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Impaired Visual Recognition Memory in Amnestic Mild Cognitive Impairment is Associated with Mesiotemporal Metabolic Changes on Magnetic Resonance Spectroscopic Imaging

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Abstract. In the early stages of Alzheimer's disease (AD), neurofibrillary tangles develop in the mesial temporal lobe (MTL), first in the anterior subhippocampal (perirhinal/entorhinal) cortex and then in the hippocampal formation. This region plays a key role in visual recognition memory (VRM). VRM has been reported to be impaired in patients with amnestic mild cognitive impairment (aMCI). The aim of the present study was to determine if an impairment of VRM is associated with metabolic changes in the MTL using magnetic resonance spectroscopic imaging and if evaluating VRM can contribute to the early diagnosis of AD. 28 patients with aMCI and 28 controls underwent a full neuropsychological assessment including an evaluation of VRM using the DMS48. NAA/mIno ratios, reduced in patients with AD and associated with the severity of pathological changes, were determined in the MTL. aMCI-patients were further divided into two subgroups according to their VRM performance. aMCI-patients showed decreased NAA/mIno levels in the right hippocampus compared with controls. aMCI-patients with impaired VRM showed decreased NAA/mIno ratios in the MTL bilaterally, including a region that sampled the left anterior subhippocampal cortex, compared to controls. No changes were found in aMCI patients with normal VRM. Performance on the DMS48 correlated with NAA/mIno levels in the anterior MTL. Clinical 6-year follow-up data (available for 78.6% of the aMCI-patients) indicates that impaired performance on the DMS48 could predict conversion to AD with a sensitivity and specificity of 81.8%. These findings provide further evidence that impaired VRM, as a hallmark of MTL dysfunction, may contribute to the early diagnosis of AD.

Keywords: Alzheimer's disease, entorhinal cortex, magnetic resonance spectroscopy, mesial temporal lobe, mild cognitive impairment, MRSI, perirhinal cortex, visual recognition memory, DMS48

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INTRODUCTION

Reliable diagnosis of Alzheimer's disease (AD) at the predementia stage is currently considered to be a priority for research, as disease modifying therapies are being evaluated. In AD, neurofibrillary tangles (NFT) initially develop in the mesial temporal lobe (MTL) in a sequential manner, initially affecting the anterior subhippocampal cortex (transentorhinal, entorhinal and perirhinal cortex) before reaching the hippocampal formation [1,2]. Although the difficulty of identifying boundaries of the entorhinal and perirhinal cortex, as well as the inter-personal variability of these anterior subhippocampal structures make it more difficult to assess than the hippocampal formation using neuroimaging techniques, potential non-invasive diagnostic tools that could reliably detect change of the subhippocampal region are currently receiving increasing interest. Using MRI, several studies demonstrated atrophy of the entorhinal cortex in incipient AD [3–8] and patients with cognitive complaints [6]. Baseline entorhinal cortex volume has been shown to be a better predictor for decline from MCI to AD than hippocampal volume [6, 8].

The function of the anterior subhippocampal cortex and its contribution to declarative memory is still debated. Meunier and collaborators demonstrated in experimental studies on non-human primates that selective lesions to the perirhinal and entorhinal cortex lead to impaired visual recognition memory (VRM) [9]. There is also increasing evidence from studies on patients with focal damage to the hippocampal formation that the anterior subhippocampal cortex plays a crucial role in VRM [10–13].

In addition, Barbeau and collaborators found VRM performance to correlate with the volume of the anterior subhippocampal cortex in aMCI patients [14]. In order to contribute to early diagnosis of AD, our group developed a delayed matching to sample task for human patients based on tasks used in experimental animals in order to evaluate VRM and assess the function of the anterior subhippocampal cortex, the DMS48. In previous studies, we reported that patients failing on the DMS 48 had both clinical [15] and neuroimaging features [14,16] of patients with early AD.

Regional pathologic metabolic change can be assessed using magnetic resonance spectroscopic imaging (MRSI). Several MRSI studies report changes in metabolic patterns in AD at the stage of dementia, with neuronal loss or dysfunction reflected by a decrease in N-acetylaspartate (NAA) levels [17] and glial cell activation reflected by increased myo-Inositol (mIno) levels [18–20]. Combining the NAA/mIno ratio has been reported to increase diagnostic accuracy in AD [18] and a correlative study recently demonstrated the antemortem NAA/mIno ratio to be associated with severity of AD pathology on postmortem brain tissue [21].

