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Research Article

Thickness in Entorhinal and Subicular Cortex Predicts Episodic Memory Decline in Mild Cognitive Impairment

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Identifying subjects with mild cognitive impairment (MCI) most likely to decline in cognition over time is a major focus in Alzheimer's disease (AD) research. Neuroimaging biomarkers that predict decline would have great potential for increasing the efficacy of early intervention. In this study, we used high-resolution MRI, combined with a cortical unfolding technique to increase visibility of the convoluted medial temporal lobe (MTL), to assess whether gray matter thickness in subjects with MCI correlated to decline in cognition over two years. We found that thickness in the entorhinal (ERC) and subicular (Sub) cortices of MCI subjects at initial assessment correlated to change in memory encoding over two years (ERC: $r = 0.34$; $P = .003$) and Sub ($r = 0.26$; $P = .011$) but not delayed recall performance. Our findings suggest that aspects of memory performance may be differentially affected in the early stages of AD. Given the MTL's involvement in early stages of neurodegeneration in AD, clarifying the relationship of these brain regions and the link to resultant cognitive decline is critical in understanding disease progression.

1. Introduction

Identifying optimal markers for subsequent cognitive decline has become a major focus in Alzheimer's disease (AD) research, especially using neuroimaging tools. Advances in neuroimaging research have led to a shift in focus from using imaging for diagnosis of AD, to the current focus of predicting conversion from preclinical states to AD. Recent results suggest subjects with mild cognitive impairment (MCI) convert to AD at an annual rate of 17% [1], although other studies have reported conversion rates in the range of 10–15% [2]. Classic MCI symptoms include deficits in the memory domain greater than expected for age, while maintaining normal overall cognition and daily functioning [2]. Amnestic MCI in particular has been identified as a precursor to AD [3]. However, predicting who among a group of MCI patients is most likely to decline in cognition within a specified period of time is more challenging. Neuroimaging biomarkers that predict likelihood of decline on an individual basis would have the greatest potential benefit for assessing risk level in single patients before the onset of the disease, including earlier therapeutic and pharmacologic intervention (and, thus, increased treatment efficacy), patient counseling, and the design of clinical trials. Developing tools to detect progression and predict conversion from MCI to AD is therefore a critical research focus.

Neuroimaging biomarkers for predicting cognitive decline or conversion to AD include [18F]fluorodeoxyglucose uptake (FDG-PET) in parietal, posterior cingulate, and temporal brain regions [4, 5] and MRI evidence of medial temporal lobe and hippocampal atrophy [6–9]. In a comparison study that examined the ability of a number of biomarkers to predict decline in a single participant pool, FDG-PET significantly predicted conversion to AD while hippocampal volume demonstrated a trend towards significance in predicting decline but did not pass threshold ($P = .06$) [1]. In a longitudinal study of MCI subjects

assessed with (11)C-PIB as an in vivo marker of brain amyloid load, PIB-positive subjects were more likely to convert to AD than PIB-negative subjects [10].

Historically results on whether medial temporal lobe (MTL) atrophy predicts conversion to AD have been mixed. Some studies have shown a relationship between smaller hippocampi and conversion from MCI to AD [6, 11, 12], while others have not [13–16]. Previous results from our lab in preclinical subjects [17] and findings from AD patients [18] indicate that measuring gray matter thickness is a more sensitive indicator of subtle structural changes related to AD than traditional volumetric methods that assess total size of a region. We suggest that increases in sensitivity among neuroimaging tools may demonstrate a clearer picture of AD pathology in its earliest stages.

Recent advances in resolution and image analysis techniques enable us to investigate regional brain characteristics previously beyond the scope of *in vivo* imaging. Our approach examines subregions of the MTL system using high-resolution MRI combined with cortical unfolding to increase visualization of the convoluted MTL and directly assess 3-dimensional anatomical integrity. Originally applied to the visual cortex [19], we have adapted this technique to the MTL structures including hippocampal subfields, ento- and perirhinal cortices, subiculum, and parahippocampal and fusiform gyri [17, 20–23]. Previous results from our lab using this approach revealed reduced cortical thickness in cognitively intact carriers of the APOE-4 variant [17]. In the current study, we hypothesized that this technique would detect a relationship between subtle structural differences and cognitive decline in MCI subjects specific to cognitive domains affected by neural loss in the MTL.

