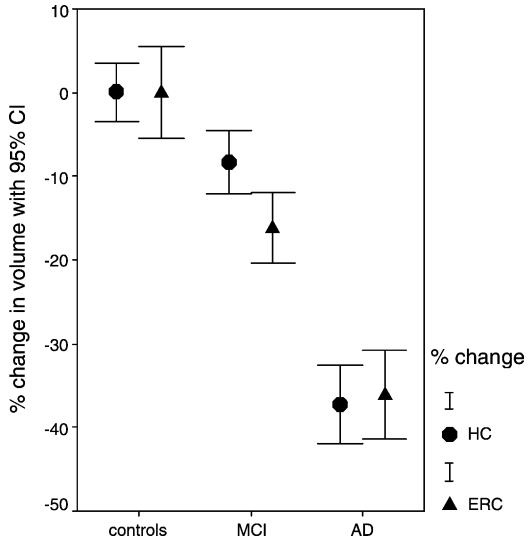

Fig. 1. Percentual decrease in volume compared to normal mean volumes of ERC and HC in controls, MCI subjects and AD patients. The 95% CI is considered. This figure shows that the most severe volume loss is the ERC in MCI, while in AD there is no significant difference in the magnitude of the volume loss of the two regions of brain.

In the classification between MCI subjects and controls, the volume of the total hippocampus yielded an overall classification of 59.7% (Wilks' $\lambda = 0.92$, $\chi^2 = 10.1$, $P = 0.01$) (Table 3), the volume of the total ERC yielded an overall classification of 66.7% (Wilks' $\lambda = 0.84$, $\chi^2 = 21.3$, $P < 0.001$) (Table 3). In the stepwise analysis, only the total ERC volume entered the model with an overall classification of 65.9% (Wilks' $\lambda = 0.84$, $\chi^2 = 21.0$, $P < 0.001$) (Table 3). In the stepwise discriminant function analysis including unilateral hippocampal and ERC volumes only left ERC volume entered the model (Wilks' $\lambda = 0.85$, $\chi^2 = 20.3$, $P < 0.001$).

Distinguishing AD patients from control subjects, the volume of total hippocampus yielded an overall classification of 90.7% (sensitivity 85.4%, specificity 94.9%, Wilks' $\lambda = 0.36$, $\chi^2 = 105.1$, $P < 0.001$), the total ERC volume yielded an overall classification of 82.1% (sensitivity 85.4%, specificity 79.3%; Wilks' $\lambda = 0.50$, $\chi^2 = 70.8$, $P < 0.001$). In the stepwise analysis both the hippocampal volume and the ERC volume entered the model (overall classification 90.6%, sensitivity 87.6%, specificity 93.1%; Wilks' $\lambda = 0.34$; $\chi^2 = 111.1$; $P < 0.001$). In the stepwise discriminant function analysis including unilateral hippocampal and ERC volumes, the left hippocampal volume and the right ERC volume entered the model (Wilks' $\lambda = 0.35$, $\chi^2 = 108.1$, $P < 0.001$).

Finally, distinguishing AD patients from MCI subjects, the total hippocampal volume showed an overall classification of 80.5% (sensitivity 77.1%, specificity 83.1%; Wilks' $\lambda = 0.52$, $\chi^2 = 72.0$, $P < 0.001$), the ERC volume yielded an overall classification of 70.8% (sensitivity 70.8%, specificity 70.8%; Wilks' $\lambda = 0.73$, $\chi^2 = 34.1$, $P < 0.001$). In the stepwise analysis only hippocampal volume entered

Table 3
Classification between the groups using discriminant function analysis with enter method including in the model hippocampus, or ERC, as well as with stepwise method including in the model both, the hippocampus and ERC

Groups and variables	Sensitivity (%)	Specificity (%)	Overall classification (%)	Explains of variance (%)	*P*
MCI-C					
Enter: HC	56.9	62.7	59.7	8	0.01
Enter: ERC	63.1	70.7	66.7	16	<0.001
Stepwise: ERC	66.2	65.5	65.9	16	<0.001
AD-C					
Enter: HC	85.4	94.9	90.7	64	<0.001
Enter: ERC	85.4	79.3	82.1	50	<0.001
Stepwise: HC + ERC	87.6	93.1	90.6	66	<0.001
AD-MCI					
Enter: HC	77.1	83.1	80.5	48	<0.001
Enter: ERC	70.8	70.8	70.8	27	<0.001
Stepwise: HC	81.3	83.1	82.3	47	<0.001