Metabolic changes in patients with MCI have also been reported using MRSI. Many studies assessed easily accessible brain regions, such as the posterior cingular cortex [21–23] or paratrigonal white matter [24]. MRSI studies focusing on the mesial temporal lobe (MTL) in MCI patients have so far been limited to the hippocampus [19,25] or extended to the MTL, without taking into account its subcomponents [26,27]. While several of these studies report metabolic changes mainly concerning NAA levels within the MTL in patients with MCI [19,25,26], one study reported no metabolic change [27]. To our knowledge, metabolic changes in the anterior subhippocampal regions, the site where NFT first appear in AD, have not specifically been investigated yet, probably because of technical challenges related to the size and the topography of the MTL leading to frequent artifacts.

The aim of the present study in aMCI-patients was twofold: 1) to determine if an impairment of VRM, a functional deficit previously reported in aMCI-patients at risk for AD, is associated with changes of the NAA/mIno-ratio within the MTL; 2) to assess NAA/mIno-ratios in subregions within the MTL including the anterior subhippocampal structures where NFT first develop in AD in patients with aMCI.

MATERIALS AND METHODS

Subjects

28 patients strictly meeting criteria for aMCI [28], consecutively enrolled into the Marseilles memory study (Mms) were included. Only patients with single domain amnestic MCI were selected, with a memory complaint, a performance of more than 1.5 SD below the mean of matched control subjects on delayed free recall of a verbal memory task, intact activities of daily living and no impairment in other cognitive domains like language, visuo-spatial skills, or executive function using normative data for matched controls. Brain imaging, routine biological survey, detailed neuropsychological evaluation, assessment of daily activities, psychiatric interview, and physical examination had been conducted prior to the inclusion into the present study

in order to exclude patients with a memory impairment subsequent to vascular disease, tumor, subdural hematoma, treatment, and concurrent diseases interfering with cognitive function. Other exclusion criteria were a history of systemic and/or neurological disease and a modified Hachinski ischemic score $\geqslant 2$ [29].

28 consecutively enrolled control subjects with normal cognitive functions and no history of systemic, mental, and neurological disorder were also included.

The procedures were done in accord with the ethical standards of the Committee on Human Experimentation of the institution and the local institutional committee approved this study. All subjects signed informed consent. Both, patients and controls underwent a full neuropsychological evaluation followed by MRSI exploration.

Assessment of VRM using the DMS48

The DMS48 (delayed matching to sample – 48 items; downloadable for research purposes at http://www.cerco.ups-tlse.fr/∼DMS48) [15] is a VRM test directly derived from tasks used in animal studies [9]. In the DMS48, stimuli to be learned consist of 48 color drawings. During the incidental learning phase, subjects are asked to look at each drawing carefully and state whether there are more or less than three colors in it. The time to complete the learning phase is recorded. Because the incidental learning phase is carefully monitored and because the subject is exposed to the stimuli twice before the long-term recognition phase, the possibility for the subjects to encode the stimuli is optimized (although encoding is not formally controlled since learning is incidental). This is followed by an interfering category fluency task. During the recognition task, each target is shown simultaneously with a distractor and the subject is asked to identify the target. Recognition is evaluated after a 3 minutes delay following the interfering task, and delayed recognition one hour later. Results reported in the present study are percentages of correct recognitions after the one-hour delay.

In order to control for deficits that could interfere with performance on the DMS48, visuo-perceptive abilities and short-term visual memory were previously evaluated during the neuropsychological assessment and only patients with normal performance were included.

aMCI subgroups

aMCI-patients were subdivided into two groups, according to their performance on the DMS48, defining the cut-off of impaired performance at 1.5 SD below the mean of age-matched controls [30].