It is wellaccepted that the MTL plays an essential role in associative memory [24]; however, the exact contribution of subregional areas to different aspects of memory formation is still under debate. It is clear that the pathological processes of AD, which begin in the entorhinal cortex (ERC) before spreading to hippocampus proper and other neocortical regions, affect the formation of new memories early in disease development. Pathologically, H. Braak and E. Braak demonstrated in post mortem brains that the hallmark neurofibrillary tangles evident in AD appear first in the prealpha transentorhinal neurons and then spread to the ERC proper [25]. From here, the tangles spread to the subiculum (Sub) and Cornu Ammonis (CA) 1, then to CA 2 and 3 [26]. It is likely, therefore, that neuronal death in ERC and potentially Sub and CA1 areas would be sensitive predictors of subsequent decline in memory encoding.

While it is clear that an intact MTL is necessary for the formation of episodic memory [27], it is still unclear how specific subregions are involved in declarative learning [24]. A recent study of AD patients by Wolk and Dickerson aimed to determine the whole-brain neural correlates of different stages of episodic memory formation and their modulation in Alzhiemer's disease [28]. The authors focused on whole-brain neural networks supporting cognitive domains and found that volumetric analysis of the hippocampus correlated with both immediate recall and delayed recall performance. We suggest that further parcelation of the MTL into substructures may help to more fully understand the contribution of separate regions within the MTL to aspects of memory performance in AD.

In the current study, we examined the relationship between MTL subregions in amnestic MCI subjects and cognitive performance two years later in key functional domains using high-resolution imaging combined with a cortical flattening algorithm applied to the MTL. We examined specific functional domains separately by combining data across several tests measuring similar cognitive functions, thereby adding power and decreasing error variance. These domains included executive functioning, processing speed, memory encoding, and delayed memory. MTL subregions are intimately tied to memory performance; specifically the entorhinal cortex is known as the gateway of the hippocampus [29], connecting the neocortex to the hippocampal formation, and is heavily involved in the encoding of new memories. We hypothesized, therefore, that thinner entorhinal cortex in MCI patients at their initial visit would correlate to greater cognitive decline in the encoding domain two years later. To test this hypothesis, we correlated thickness metrics in subregional areas of the MTL to memory performance in the encoding domain. To the best of our knowledge, this is the first study to assess anatomical integrity within MTL subregions in MCI subjects and the correlation to performance in encoding new associations at a two-year follow-up.

2. Methods

2.1. Subjects. Participants were drawn from a pool of potential subjects (age range: 45 to 84 years) recruited through local advertising of a study of mild memory impairment, media coverage of the study, and referrals by physicians and families for investigations intended to examine brain structure and function using neuroimaging techniques in cognitively healthy people, subjects with MCI, and AD, from 2004 to 2008. Subjects with a history of psychiatric or neurologic disorder, alcohol or substance abuse, head trauma or other major systemic disease that affects brain function, as well as hypertension and cardiovascular disease were excluded. At both their initial and follow-up visits, all subjects underwent diagnostic evaluation including physical and medical examination, laboratory screening blood tests that ruled out medical conditions possibly affecting cognition, medical history assessment, and neuropsychological testing. Subjects were asked to return for follow-up at a two-year interval (average time between visits = 25.9 ± 7.9 months for MCI subjects; 24.3 ± 6.5 months for normal control (NC)). From this potential pool of participants we identified 45 subjects who had received a follow-up neuropsychological assessment as of 2010 and who had good quality MRI data at their initial visit. This resulted in a subject pool of 25 MCI and 20 NC subjects. All subjects had baseline MRI scans, while 20 MCI subjects and 19 control subjects also had follow-up MRI exams. Five MCI subjects converted to AD and 3 NC converted to MCI over the timecourse of the study. Three of the MCI subjects who

converted to AD were unable to complete a follow-up MRI due to an inability to lie still inside the scanner, while two additional MCI patients who did not convert and 1 NC who converted to MCI refused an MRI at the time of follow-up. All MRI scans were reviewed by a neuroradiologist to rule out medical conditions that would preclude subjects from participating in the study. Investigators were unaware of the clinical data when deciding whether to exclude scans for poor image quality. Demographic and clinical characteristics are presented in Table 1. There were no significant differences between MCI and NC groups for any of the characteristics presented in Table 1. The study procedures were performed at the Semel Institute for Neuroscience and Human Behavior at the University of California, Los Angeles. Subjects gave written informed consent according to the UCLA Human Subjects Protection Committee procedures.

Table 1: Clinical and demographic characteristics of MCI (mild cognitive impairment) and NC (normal control) subjects. Scores are listed as mean (standard deviation).