The analyses included the bilaterally summed and normalized volumes adjusted for age. HC: hippocampus; ERC: entorhinal cortex; C: controls; MCI: mild cognitive impairment; AD: Alzheimer's disease.

the model (overall classification 82.3%, sensitivity 81.3%, specificity 83.1%; Wilks' $\lambda = 0.53$, $\chi^2 = 69.3$, $P < 0.001$). In the stepwise discriminant function analysis including unilateral hippocampal and ERC volumes, only left hippocampal volume entered the model (Wilks' $\lambda = 0.55$, $\chi^2 = 66.0$, $P < 0.001$).

4. Discussion

The definition of MCI has been in constant flux. After the subjects for this study were identified, the criteria have been modified in several details. The previous "memory complaint by patient, family or physician" has been modified as "memory complaint, preferably corroborated by an informant." The specific amnestic MCI has been defined [41] to replace previous inclusion of subjects with impairment in other cognitive domains also. All subjects with MCI in the present study had impairment of memory, and some of them had other cognitive impairment as well but were not demented. In previous Mayo Clinic papers all MCI subjects were described to score 0.5 on the CDR, but this has not been incorporated in the later modification of amnestic MCI criteria. In our study, the CDR score of 0.5 was used as inclusion criteria. We believe that this makes the MCI population more homogeneous while ensuring that in addition to psychometric aspects also the clinical status of subjects was taken into account in the diagnosis. Contrary to the MCI studies by Mayo Clinic we did not use education specific norms for neuropsychological tests when determining the objective cognitive impairment. This may have some influence on results but in Finland in this age group the education varied quite little. Consequently, persons with little formal education may also have held a demanding professional position before retirement. The former occupation would be more exact indication for subjects' personal "cognitive history" but it would be difficult to take occupation into account in determining the cut off points for tests.

Previous studies have shown promising results when MRI volumetry of the medial temporal lobe structures has been used in the early diagnosis of AD as well as in the prediction of MCI converting into AD. The strength of the present study is the large sample size of MCI and controls deriving from population-based cohorts. Our major findings were: first, the hippocampal and ERC volumes were significantly reduced in the following order: control > MCI > AD. Second, in cases with MCI, the magnitude of the ERC atrophy was more prominent than that of the hippocampal atrophy, whereas in AD patients the ERC atrophy was of the same magnitude as that of the hippocampal atrophy. Thus, the discrimination of MCI subjects from controls was best achieved using ERC volume measurements; and discrimination of MCI subjects from AD patients by hippocampal volumes.

4.1. Volumetric measurements and discriminant classification

Neurodegenerative changes in AD are accompanied by brain atrophy, which is the main gross pathological feature of AD [36]. The neurofibrillary pathology, first seen in the entorhinal/transentorhinal area, spreads to other limbic structures such as the hippocampus, and thereafter to the isocortical areas, permitting differentiation of six stages in the degenerative process occurring in AD [6]. Furthermore, no significant neuronal loss in ERC was detectable in a study on cognitively normal aged people (between the age of 60 and 90), while a very severe neuronal loss is seen in ERC even in the very mild AD cases [17]. Kordower et al. showed that the atrophy and the loss of neurons in the layer II of ERC occur in individuals with MCI prior to the onset of dementia and this atrophy is significantly correlated with the MMSE scores [31]. With respect to the hippocampus,

strong correlations were found between MRI-based volumetric measurements and neuronal [4] and tangle counts [21]. These facts support the rationale for our MRI volumetric study to concentrate on medial temporal lobe structures.