MRSI

MR explorations were performed on a 1.5T MR scanner (Magnetom Vision Plus system, Siemens, Erlangen, Germany). The spectroscopic examination consisted of two 2D-MRSI slices acquired in the axial bihippocampal plane (Fig. 1) at two levels: the first slice was centered on the hippocampal formation (10 mm thickness), and the second was contiguous to the first slice but located 10 mm below including anterior subhippocampal tissue (Fig. 1). The MRSI acquisitions consisted in home-designed Hamming shape acquisition–weighted, inversion recovery (IR) 2D-SE pulse sequences (TI/TE/TR=150ms/22ms/1500ms; slice thickness 10mm; FOV=240 mm, 21×21 encoding steps leading to 524 free induction decays; acquired in 11 min 27 s) with outer volume suppression scheme [31, 32]. The IR scheme was used in order to minimize the contribution of fat scalp in the spectra. All radiofrequency pulses were optimized and generated using the Matpulse software [33] The spatial resolution was defined as the width of the spatial response function at 64% of maximum voxel volume of 5.7 mL [34,35]. Due to the pseudo circular spatial k sampling, voxel shape was cylindrical, resulting in an effective resolution of 22 mm of diameter. The water/lipid suppression scheme was implemented according to Tkac et al. [36] with outer volume saturation and chemical selection saturation scheme to suppress scalp fat and water signals, respectively. Automatic (mapshim) and manual shimming was performed on the water signal in the slice of interest. Measurement was conducted after optimization (frequency and pulse intensity) of the water suppression scheme.

Regions of interest (ROIs) for spectroscopic analyses within the mesial temporal lobe were delineated in ROIs in the bi-hippocampal plane at two levels. The first slice was centered on the hippocampal formation because of evidence for metabolic change in this area from studies in AD [17,27] and MCI [19, 25] using MRSI and because NFT in the hippocampal formation have been reported in patients with AD at the stage of MCI [37]. Because of evidence for an antero-posterior gradient in the distribution of NFT within the hippocampal formation, NFT first appearing in the CA1 subfield of the hippocampus [38], most represented in the head of the hippocampus, and studies suggesting an antero-posterior gradient concerning

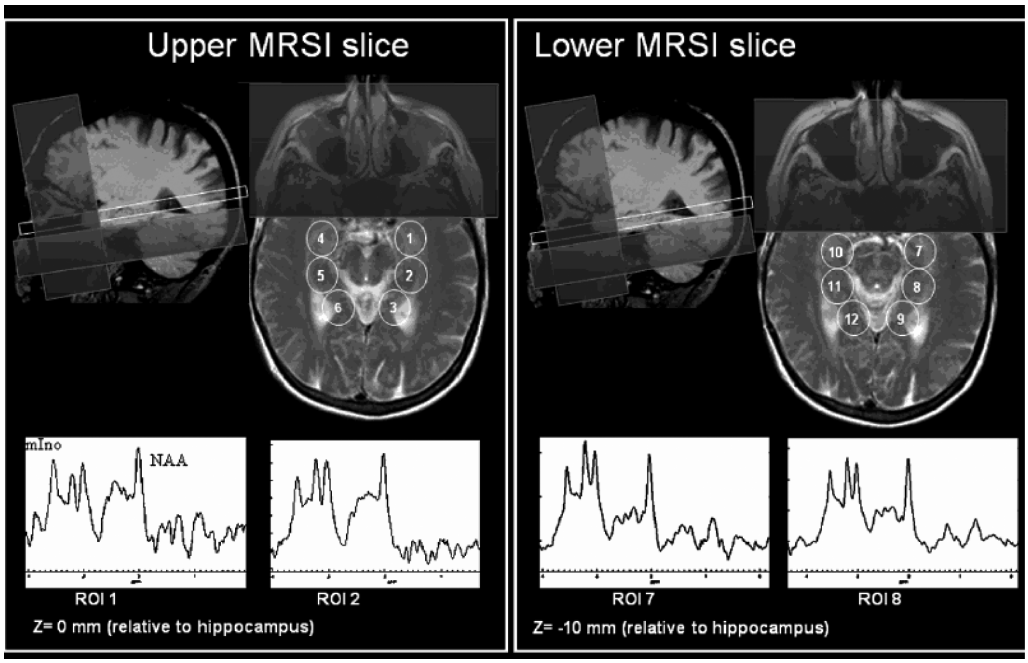

Fig. 1. MRSI slices acquired to sample medial temporal lobe structures in aMCI patients and controls. Schematic representation of the MRSI ROIs including real point spread function. The gray shade represents the saturation bands used to limit fat bone contamination. The first MRSI slice was centered on the hippocampus to sample the amygdala and the head of the hippocampus (ROIs 1 and 4), the body of hippocampus (ROIs 2 and 5) and the tail of the hippocampus (ROIs 3 and 6). The second MRSI slice was contiguous to the first and positioned just below in order to sample perirhinal/entorhinal cortices and part of the head of the hippocampus (ROIs 7 and 10), the medial parahippocampal cortex and part of the body of the hippocampus (ROIs 8 and 11), the posterior parahippocampal cortex, and part of the tail of the hippocampus (ROI 9 and 12).