Characteristic	MCI	NC
No. enrolled (baseline)	25	20
M/F	15/10	7/13
Age, y (baseline)	63.7 (10.7)	63.6 (11.9)
Education, y	17.1 (3.3)	16.6 (2.9)
Mini_mental state examination	28.4 (1.4)	29.3 (0.9)
Hamilton depression scale score	2.04 (2.6)	1.45 (2.1)
Follow-up MRI completed	20	19

2.2. Neuropsychological Testing. All subjects were given the Mini-Mental State Examination [30], the Hamilton Rating Scale for Depression [31] to assess mood, and a clinical interview at both initial and follow-up assessments. We administered a battery of neuropsychological tests at both visits [32] and divided scores into the following domains of cognitive functioning: *Processing Speed* (Wechsler adult intelligence scale-III Digit Symbol; Trailmaking test part A; Stroop test, word reading speed), *Executive Functioning* (Verbal fluency FAS and animal naming tests; Trailmaking test part B; Stroop test, interference), and two memory domains, based on subtests involved primarily in forming new associations and those measured retrieval after a delay period: *Memory Encoding* (Wechsler memory scale-III: Logical Memory I and Verbal Paired Associates I; Buschke-Fuld selective reminding test, consistent long-term retrieval), and *Delayed Memory* (Rey Osterrieth Complex Figure, delayed recall; Wechsler memory scale: Logical Memory II and Verbal Paired Associates II; Buschke-Fuld selective reminding test, delayed recall). To ascertain cognitive change in subjects, for each of the cognitive measures, we first calculated change scores (follow-up − baseline). These raw change scores were converted to Z scores (Z = (raw score − mean)/standard deviation)), and a domain Z score was obtained by averaging those Z scores belonging to the cognitive tests in that domain. In addition, an overall cognitive change Z score was obtained by averaging the 5 domain Z scores. The overall and domain Z scores were used to examine associations with subregional cortical thickness [33, 34].

In order to diagnose MCI, we used recent standard diagnostic criteria [35], which include (1) patient awareness of memory decline, preferably confirmed by another person; (2) greater-than normal cognitive impairment on standardized tests; (3) normal daily activities performance; and (4) no dementia. We included MCI subjects who scored ≥1 SD below age-corrected norms, as this impairment threshold yields high sensitivity for predicting dementia [36]. To balance increased sensitivity with specificity we required impairment on at least two neuropsychological tests per cognitive domain. We documented subjective memory complaints using the Memory Functioning Questionnaire [37] and clinical interview.

2.3. Neuroimaging. All MRI scans were performed on a Siemens Allegra 3T head-only MRI scanner. We acquired that sagittal T1-weighted magnetization prepared rapid acquisition gradient-echo (MPRAGE) volumetric scans (TR 2300 ms, TE 2.93 ms, slice thickness 1 mm, 160 slices, inplane voxel size 1.3 × 1.3 mm, FOV 256 mm) for volumetric measurements and high-resolution oblique coronal T2-weighted fast spin echo (FSE) sequences for structural segmentation and unfolding procedures (TR 5200 ms, TE 105 ms, slice thickness 3 mm, spacing 0 mm, 19 slices, in-plane voxel size 0.39 × 0.39 mm, FOV 200 mm).

We used cortical unfolding to enhance the visibility of the convoluted MTL cortex by flattening the entire MTL gray matter volume to 2D-space [20, 21]. First we manually defined white matter and cerebrospinal fluid (CSF) on the oblique coronal T2 FSE structural MRI sequence with high in-plane resolution. In order to maximize visibility of the images for manual segmentation, high in-plane resolution (0.39 × 0.39 mm) is critical. Our approach uses greater slice thickness (3 mm) to increase signal to noise and tissue contrast; to minimize the effect of this larger through-plane resolution across slices on boundary changes, we acquired images perpendicular to the long axis of the hippocampal system where anatomical variability in hippocampal structures is smallest, thereby minimizing variability from slice to slice while maximizing resolution in-plane where anatomic variability is greatest. Once segmentation is complete, the original images are interpolated by a factor of 7, resulting in a final voxel size of 0.39 × 0.39 × 0.43 mm. Next, up to 18 connected layers of gray matter are grown out from the boundary of white matter, using a region-expansion algorithm to cover all pixels defined as gray matter. This produces a gray matter strip containing cornu ammonis (CA) fields 1, 2, and 3, the dentate gyrus (DG), subiculum (Sub), entorhinal cortex (ERC), perirhinal cortex (PRC), parahippocampal cortex (PHC), and the fusiform gyrus (FUS). We are unable to distinguish between CA fields 2, 3, and DG due to limits in resolution; thus we treat these regions as a single entity (CA23DG). It is this strip of gray matter that is the input for the unfolding procedure, an iterative algorithm based on multidimensional scaling

(http://airto.hosted.ats.ucla.edu/Hippocampus/) [19]. We delineated boundaries between subregions on the original in-plane MRI images, based on histological and MRI atlases [38–40] and then projected them mathematically to their corresponding coordinates in flat map space (Figure 1). We calculated cortical thickness in all MTL subregions (CA23DG, CA1, SUB, ERC, PRC, PHC, and FUS), averaged over left and right hemispheres, as well as overall MTL cortical thickness (Global) by averaging thickness across these subregions. To calculate thickness, for each gray matter voxel we computed the distance to the closest nongray matter voxel. In 2D-space, for each voxel, we took the maximum distance value of the corresponding 3D voxels across all layers and multiplied by two. Mean thickness in each subregion was calculated by averaging thickness of all 2D voxels within each region of interest.