4.1.1. MCI subjects versus controls

Our study confirmed the findings from some earlier studies [9,14,28,51] showing that compared with controls, the volumes of the ERC and hippocampus are significantly reduced in individuals with MCI. In addition, we showed that the ERC volume loss (16%) was significantly greater than the hippocampal volume loss (8%) in MCI versus controls. This differs from the results of another cross-sectional study by Du et al. [14], who found no differences in the magnitude of the volume loss between ERC and hippocampus in MCI. Our results are of interest, given that MCI refers to a transitional stage between cognitive changes of normal aging and AD, and may represent a preclinical stage of AD. Therefore, our data is well in line with the assumption that the pathology of AD starts in ERC, providing in vivo evidence for the Braak stages [6,7]. The explanations for the relatively modest average ERC volumes losses, 16%, may be that the MCI subjects derived from a population-based cohort, and not from a group of individuals seeking help for the memory problems. It should be noted that mildly impaired non-demented subjects form a heterogeneous group that includes both stable subjects and subjects who will develop AD [39–41], but at this point the follow-up data of our cohort is not available yet.

We showed that the ERC volume, but not the hippocampal volume, best discriminated MCI subjects from controls. Although this finding was theoretically expected [11], to our knowledge it has not been demonstrated previously using the same protocol for ERC volumetric measurements [22] applied to MCI subjects as defined by Petersen et al. [39,40]. In a longitudinal study by Dickerson et al. [13], the authors showed that ERC volume is better than hippocampal volume in distinguishing (69%) non-demented individuals (ND) ($n = 28$) from controls ($n = 34$). However, the concepts of ND and MCI overlap only partially and their protocol used for ERC volumetric measurements [18] was different from ours. In another longitudinal study, Killiany et al. [29] showed that only ERC could discriminate normals from "questionables" (83% accuracy) and from converters (84% accuracy), while the comparable discriminant analysis with the hippocampus was in both cases not significant. But the "questionables" and converters could not be discriminated by any of the two measured regions. For the classification of "questionables" a clinical dementia rating of 0.5 was considered and not the MCADRC criteria of MCI [39,40]. Retrospectively shown, only a part of them met the criteria for MCI. Additionally the ERC protocol was different from ours: they measured only the midregion of the ERC from three slices of 1.5 mm thickness each. Based on these facts, it is difficult to make a comparison between our and their results. A high classification between the two groups was achieved in another longitudinal study by Killiany et al. [28], where more than 85% of MCI subjects were distinguished from controls using ERC volume. However, that study did not report any data on hippocampal volumetry. The poor overall classification achieved in our study underlines the heterogeneity of MCI subjects identified from the population based cohort. In our study, the overall classification of about 66% between MCI subjects and controls is poor, but it is consistent with the findings of the cross-sectional study by Xu et al. [51] who reported less than 70% overall classification when using the ERC volume. In their study, the hippocampal volume appeared to be the most powerful in discriminating MCI subjects from controls. It should be noted, however, that as far as perfect discrimination goes, such a marker is not likely to be found because of the heterogeneity of the MCI group. And, perhaps, this is not even the very issue. The bottomline perhaps rather is that this finding theoretically supports the hierarchical distribution of AD pathology from the rhinal cortices to the hippocampus, and thus is in vivo support of the Braak and Braak concept. Whether or not the atrophy has diagnostic value in terms of conversion to AD is another issue, but this cannot be established here, but only when the follow-up data is available.

4.1.2. AD patients versus controls

Our findings of significantly reduced ERC and hippocampal volumes in patients with AD compared with controls are comparable, in general, to those of previous MRI volumetric studies. Juottonen et al. [25] found a 40% reduction of ERC volume in patients with AD compared with controls. Our study revealed a 36% ERC volume loss in the AD group compared with controls. In the study of Laakso et al. [33], the right and left hippocampal volumes decreased by 38% in patients with AD ($n = 32$) compared with controls ($n = 16$). Similarly, our findings show 37% total hippocampal volume loss and a reduction of 36% in the right and 39% in the left hippocampus, in the AD group compared with the controls. The magnitude of the ERC changes was similar to that of the hippocampal changes in AD patients. However, the variability in measurements of ERC is greater than that of the hippocampus, and anatomical ambiguity exists when depicting the ERC boundaries on MRI, especially in the AD patients [26].