declarative memory [39], several ROIs were placed along its long axis. The rostral ROI included signal from both the amygdala and part of the head of the hippocampus (ROI 1 and ROI 4). Another ROI was placed in the hippocampal body (ROI 2 and ROI 5) and a caudal ROI was placed in the tail of the hippocampus (ROI 3 and ROI 6). The second MRSI slice was contiguous to the first and positioned just below in order to sample metabolites in the perirhinal/entorhinal cortices because of evidence for neurofibrillary tangles in transentorhinal, perirhinal and entrorhinal cortex in Braak and Braak's stage I of AD [1,2] and evidence for the involvement of these anterior subhippocampal areas in visual recognition memory [9,11,13,14] (ROI 7 and 10). For comparative reasons, we also sampled the medial parahippocampal cortex (ROI 8 and 11) and the posterior parahippocampal cortex (ROI 9 and 12), since modular models of declarative memory suggest that more posterior parts of the parahippocampal gyrus are not involved in visual recognition memory [40,41]. However, because of the technical challenge related to the assessment of the anterior subhippocampal areas and the parahippocampal gyrus with MRSI due to their small size and neighboring structures causing artifacts, all ROIs of the second MRSI slice included some additional signal from the hippocampal formation.

MRSI data were post-processed and quantified with a home-written software (CSIAPO) [42] based on the IDL platform (Interactive Data Language, Research System Inc., Boulder, CO). Time domain post-processing was conducted on spectroscopic raw data. The remaining water signal was removed using the Hankel Lanczos singular value decomposition (HLSVD) method [43]. Time domain fitting was performed with AMARES (advanced method for accurate, robust, and efficient spectral fitting) [44] to quantify the areas of signals corresponding to NAA and mIno. Quality of fits was evaluated visually by looking at the residuals of the difference between the modeled and the real spectra and validated by Cramer-Rao of AMARES analysis. Meaningless fitting results were discarded. The metabolic ratio NAA/mIno was determined because of evidence for a high diagnostic accuracy using this ratio in AD [18] and the association of NAA/mIno ratio with Braak and Braak stages in a correlative neuro-pathological study [21], as well as correlations of cognitive measures reflecting disease severity with this ratio [17,18].

Table 1
Demographic and neuropsychological data of patients and controls at the time of scan

	Controls	aMCI	aMCI subgroup DMS48+	aMCI subgroup DMS48 –
n	28	28	12	16
Women/men	13/15	16/12	7/5	9/7
Age in years	63.3 (7.2)	69.3 (8.6)*	72.1 (9.1)*	67.3 (7.8)
MMSE	28.9 (1.0)	27.4 (1.4)*	27.7 (1.7)*	27.2 (1.2)*
Assessment of memory				
DMS48 (max = 100%)	98% (3%)	87% (10%)*	95% (3%)*	82% (9%)*,a
Delayed free recall of a word list (max = 16)	13.1 (1.7)	5.3 (3.1)*	6.8 (2.5)*	4.2 (3.1)*,a
Cueing efficiency (max = 100%)	89% (22%)	74% (22%)*	86% (12%)	66% (24%)*,a
Intrusions during recall	0.8 (1.2)	4.3 (5.2)*	1.8 (2.1)	8.2 (8.5)*,a
Assessment of executive functions				
WMS-III digit span scaled score	10.6 (3.2)	9.4 (2.9)	9.7 (3.1)	9.1 (2.7)
Verbal Fluency "P" in 2 mn	26.9 (7.1)	17.4 (4.7)*	19.5 (4.5)*	15.8 (6.2)*,a
Verbal Fluency repetition	0.37 (0.69)	0.61 (0.96)	1.17 (1.19)*	0.19 (0.40)
MCST – categories (max = 6)	n/a	4.8 (1.2)	4.8 (1.4)	4.7 (1.0)
MCST – errors	n/a	19.39 (12.1)	21.5 (12.8)	17.8 (11.6)
Picture naming (max = 80)	79.7 (0.6)	79.3 (1.3)	78.7 (1.8)	79.6 (0.9)

Mean, SD in brackets. n/a: not available. $^{*}p < 0.05$ relative to controls; $^{a}p < 0.05$ aMCI succeeding on the DMS48 relative to MCI failing on the DMS48. DMS48 +: aMCI subgroup with normal performance on the DMS48. DMS48-: aMCI subgroup with impaired performance on the DMS48. MCST: Modified Card Sorting Test.