Manual segmentations were finalized and readied for unfolding procedures by the same person. This investigator was unaware of all demographic and clinical information. All manual segmentations were performed in native space in line with previous studies using the cortical unfolding technique [17, 22, 23, 41]. We have previously reported interrater and test-retest reliability analyses for the manual procedures involved [17, 22, 41].

2.4. Statistical Analyses. The two sample t-test was used to compare the continuous variables of cognitive groups, first for total thickness averages across all subregions studied within the MTL. Once significance was established for a cognitive domain and total MTL thickness, t-tests were then used to compare subregions within the MTL to that particular cognitive domain, in order to determine the subregions that contributed to the significant findings. Only those domains found to have that significant associations were further analyzed to determine region-specific associations. For significant associations, findings are presented as Pearson correlation coefficients (r). Statistical analyses used a significance level of $P < .05$ (two-tailed).

3. Results

Table 2 summarizes the neuropsychological test results from NC and MCI subjects, while Table 3 summarizes the associations between each of the four cognitive domains and cortical thickness. Five of the MCI subjects converted to AD within the 2-year interval between initial and follow-up visits (20%). Three NC subjects converted to MCI over the duration of the study. Within the MCI group, total averaged thickness was correlated to averaged Z-change score in the Memory Encoding Domain. Further investigation revealed that lower cortical thickness in the ERC ($r = 0.34$; $P = .003$) and Sub ($r = 0.26$; $P = .011$) at the subject's initial visit correlated to decline in averaged Z-scores in the Memory Encoding Domain, but not the Delayed Memory Domain, over the 2-year study interval (Figure 2). Results were similar whether Rey-O scores were included or not. Additionally, these findings were specific to the ERC and Sub regions; no other subregions were significantly related to a decline in Z-scores over time in either the Encoding or Delayed Memory Domains at their follow-up assessment. These analyses included subjects who converted to AD from the MCI group, or to MCI from the NC group. When converters in both groups were excluded, there were no significant associations, demonstrating that the associations in the MCI group were largely driven by converters. Total thickness in the MTL was also significantly correlated to Z-change score in the Processing Speed Domain, specifically that the correlation was driven by an association between subicular cortex and change over time in the Z-score Processing Speed Domain. Also in MCI subjects, there were no significant correlations between thickness in any subregions at their initial assessment and change in scores in the Executive Functioning Domain over time. NC did not demonstrate an association between cortical thickness in any subregion at initial assessment and change in performance over time in any of the four cognitive domains (Figure 2). Additionally, when all MCI and NC subjects were pooled together, there were no significant associations between baseline cortical thickness and change in cognitive scores in any of the four domains.

Longitudinal assessment of structural change over time revealed that MCI subjects, compared to NC subjects, showed significantly greater cortical thinning in CA23DG, CA1, Sub, and ERC and Total Thickness (averaged across all the subregions). Subjects in the MCI group declined an average of 2.3% in CA23DG, 3.4% in CA1, 4.6% in Sub, and 6.1% in ERC over the duration of the study (see Figure 3 for full results). Subjects in the normal group changed 0.9% in CA23DG, 1.7% in CA1, 3.7% in Sub, and 4.8% in ERC. When the 2/5 MCI subjects who converted to AD were excluded (3 were unable to complete follow-up MRI), the rate of change in ERC was 5.7% and 4.4% in Sub. When the 2/3 NC subjects who converted to MCI (1 refused follow-up MRI) were excluded, ERC change dropped to 2.8% and Sub change was 0.6%. Thinning over time was significantly different for the two groups in averaged total hippocampus ($P = .007$) and the following subregions: CA23DG ($P = 5.0 \times 10^{-4}$), CA1 (2.0×10^{-4}), Sub (4.2×10^{-3}), and ERC ($P = 8.2 \times 10^{-3}$). Thinning over time was not, however significantly correlated to cognitive change over time.