The stepwise discriminant analysis, while comparing AD patients with controls, was entered by both the hippocampal and the ERC volumes. This yielded an overall classification of 90.6% which was similar to the 90.7% classification achieved using hippocampus alone, thus the contribution of ERC was negligible. Du et al. [14] found no significant differences in the power of ERC and that of the hippocampus for the distinction of AD from controls, but they improved the classification between the two groups using a combination of the volumes of ERC and hippocampus. In separating controls from AD patients, Lehéricy et al. [35] reached 100% accuracy, combining the volumes of hippocampus with the volumes of amygdala, but using the hippocampus alone they

achieved an accuracy of only 89% in the correct diagnosis of 26 subjects. Laakso et al. [34] also achieved a correct classification of 89% with the hippocampus between 55 AD patients and 42 controls.

4.1.3. AD patients versus MCI subjects

Both the ERC and hippocampal volumes were reduced significantly in AD patients compared with MCI subjects. AD patients showed significantly greater volume loss in hippocampus (32%) than in ERC (23%) compared with MCI. This is in contrast to the finding of Du et al. [14] showing that, in the AD group compared with the MCI group, the ERC volume loss (30%) was significantly greater than that of the hippocampus (19%). We account for these discrepancies by the differences in the study population and by the variability in depicting very atrophied ERC on MRI scans.

When AD patients were compared with MCI subjects, the loss of hippocampal volume was greater than that of the ERC and discrimination analysis also highlighted the greater power of discrimination with the hippocampus than with the ERC. An overall classification of 80.5% was obtained with hippocampus, while adding ERC to the model did not improve the classification between MCI subjects and AD patients. The only significant variable that entered into the model in a stepwise discriminant analysis was the total hippocampal volume, which yielded a classification of 82.3%. In the study by Killiany et al. [29] the ERC alone gave a better accuracy in discriminating mild AD from questionables (81%) or from converters (85%) than did the hippocampus alone (75%, respectively 76%). Our findings are similar to the results from the study by Dickerson et al. [13] in classifying non-demented subjects from AD patients. Because of the different protocols used in the latter two studies, we must be cautious when comparing them with our study. In contrast to our findings, in the study by Du et al. [14] the volume of ERC had a greater power of discrimination than that of hippocampus, while Xu et al. [51] identified no differences in the power of ERC and hippocampal volumes in classifying the MCI and AD groups.

Our findings suggest that ERC volume offers no advantage over hippocampal volume in differentiating MCI subjects from AD patients. It should be noted that the ERC is a very small region, which when atrophied, may be difficult to delineate [26,51]. On the other hand, the hippocampus does not pose any such problems with its boundaries or with depiction of the ROI, even if atrophied. This could explain the differences across structural MRI studies.

In conclusion, although both the measurements of ERC and those of the hippocampus differed significantly between AD patients and controls and between AD patients and MCI subjects, the discrimination by hippocampal volume measurements was slightly better between AD patients and control subjects, and significantly better between AD patients and MCI subjects. Instead, we showed that volumetric measurements of the ERC were more powerful than those of the hippocampus in discriminating MCI subjects from controls, in line with the fact that the pathology of AD originates in ERC. Discriminant function analysis including unilateral measurements yielded comparable power as the total volumes. Our findings showed that, although MCI subjects form a heterogeneous group which is difficult to distinguish from the group of controls by using only structural MRI volumetric measurements, this technique remains a useful tool in identifying the anatomical markers for incipient AD.

Acknowledgments

The authors wish to express their gratitude to Pirjo Halonen from the Computing Centre of the University of Kuopio, Finland, for statistical consultations. The study was supported by the Aging Program of the Health Research Council of the Academy of Finland and the EVO grants (5510, 5152) from the Kuopio University Hospital.