Clinical follow-up

In order to evaluate outcome, 22 patients were assessed 6 years after the inclusion into the study. Four of the remaining patients will be assessed at 6 year follow-up over the coming year. Two patients died before the 6 year follow-up due to acute diseases that were unrelated to their memory dysfunction.

Data analysis

Statistical analyses were performed with SPSS Version 17.0 (Chicago, IL). Comparisons of metabolic peak ratios between aMCI ($n = 28$) and controls ($n = 28$) were assessed using ANOVA adjusted for age. Nonparametric Mann-Whitney U test was used to compare age and neuropsychological data, as well as for comparisons of metabolic peak ratios between the subgroup of aMCI failing ($n = 16$) and the subgroup succeeding on the DMS48 ($n = 12$), as well as for the comparison of both subgroups with controls. Khi square test was used to compare age and gender between aMCI and controls, as well as across aMCI subgroups. Non parametric Spearman rank correlations were used to determine relationships between metabolic ratios and VRM performance for all subjects. Because each hypothesis test addresses a distinct, although related, neuropsychological, clinical and neuropathological question of interest according to a pre-established hypothesis, we did not correct reported p-values for multiple comparisons [21,23,45]. The level of significance was set at 0.05.

RESULTS

aMCI subgroups

12 aMCI-patients had normal DMS48 scores (DMS+), while performance on the DMS48 was impaired in 16 patients (DMS-).

Demographics and neuropsychological evaluation. Demographical and clinical data is displayed in Table 1.

aMCI-group compared to controls

Patients with aMCI were significantly older than controls ($p = 0.012$). There was no difference concerning gender between the aMCI group and controls. As expected, aMCI patients differed significantly from controls on the Mini Mental State Examination (MMSE) and all tasks evaluating memory. Compared with controls, the aMCI group also produced significantly less words beginning with the letter p on a verbal fluency task [46]. The performance of aMCI-patients did not differ from controls on other tasks evaluating executive functions like the Modified Card Sorting test [47], the digit span [48], or a picture naming task [49].

aMCI DMS- subgroup compared to controls

The aMCI-patient subgroup with impaired performance on the DMS48 (DMS-) did not differ from that of controls concerning age or gender. MMSE differed from controls, as expected. Concerning the assessment

Table 2
Follow-up data on conversion 6 years after the inclusion into the study

	aMCI subgroup DMS48+	aMCI subgroup DMS48-
n	12	16
Converters	2	9
Non-converters	9	2
Follow-up scheduled	0	4
Not available for follow-up	1	1

Converters: patients with probable AD fulfilling NINCDS-ADRDA criteria for AD [51]. Non-converters: patient not fulfilling NINCDS-ADRDA criteria for AD [51]. DMS48 +: aMCI subgroup with normal performance on the DMS48. DMS48-: aMCI subgroup with impaired performance on the DMS48.

of memory, the aMCI DMS- subgroup differed from controls on delayed free recall on the Free and Cued Selective Reminding Test (FCSR) [50] and the delayed recognition trial of the DMS48. Significantly more intrusions and less benefit from cueing on the FCSR were also observed. Concerning executive functions, the aMCI DMS- subgroups performed significantly worse than controls on a verbal fluency task (letter p). No difference between controls and the aMCI DMS- subgroup was found on other tasks evaluating executive functions or naming.

aMCI DMS+ subgroup compared to controls

While the aMCI subgroup with normal performance on the DMS48 (DMS+) was significantly older than controls ($p < 0.01$), there was no difference concerning gender. MMSE differed from controls. The aMCI DMS+ subgroup differed from controls on delayed free recall on the Free and Cued Selective Reminding Test (FCSR) and the delayed recognition trial of the DMS48. Concerning executive functions, the aMCI DMS+ subgroup performed significantly worse than controls on a verbal fluency task (letter p) and more repetitions in the verbal fluency task compared with controls were also found. There were no differences on other tasks evaluating executive functions or naming.

aMCI subgroup DMS – compared to aMCI subgroup DMS+

The two subgroups of aMCI-patients did not differ concerning age or gender. MMSE did not differ between the subgroups either. Compared with the DMS+ subgroup, the DMS48- aMCI subgroup performed significantly worse on delayed free recall of the FCSR, benefited less from cueing on this task and made more intrusions. As expected, the DMS48- subgroup performed significantly worse on the DMS48 than the DMS48+ subgroup. aMCI-patients failing on the DMS48 produced significantly less words beginning with the letter p than aMCI patients succeeding on the task. No other differences were found on tasks evaluating executive functions or naming comparing the two subgroups.