4. Discussion

The goal of the current study was to investigate whether structural thinning in regions affected in the early stages of AD pathology predicted decline over time on cognitive tests known to involve these same brain regions. Our findings show that reduced cortical thickness within the ERC and Sub of MCI patients predicts decline over time on tests of memory encoding but not delayed memory performance.

ERC is the gateway of cortical input to the hippocampus [21, 25], enabling the binding of novel associations and shuttling bound information to neocortex [38]. The dissociation of encoding and retrieval processes along the hippocampal axis has been demonstrated by several studies [21, 42] suggesting the involvement of anterior regions, such as ERC

Table 2: Neuropsychological test performance: MCI and NC subjects scores on individual neuropsychological tests. Averages across specified tests provide Domain scores. Both initial and follow-up raw scores, with standard deviation in parentheses, as well as average Z scores are provided for both groups.

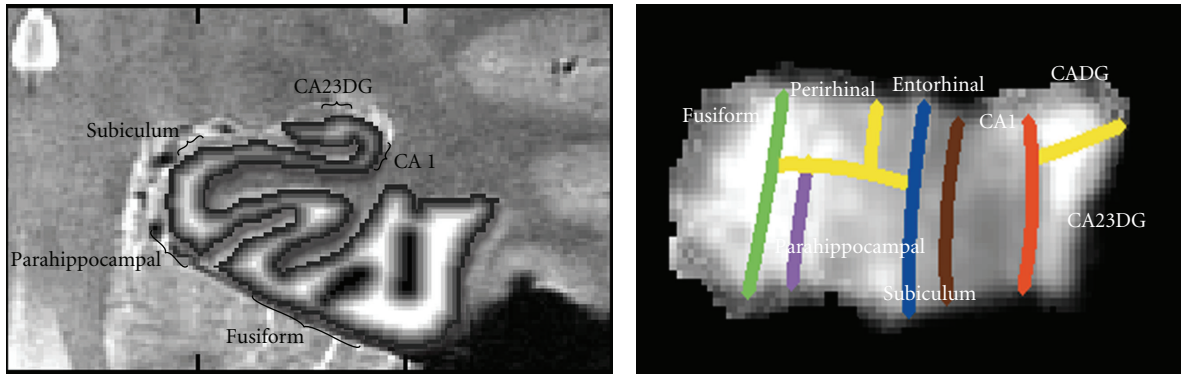
		MCI subjects ($N = 25$)			NC subjects ($N = 20$)		
		Initial visit average raw score	Follow-up visit average raw score	Z-scaled change over time	Initial visit average raw score	Follow-up visit average raw score	Z-scaled change over time
Domain	Neuropsychological test						
Processing speed	Weschler adult intelligence scale-III Digit Symbol	59.0 (14.5)	54.8 (16.0)	−0.19 (1.1)	72.2 (14.6)	72.6 (19.7)	0.28 (0.8)
	Trailmaking test part A**	36.0 (12.7)	36.7 (14.0)	−0.01 (1.1)	29.3 (7.1)	30.5 (9.6)	−0.01 (1.0)
	Stroop test, word reading speed**	50.0 (8.7)	51.9 (8.4)	−0.21 (1.0)	47.5 (5.9)	47.7 (8.5)	0.28 (1.0)
	Average for Processing Speed Domain			−0.14			0.18
Executive functioning	Boston naming test letter fluency (F.A.S)	39.6 (11.7)	35.6 (10.0)	−0.39 (0.9)	40.0 (9.9)	45.6 (9.9)	0.46 (1.0)
	Boston naming test category fluency (Animal naming)	18.3 (4.03)	17.0 (4.8)	−0.16 (1.0)	20.8 (4.0)	21.8 (4.5)	0.20 (1.0)
	Trailmaking test part B**	92.9 (47.3)	103.8 (47.6)	−0.13 (1.1)	63.6 (16.9)	72.3 (29.8)	0.14 (0.9)
	Stroop test, Interference (Kaplan version)**	135.2 (30.4)	148.5 (42.1)	−0.28 (1.0)	118.3 (31.1)	119.1 (34.7)	0.39 (0.9)
	Average for Executive Functioning Domain			−0.24			0.30
Memory encoding	Weschler memory scale-III, logical memory I	37.1 (11.9)	36.5 (11.4)	−0.15 (1.2)	44.5 (8.2)	45.5 (10.5)	0.17 (0.6)
	Weschler memory scale-III, verbal paired associates I	16.8 (8.5)	16.8 (6.9)	−0.19 (1.2)	23.7 (6.0)	24.4 (6.6)	0.24 (0.6)
	Buschke-Fuld selective reminding test, consistent long-term retrieval	48.3 (34.3)	36.2 (35.1)	−0.35 (0.94)	57.3 (32.5)	63.4 (34.5)	0.39 (0.9)
	Average for Memory Encoding Domain			−0.23			0.27
Delayed memory	Rey-Osterreith Complex Figure, delayed recall	9.9 (4.6)	9.2 (6.0)	−0.01 (1.0)	15.2 (5.7)	14.6 (6.7)	0.01 (1.0)
	Weschler memory scale-III, logical memory II	18.4 (9.8)	20.8 (10.7)	0.07 (1.1)	26.9 (7.8)	27.3 (8.3)	−0.12 (0.8)
	Weschler memory scale-III, verbal paired associates II	5.1 (2.8)	5.7 (2.2)	−0.01 (1.3)	7.2 (1.5)	7.3 (1.4)	0.04 (0.6)
	Buschke-Fuld selective reminding test, delayed recall	5.9 (3.7)	5.3 (4.2)	−0.10 (1.0)	8.2 (2.4)	7.8 (3.4)	0.14 (1.0)
	Average for Delayed Memory Domain			0.01			0.02