References

[1] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association; 1994.
[2] Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 1992;42:631–9.
[3] Berg L. Clinical dementia rating (CDR). Psychopharmacol Bull 1988;24:637–9.
[4] Bobinski M, de Leon MJ, Wegiel J, DeSanti S, Convit A, Saint Louis LA, et al. The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. Neuroscience 2000;95:721–5.
[5] Borkowski JG, Benton AL, Spreen O. Word fluency and brain damage. Neuropsychologia 1967;5:135–40.
[6] Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl) 1991;82:239–59.
[7] Braak H, Braak E, Bohl J. Staging of Alzheimer-related cortical destruction. Eur Neurol 1993;33:403–8.
[8] Butters N, Granholm E, Salmon DP, Grant I. Episodic and semantic memory: a comparison of amnesic and demented patients. J Clin Exp Neuropsychol 1987;9:479–97.
[9] Convit A, de Leon MJ, Tarshish C, De Santi S, Tsui W, Rusinek H, et al. Specific hippocampal volume reductions in individuals at risk for Alzheimer's disease. Neurobiol Aging 1997;18:131–8.
[10] de Leon MJ, Convit A, DeSanti S, Bobinski M, George AE, Wisniewski HM, et al. Contribution of structural neuroimaging to the early diagnosis of Alzheimer's disease. Int Psychogeriatr 1997;9(Suppl 1):183–90 [discussion 247–52].
[11] de Leon M, Bobinski M, Convit A, Wolf O, Insausti R. Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. Neurology 2001;56:820–1.
[12] De Santi S, de Leon MJ, Rusinek H, Convit A, Tarshish CY, Roche A, et al. Hippocampal formation glucose metabolism and volume losses in MCI and AD. Neurobiol Aging 2001;22:529–39.
[13] Dickerson BC, Goncharova I, Sullivan MP, Forchetti C, Wilson RS, Bennett DA, et al. MRI-derived entorhinal and hippocampal atrophy in incipient and very mild Alzheimer's disease. Neurobiol Aging 2001;22:747–54.
[14] Du AT, Schuff N, Amend D, Laakso MP, Hsu YY, Jagust WJ, et al. Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. J Neurol Neurosurg Psychiatry 2001;71:441–7.