Clinical follow-up data

22 of the 28 patients that were included into this study were re-examined 6 years after the initial assessment (78.6%). At follow-up, 11 patients fulfilled NINCDS-ADRDA criteria for AD [51]. 6 of the patients still fulfilled criteria for MCI (Table 2). 5 patients, although still complaining of memory loss, regained normal scores on tasks evaluating memory. Below cut-off performance on the DMS48 at baseline (i.e., 1.5 SD below the mean of controls) predicted conversion to AD at 6 year with a sensitivity of 81.8%, and a specificity of 81.8%.

Metabolic MRSI profiles

aMCI- group compared to controls

Compared with controls and controlling for age, we found a decreased NAA/mIno ratio in patients with aMCI in the right body of the hippocampus (ROI 5) (F-test $p = 0.05$ – group effect $p = 0.02$ – age effect $p = 0.98$) (Table 3).

aMCI DMS- subgroup compared to controls

Compared to controls, the subgroup of aMCI-patients with impaired VRM showed a decrease in NAA/mIno in the right hippocampal body (ROI 5: $p = 0.019$) and in the region containing the left head of the hippocampus and the amygdala (ROI 1: $p = 0.049$), as well as in the region containing the left anterior subhippocampal cortex (ROI 7: $p = 0.049$) (Table 3).

aMCI DMS+ subgroup compared to controls

aMCI-patients with normal performance on the VRM task showed no significant difference of NAA/mIno levels relative to controls (Table 3).

Correlations between performance on the DMS48 and NAA/mIno

A correlation between performance on the DMS48 and NAA/mIno-ratios was observed in the ROI containing anterior subhippocampal cortex on both the left (ROI 7) ($p = 0.029$, rho: 0.323) and the right side (ROI 10) ($p = 0.037$, rho: 0.309), as well as for the left anterior hippocampus (ROI 1) ($p = 0.013$, rho: 0.351).

Table 3
Metabolic parameters

	Regions	Controls	all aMCI	aMCI DMS –	aMCI DMS +
#	Location	($n = 28$)	($n = 28$)	($n = 16$)	($n = 12$)
1	L amygdala/ head of HF	1.60 (0.72)	1.3 (0.56)	1.25 (0.28)**	1.58 (0.78)
4	R amygdala/head of HF	1.48 (0.48)	1.38 (0.45)	1.34 (0.53)	1.44 (0.32)
2	L body of HF	2.29 (0.48)	2.19 (0.44)	2.33 (0.44)	2.16 (0.39)
5	R body of HF	2.48 (0.50)	2.16 (0.49)*	2.07 (0.49)**	2.29 (0.48)
7	L PC – ER cortices	1.74 (0.40)	1.53 (0.33)	1.48 (0.30)**	1.63 (0.41)
10	R PC – ER cortices	1.80 (0.47)	1.64 (0.34)	1.60 (0.35)	1.74 (0.30)
8	L medial PH cortex/body of HF	2.26 (0.30)	2.41 (0.44)	2.44 (0.51)	2.38 (0.35)
11	R medial PH cortex/body of HF	2.43 (0.36)	2.49 (0.76)	2.60 (0.94)	2.36 (0.41)
3	L tail of HF	2.74 (0.42)	2.75 (0.41)	2.86 (0.43)	2.61 (0.32)
6	R tail of HF	2.90 (0.48)	2.75 (0.56)	2.77 (0.70)	2.71 (0.30)
9	L post PH cortex/tail of HF	2.96 (0.40)	2.83 (0.32)	2.90 (0.33)	2.75 (0.32)
12	R post PH cortex / tail of HF	3.07 (0.46)	2.98 (0.98)	3.13 (0.99)	2.77 (0.99)

DMS48 +: aMCI subgroup with normal performance on the DMS48. DMS48-: aMCI subgroup with impaired performance on the DMS48. L = left, R = right, EC = entorhinal cortex, PC = perirhinal cortex, HF = hippocampal formation, PH = parahippocampal cortex.
*Significantly different from controls using ANOVA with group and age as variables.
**Significantly different from controls (Mann-Whitney U test).