** Larger number indicates longer time to complete test, thus poorer performance. Z-score ± sign is inverted to represent this.

Table 3: Pearson Correlation Coefficients (r) and P-values for correlations between Cognitive Domains and Total Thickness averaged across all subregions studied in the MTL as well as the subregions within the MTL that were significantly correlation to the Domain listed.

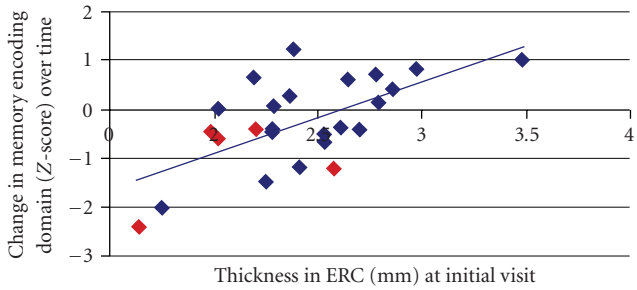
Cognitive domain	Region of interest	r	P-value
Processing speed	Total thickness	0.38	.001
	Subicular cortex	0.35	.002
Executive functioning	Total thickness	0.07*	.203*
Memory encoding	Total thickness	0.17	.044
	Entorhinal cortex	0.34	.003
	Subicular cortex	0.26	.011
Delayed memory	Total thickness	0.06*	.253*

* Not significant.

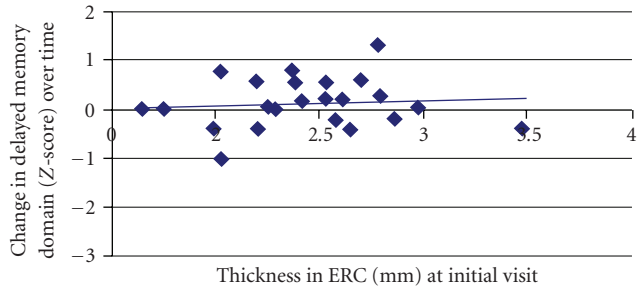


(a) (b)

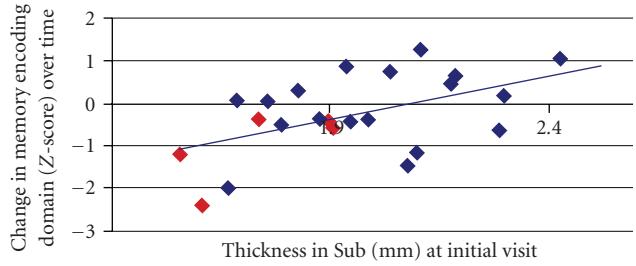
FIGURE 1: Segmentation and Unfolding of Hippocampal Subregions. High-resolution images were segmented (a) defining gray matter within the MTL. Boundary demarcations were drawn on the in-plane image and projected to flat map space (b) including CA fields 2, 3 and the dentate gyrus (CA23DG), subiculum (Sub), entorhinal Cortex (ERC), perirhinal cortex (PRC), parahippocampal cortex (PHC), and fusiform cortex (FUS).