[15] Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. J Psychiatry Res 1975;12:189–98.
[16] Frisoni GB, Laakso MP, Beltramello A, Geroldi C, Bianchetti A, Soininen H, et al. Hippocampal and entorhinal cortex atrophy in frontotemporal dementia and Alzheimer’s disease. Neurology 1999;52:91–100.
[17] Gomez-Isla T, Price JL, McKeel Jr DW, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer’s disease. J Neurosci 1996;16:4491–500.
[18] Goncharova II, Dickerson BC, Stoub TR, deToledo-Morrell L. MRI of human entorhinal cortex: a reliable protocol for volumetric measurement. Neurobiol Aging 2001;22:737–45.
[19] Hänninen T, Hallikainen M, Koivisto K, Partanen K, Laakso MP, Riekkinen Sr PJ, et al. Decline of frontal lobe functions in subjects with age-associated memory impairment. Neurology 1997;48:148–53.
[20] Hänninen T, Hallikainen M, Tuomainen S, Vanhanen M, Soininen H. Prevalence of mild cognitive impairment: a population-based study in elderly subjects. Acta Neurol Scand 2002;106:148–54.
[21] Huesgen CT, Burger PC, Crain BJ, Johnson GA. In vitro MR microscopy of the hippocampus in Alzheimer’s disease. Neurology 1993;43:145–52.
[22] Insausti R, Juottonen K, Soininen H, Insausti AM, Partanen K, Vainio P, et al. MR volumetric analysis of the human entorhinal, perirhinal, and temporopolar cortices. AJNR Am J Neuroradiol 1998;19:659–71.
[23] Jack Jr CR, Petersen RC, Xu YC, O’Brien PC, Smith GE, Ivnik RJ, et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology 1999;52:1397–403.
[24] Jack Jr CR, Petersen RC, Xu Y, O’Brien PC, Smith GE, Ivnik RJ, et al. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. Neurology 2000;55:484–9.
[25] Juottonen K, Laakso MP, Insausti R, Lehtovirta M, Pitkanen A, Partanen K, et al. Volumes of the entorhinal and perirhinal cortices in Alzheimer’s disease. Neurobiol Aging 1998;19:15–22.
[26] Juottonen K, Laakso MP, Partanen K, Soininen H. Comparative MR analysis of the entorhinal cortex and hippocampus in diagnosing Alzheimer disease. AJNR Am J Neuroradiol 1999;20:139–44.
[27] Kaplan EF, Goodglass H, Weintraub S. The Boston Naming Test. Boston: E. Kaplan & H. Goodglass; 1991.
[28] Killiany RJ, Gomez-Isla T, Moss M, Kikinis R, Sandor T, Jolesz F, et al. Use of structural magnetic resonance imaging to predict who will get Alzheimer’s disease. Ann Neurol 2000;47:430–9.
[29] Killiany RJ, Hyman BT, Gomez-Isla T, Moss MB, Kikinis R, Jolesz F, et al. MRI measures of entorhinal cortex versus hippocampus in preclinical AD. Neurology 2002;58:1188–96.
[30] Kivipelto M, Helkala E-L, Hänninen T, Laakso MP, Hallikainen M, Alhainen K, et al. Midlife vascular risk factors and late-life mild cognitive impairment: a population-based study. Neurology 2001;56:1683–9.
[31] Kordower JH, Chu Y, Stebbins GT, DeKosky ST, Cochran EJ, Bennett D, et al. Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. Ann Neurol 2001;49:202–13.
[32] Kluger A, Ferris SH, Golomb J, Mittelman MS, Reisberg B. Neuropsychological prediction of decline to dementia in nondemented elderly. J Geriatr Psychiatry Neurol 1999;12:168–79.
[33] Laakso MP, Soininen H, Partanen K, Helkala E-L, Hartikainen P, Vainio P, et al. Volumes of hippocampus, amygdala and frontal lobes in the MRI-based diagnosis of early Alzheimer’s disease: correlation with memory functions. J Neural Transm Park Dis Dement Sect 1995;9:73–86.
[34] Laakso MP, Soininen H, Partanen K, Lehtovirta M, Hallikainen M, Hänninen T, et al. MRI of the hippocampus in Alzheimer’s disease: sensitivity, specificity, and analysis of the incorrectly classified subjects. Neurobiol Aging 1998;19:23–31.
[35] Lehéricy S, Baulac M, Chiras J, Pierot L, Martin N, Pillon B, et al. Amygdalohippocampal MR volume measurements in the early stages of Alzheimer disease. AJNR Am J Neuroradiol 1994;15:929–37.
[36] Mann DM. The topographic distribution of brain atrophy in Alzheimer’s disease. Acta Neuropathol (Berl) 1991;83:81–6.
[37] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 1984;34:939–44.
[38] Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The consortium to establish a registry for Alzheimer’s disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer’s disease. Neurology 1989;39:1159–65.
[39] Petersen RC, Smith GE, Ivnik RJ, Tangalos EG, Schaid DJ, Thibodeau SN, et al. Apolipoprotein E status as a predictor of the development of Alzheimer’s disease in memory-impaired individuals. J Am Med Assoc 1995;273:1274–8.
[40] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999;56:303–8.
[41] Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2001;56:1133–42.
[42] Reitan RM. Validity of the Trail Making Test as an indicator of organic brain damage. Percept Mot Skills 1958;8:271–6.
[43] Russel E. A multiple scoring method for the assessment of complex memory functions. J Cons Clin Psychol 1975;43:800–9.
[44] Smith GE, Petersen RC, Parisi JE, Ivnik RJ, Kokmen E, Tangalos EG, et al. Definition, course, and outcome of mild cognitive impairment. Aging Neuropsychol Cogn 1996;3:141–7.
[45] Van Hoesen GW, Hyman BT. Hippocampal formation: anatomy and the patterns of pathology in Alzheimer’s disease. Prog Brain Res 1990;83:445–57.
[46] Van Hoesen GW, Hyman BT, Damasio AR. Entorhinal cortex pathology in Alzheimer’s disease. Hippocampus 1991;1:1–8.
[47] Visser PJ, Scheltens P, Verhey FR, Schmand B, Launer LJ, Jolles J, et al. Medial temporal lobe atrophy and memory dysfunction as predictors for dementia in subjects with mild cognitive impairment. J Neurol 1999;246:477–85.
[48] Visser PJ, Verhey FR, Hofman PA, Scheltens P, Jolles J. Medial temporal lobe atrophy predicts Alzheimer’s disease in patients with minor cognitive impairment. J Neurol Neurosurg Psychiatry 2002;72:491–7.
[49] Wechsler D. Wechsler Adult Intelligence Scale-Revised. Cleveland: Psychological Corporation; 1981.
[50] Wechsler D. Wechsler Memory Scale-Revised manual. San Antonio, TX: The Psychological Corporation; 1987.
[51] Xu Y, Jack Jr CR, O’Brien PC, Kokmen E, Smith GE, Ivnik RJ, et al. Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. Neurology 2000;54:1760–7.