DISCUSSION

The aim of the present study was to evaluate, within a group of amnestic MCI-patients at risk for AD, if an impairment of visual recognition memory (VRM) is associated with changes of the NAA/mIno ratio within the MTL including the anterior subhippocampal structures where NFT first develop in AD. The main findings of the study are: 1) Reduced NAA/mIno levels were detected in the MTL of aMCI patients; 2) Reduced NAA/mIno ratios in several regions of the MTL were only found in a subgroup of aMCI patients failing on a VRM task designed to assess the function of the anterior subhippocampal region; 3) Performance on the VRM task was correlated with the NAA/mIno ratio of anterior MTL structures. Finally, and from a practical point of view, although we were able to sample brain metabolites in a region containing the anterior subhippocampal (perirhinal and entorhinal) cortex using 2D MRSI at two levels, there was an overlap with the hippocampus because of the technical difficulty of sampling the anterior subhippocampal cortex exclusively.

Metabolic changes on MRSI in the MTL of aMCI patients

Metabolic change within the MTL has inconsistently been reported in patients with MCI using MRSI. Chantal et al. [19] and Ackl et al. [25] both reported reduced NAA in the hippocampus in a population that included patients with both, single domain and multidomain aMCI. The latter, although considered to have a high likelihood of progression to AD [52] may represent a later stage of AD than single domain MCI, since the memory impairment is associated with impairment in other cognitive domains and, probably, more widespread pathology. In a recent multicenter study, the authors attributed the lack of metabolic change in the MTL in MCI-patients partly to a selection bias, suggesting that the low threshold in the inclusion criteria consisting in performance below one standard deviation on any cognitive task might have led to the inclusion of a large proportion of patients without an underlying degenerative disease [27]. The present study confirms that metabolic changes can be detected in the MTL as early as the stage of single domain amnestic MCI (or in cognitively impaired but not-demented patients), as previously reported by Chao and collaborators for NAA [26].

Metabolic changes on MRSI in aMCI subgroups

Patient selection using appropriate neuropsychological tasks also appears to be crucial. When aMCI-patients were separated into subgroups on the basis on their performance on a VRM-task, metabolic change in several regions of the MTL was only found in the aMCI subgroup with impaired VRM, compared to controls. In addition, while reduced NAA/mIno levels were detected in the right hippocampal body in the aMCI group as a whole, there was metabolic change in several subregions of the MTL in the subgroup of aMCI patients who failed on the VRM task. In this subgroup, reduced NAA/mIno levels were detected in the left anterior hippocampus and a region that contains the anterior subhippocampal region, where NFT first appear in AD. If

metabolic change had been restricted to this lower region containing anterior subhippocampal metabolites, this could have been in favor of specific subhippocampal changes. However, because of the overlap with signal emerging from the hippocampal formation in this region of interest, and since reduced NAA/mIno levels were also found in the adjacent region sampling the head of the hippocampus, it is uncertain whether these changes are related to the anterior subhippocampal region only. Further MRSI studies using higher magnetic fields, with a better spatial resolution, may be able to identify metabolic changes related to neuropathological lesions in the entorhinal and perirhinal cortex more reliably. Within the hippocampus, reduced NAA/mIno ratios within the left head and the right body could be related to neuropathological change in the CA1 field of the hippocampus, which is overrepresented in the head and the body and where changes have been described in the early stages of AD in both neuropathological [53] and neuroimaging studies [54]. As expected, there was no reduction of the NAA/mIno ratio in the tail of the hippocampus and the posterior parahippocampal gyrus. No metabolic anomalies were found in the aMCI subgroup succeeding on the VRM task compared with controls. Although this subgroup was significantly older than the controls, age is unlikely to account for the present findings, since pathological changes would be expected to increase with age. Also, there was no correlation of metabolic ratios with age. Distinct metabolic patterns in the two subgroups of aMCI-patients could not be explained by a difference in severity of cognitive decline, since mean MMSE scores did not differ either.