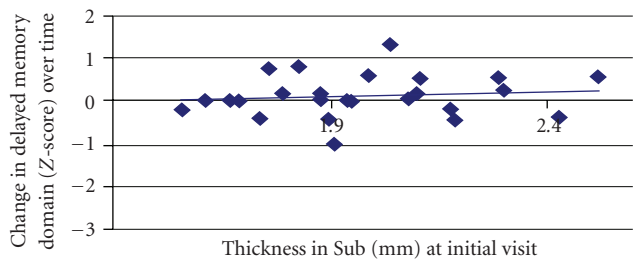
(a) Memory encoding domain correlated to entorhinal thickness in MCI subjects



(b) Delayed memory domain correlated to entorhinal thickness in MCI subjects



(c) Memory encoding domain correlated to subiculum thickness in MCI subjects



(d) Delayed memory domain correlated to subiculum thickness in MCI subjects

FIGURE 2: Correlation of subregional thickness in MCI subjects at their initial visit to decline in averaged Z score performance within cognitive domain over time. Scatterplots display: ERC thickness related to decline in Memory Encoding Domain ($r = 0.34$; $P = .003$, (a)); ERC thickness related to Z-change in Delayed Memory Domain ((b); not significant); Sub thickness related to decline in Memory Encoding Domain ($r = 0.26$; $P = .011$, (c)); Sub thickness related to Z-change in Delayed Memory Domain ((d); not significant). MCI subjects who converted to AD are highlighted in red on the Encoding Domain charts (a, c) to illustrate the relative thickness in ERC and Sub cortex compared to subjects who did not convert.

in encoding and posterior regions in retrieval. AD pathology begins primarily in the ERC, followed by immediate progression across projections through Sub to the hippocampus proper [25]. The specificity of this pattern predicts specific cognitive deficits related to disease-related regionally specific neuronal death. This hypothesis is confirmed in the current study, demonstrating that information in the initial encoding stage is most susceptible to early AD-related pathology and related to thinning in ERC and Sub.

In this study we separated out tests that measured the initial memory-encoding episode from those that involved delayed retrieval of information that had been learned initially. These tests, which included the Buschke-Fuld selective reminding test consistent long-term retrieval section,

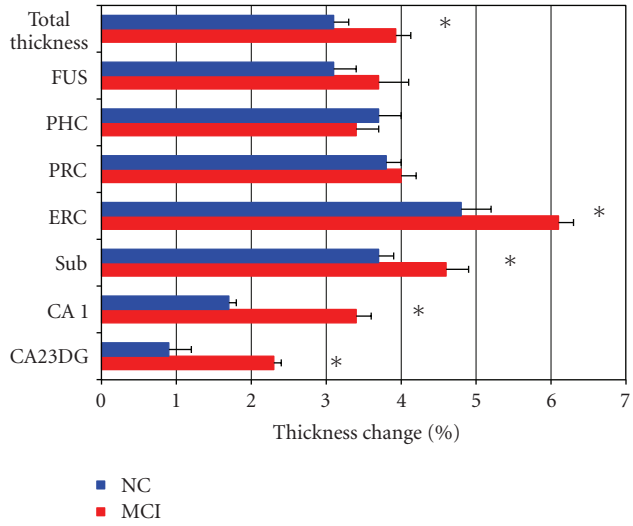


Figure 3: Cortical thickness change over time (%) across subregions. MCI subjects ($N = 20$) compared to normal control ($N = 19$) showed significantly greater cortical thinning in CA23DG, CA1, Sub, and ERC and Total Thickness (CA23DG: CA fields 2, 3 and the dentate gyrus, CA1: CA field 1, Sub: Subiculum, ERC: entorhinal cortex, PRC: perirhinal cortex, PHC: parahippocampal cortex, Fus: Fusiform gyrus).

immediate recall of word pairs, and immediate recall of a prose passage, are challenging and require that subjects form novel associations in the case of word paired learning, learn novel information that exceeds immediate working memory capacity, and consolidate that information dynamically over multiple learning trials [43]. The formation of novel associations is considered a primary role of the hippocampus, and in particular, the ERC. The delayed memory tests, which require recalling information that had been encoded initially, are also clearly related to memory encoding (since only information encoded can be successfully recalled), however these scores are also affected by additional processes such as initiating a search for information, spontaneously retrieval information, which may relate to frontal as well as hippocampal systems. These measures were correlated in our study, however, only decline in the encoding tests related to ERC thickness.

This suggests that measures of structural differences in MTL subregions may predict change within cognitive domains that involve these same brain regions. The specificity of this relationship suggests that subregional analysis may be able to measure AD-specific pathology, though that needs to be corroborated in studies with a more diverse MCI (nonamnestic) and non-AD dementia populations. While these measures are group averages of MCI patients compared to normal subjects, they suggest that individual assessment of structural changes within the MTL may identify those subjects who are at greatest risk of longitudinal decline in cognitive performance. Analysis of longitudinal change rather than static measures of structural change may more clearly differentiate subjects at the greatest risk of subsequent decline, as we further refine the profile of individuals at the greatest risk for progression into AD [18, 44, 45]. Of the 25 MCI subjects we followed longitudinally, five converted to AD over the duration of the study; all five had lower-than-average ERC and Sub thickness compared to the average for MCI subjects.

Additionally three NC subjects converted to MCI over the course of the study; all three had lower-than average ERC and Sub thickness for the NC group average. This supports previous studies that reduced cortical thickness in MTL subregions predicts cognitive decline related to AD-pathology [6, 11, 12]. However, it is noteworthy that some subjects who did not convert to either AD from the MCI pool or MCI from the NC pool also had lower than average cortical thickness in ERC and Sub. Among the MCI group, it is possible that these subjects have not yet converted to AD but only future longitudinal studies will reveal the conversion rates in these subjects. These findings suggest the importance of continued longitudinal tracking of subjects at-risk for AD. However, the small sample size of the current study precludes definitive findings based on this sample. It is of note that the rate of change in ERC thickness is nearly equal for both the MCI group (4.1%) and the NC group (3.2%). At follow-up testing, 3 of the 5 subjects who converted to AD and 1 of the 3 NC who converted to MCI did not complete follow-up MRI testing making it difficult to draw conclusions on cortical thickness differences in convertes compared to nonconverters in this particular sample. A large portion of those MCI subjects most susceptible to subsequent cognitive decline and conversion to AD did not have follow-up MRI data available. However, when the two NC subjects who converted to MCI were excluded from the thickness calculations, the rate of decline in ERC dropped to 1.8%. Thus, the change in ERC thickness in NC over time was mainly driven by those subjects who converted to MCI.

An intact MTL is necessary for the formation of episodic memory [27] and among the earliest manifestations of AD pathophysiology is a declining ability to form new memories, even when patients are able to retrieve previously encoded ones. While it is clear that the ability to encode new memories is required for successful delayed retrieval of these same items, we are arguing that there may be a dissociation of these processes in early stages of the disease, perhaps related to differential degradation of anterior MTL subregions (entorhinal cortex) from posterior MTL subregions. While the subiculum is present in both anterior and posterior sections of the MTL, the ERC is clearly defined to anterior MTL [25]. It is possible that the subiculum, or another region, is more involved in the delayed memory effects of AD and later chronological pathophysiological effects in the course of the cascade of the disease. Thus, the pathophysiological AD-process that begins in the ERC [25] before progressing to other MTL regions may be more significantly affecting Memory Encoding than Delayed Memory. One other possibility is that our delayed memory tests merely tested a specific type of long-term memory and that further tests will have to clarify the relationship between ERC and memory subtypes. It is also possible that our sample size is too small to detect subtle changes to Delayed Memory and we suggest in the discussion that further studies with larger sample sizes may aid in investigations of the correlation

between AD-pathophysiology and neuropsychological test performance.

In prior studies we argued that more subtle structural MRI measures, such as the one used in the current study, may reveal brain changes that are obscured in less sensitive techniques and may account for previously mixed results on the ability of the MTL to predict cognitive decline in AD [17]. Early structural changes in AD are limited to specific laminae within neocortex, thus measures of thickness may be more sensitive than whole-region volumetric assessments, blurring regionally specific changes with regions that have yet to experience neuronal degeneration due to disease pathology. By focusing structural analyses on regions known to be first affected in AD we may better identify those individuals at greatest risk for future memory decline.

There are several potential limitations of the present study including a relatively small N and the time consuming nature of this study, which makes it difficult to generalize to clinical practice. However, as we continue to develop these techniques and identify optimal predictive variables, we may be able to automate those procedures most likely to show changes over time. Additionally there are limitations to the use of cognitive domains as measures of isolated cognitive impairment. Delayed recall is clearly impaired in patients with hippocampal damage, but the presumption is that this is due to a failure for information to fully consolidate during the initial encoding episode. These results provide support for the notion that the ERC is particularly important for this initial episodic encoding. No cognitive domains can be tested in isolation, and by necessity executive functions are important in both encoding and retrieval; similarly initial encoding tests are correlated with delayed recall, as the latter is dependent on the former; thus these processes can never be entirely isolated. However by looking across a range of tests with some similar characteristics, the domain approach tends to remove variability due to specific aspects of a single test and will better emphasize underlying, shared cognitive processes. This method also offers the advantage of reducing the number of comparisons, which can lead to spurious results.

Our results demonstrate a relationship in MCI patients between cortical thinning in ERC and Sub and decline in the ability to encode new memories over time. Given the involvement of the ERC and surrounding neocortices in the early stages of neurodegeneration in AD, clarifying the roles of these brain regions in associative memory is a critical goal in understanding the link between disease pathophysiology and resultant cognitive decline.

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