Metabolic changes on MRSI and AD

It is likely that reduced NAA/mIno ratios in the present study are AD related, since a reduction of the NAA/mIno ratio has been shown to be associated with severity of AD pathology on postmortem brain tissue [21]. Decreased NAA is a marker of neuronal dysfunction or axonal injury, due to impaired mitochondrial metabolism, while elevated mIno levels are associated with glial activation [18]. In AD, a decrease of NAA combined with an increase in mIno is thought to reflect neuronal loss combined with replacement through gliosis. Reductions of the NAA ratio or of NAA/Cr levels have been reported in several studies on patients with MCI who later declined to AD [55–57] and in patients with probable AD [17–19,25,27]. Increased mIno has been reported in the white matter of patients with MCI and AD [24,58,59]. Recently, it has been suggested that NAA/mIno ratios [21] or NAA measures [27] could be additional biomarker candidates for AD.

Performance on the DMS48 and NAA/mIno levels in the MTL

The present findings support the role of anterior MTL structures in VRM. Performance on a visual recognition memory task was correlated with NAA/mIno in the MTL lobes, with both, the region containing metabolites from the anterior subhippocampal cortex and the anterior hippocampal formation. While a previous MRI-study using Volume Based Morphometry found a correlation of performance on the DMS48 with the volume of the anterior subhippocampal cortex only, in line with the crucial role of the perirhinal cortex in VRM [11–13,41,60,61], using MRSI, there was also a correlation with the anterior hippocampus. While a functional relationship between NAA/mIno ratios or the NAA/Cr-ratio with cognitive function or disease severity is supported by several studies [17,18,62], it could be that the correlation with the hippocampal formation reflects a confounding factor related to neuropathological changes in the hippocampus and subhippocampal region of the aMCI patients with AD enrolled into the present study. However, we cannot exclude that the association with metabolite levels in MTL reflects a functional relationship supporting an alternative model of declarative memory in which all MTL structures contribute to VRM [63]. That no correlation was found in the posterior parahippocampal gyrus and more posterior structures within the hippocampal formation is consistent with models based on studies with experimental animals [40] and patients with focal lesions suggesting that the posterior MTL is not involved in VRM [41].

Implications of the assessment of visual recognition memory for diagnosis of AD in patients with aMCI

The results of the present study are consistent with previous findings that suggested that aMCI patients with impaired VRM may be at particularly high risk for AD [14–16]. On a clinical level, Dubois and collaborators suggested that memory impairment with a recall deficit and a reduced benefit from cueing, highly indicative of MTL dysfunction, ought to be considered as a “core diagnostic criterion” for the diagnosis of AD [64]. The memory profile of the aMCI patients with impaired VRM of the present study was also characterized by reduced delayed free recall and reduced cueing efficiency on the Free and Cued Selective Reminding Test [50]. There is also evidence from imaging studies that patients with impaired VRM present

changes that are consistent with early AD. On SPECT, Guedj and collaborators found hypoperfusion in the MTL, the posterior cingulate and the temporo-parietal cortices in a subgroup of aMCI patients with impaired VRM [16], a profile that is commonly found in early AD [65,66]. Using Voxel Based Morphometry, aMCI-patients who fail on the VRM-task have been found to display gray matter loss in the MTL and temporo-parietal cortex [14], an imaging profile also reported in early AD [67–69]. Although not specifically investigated, a number of studies report deficits on tasks evaluating visual recognition memory in patients with AD [70] including the early stages of AD [71–73] and in patients with aMCI [74]. In familial AD, Fox and collaborators observed that patients who later developed AD scored below controls on recognition memory tasks before the clinical onset of the disease [75].

Clinical follow-up for 22 patients who have been followed over 6 years indicates that assessing VRM using the DMS48 can identify early AD in patients with aMCI with a sensibility and specificity of both 81.8%. Considering the small group of subjects, the predictive value appears high. This adds to neuropsychological and imaging findings that suggest that these patients may be those who will ultimately develop AD. Among the limitations of the present study are the relatively small number of subjects included, the absence of available CSF to assess biomarkers for AD, as well as amyloid PET or neuropathological data that could provide additional evidence for AD pathology in these patients, since the combined use of cognitive tasks with several neuroimaging techniques, as well as CSF biomarkers, is most likely to predict AD in patients with aMCI [52]. However, the findings of the present study converge with efforts to integrate the type of memory impairment in early diagnosis of AD [64]. They also suggest that early diagnosis of AD should not only take into account changes concerning the hippocampus, but also changes resulting from dysfunction of the subhippocampal region. Neuropsychological tasks designed to evaluate brain areas where degenerative change causes dysfunction could be considered as biomarkers reflecting neural dysfunction on a clinical level. In this context, assessing the function of the subhippocampal region through VRM may critically contribute to early diagnosis of AD.

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