Longitudinal fMRI in elderly reveals loss of hippocampal activation with clinical decline

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ABSTRACT

Background: Previous cross-sectional fMRI studies in subjects with prodromal Alzheimer disease (AD) have reported variable results, ranging from hypoactivation, similar to patients with AD, to paradoxically increased activation or hyperactivation compared to cognitively normal older individuals. We have hypothesized that subjects in early phases of prodromal AD may experience a period of hippocampal hyperactivation, followed by loss of hippocampal activation as the disease progresses.

Methods: We studied 51 older individuals without dementia (Clinical Dementia Rating [CDR] at baseline of 0, n = 21, and 0.5, n = 30) with longitudinal clinical and neuropsychological assessments, as well as fMRI during a face-name associative memory paradigm. Whole brain and region-of-interest analyses were applied to the longitudinal fMRI data.

Results: Subjects classified as CDR 0 at baseline showed no difference in fMRI activity over 2 years, whereas those who were CDR 0.5 at baseline demonstrated a decrease in fMRI activity in the right hippocampus (p < 0.001). Dividing the subjects on the basis of their clinical and neuropsychological change over the 2 years, we found that subjects with more rapid decline demonstrated both the highest hippocampal activation at baseline, and the greatest loss of hippocampal activation. These findings remained significant after accounting for age, hippocampal volume, and APOE ε4 carrier status.

Conclusions: Clinical decline is associated with loss of hippocampal activation in older subjects. Longitudinal fMRI provides a reliable indicator of brain activation over time, and may prove useful in identifying functional brain changes associated with cognitive decline on the trajectory toward clinical Alzheimer disease.

Neurology® 2010;74:1–1

GLOSSARY

AD = Alzheimer disease; ANOVA = analysis of variance; BOLD = blood oxygen level dependent; CDR = Clinical Dementia Rating; CDR-SB = Clinical Dementia Rating Sum of Boxes; CVLT = California Verbal Learning Test; EPI = echo-planar imaging; MCI = mild cognitive impairment; MTL = medial temporal lobe; NvR = novel vs repeated; RAVLT = Rey Auditory Verbal Learning Test; ROI = region of interest; SPM = statistical parametric mapping.

The pathophysiologic process of Alzheimer disease (AD) begins many years prior to the clinical diagnosis of dementia. Tools such as fMRI have the potential to detect early alterations in brain function that may precede cognitive decline. While several studies have suggested that baseline patterns of fMRI activity may predict clinical decline, there have been no longitudinal fMRI studies to document that clinical decline is accompanied by a measurable loss of functional activation.

There has been much debate surrounding functional activation patterns in the hippocampus and related medial temporal lobe (MTL) regions among subjects in early preclinical phases of AD. While some studies have reported decreased activation in subjects with mild cognitive impairment (MCI) compared to normal elderly controls, other studies have noted that...
subjects in the earliest stages of MCI demonstrate hyperactivation relative to elderly controls. We have postulated that the discrepant findings may relate to the heterogeneity of the subjects under the broad rubric of MCI and prodromal AD. Increased MTL activation has also been reported in asymptomatic individuals at genetic risk. We hypothesize that hyperactivation might represent an early response to AD pathology, which may predict impending hippocampal failure and memory decline. Longitudinal fMRI research is needed to determine the evolution of fMRI activity over the course of clinical decline. In this study, we acquired fMRI and clinical assessments in older individuals without dementia at baseline and 2-year follow-up to investigate the relationship of brain function activity to clinical change over time.

METHODS Fifty-one elderly subjects without dementia (74.8 ± 5.4 years) were recruited from ongoing longitudinal studies on cognitive aging and prodromal AD.

At the baseline visit, subjects were evaluated clinically with the Clinical Dementia Rating (CDR) scale and assigned a global CDR rating and CDR Sum of Boxes (CDR-SB) score. Subjects also underwent neuropsychological assessment, including the California Verbal Learning Test (CVLT) and/or Rey Auditory Verbal Learning Test (RAVLT). Subjects were eligible for the longitudinal fMRI study if they were clinically normal (CDR = 0) or had very mild impairment (CDR = 0.5). The CDR 0.5 subjects selected for this study had subjective memory complaints corroborated by their study partner, but did not yet meet research diagnostic criteria for MCI with significant objective memory impairment (performing at least 1.5 standard deviations below age- and education-adjusted norms at baseline). Subjects returned 2 years after the baseline visit for follow-up scanning and repeat clinical assessment. As the tests of episodic memory evolved in the longitudinal cohorts over the course of the follow-up period (i.e., some subjects received the CVLT at baseline and RAVLT at follow-up), we calculated Z scores to allow for a standardized comparison of neuropsychological test scores.

MRI procedures. Subjects were scanned using a Siemens (Iselin, NJ) Trio 3.0-T scanner. Functional data were acquired using a T2*-weighted gradient echoplanar imaging (EPI) sequence (repetition time/echo time, 2,000/30 msec; flip angle, 90°). Twenty-eight slices were acquired in an oblique coronal orientation, perpendicular to the anterior-posterior commissural line (5 mm with 1 mm gap; voxel dimensions, 3.125 × 3.125 × 6 mm). Six functional runs (each 127 timepoints) were acquired for each subject.

fMRI memory paradigm. fMRI data were collected serially at baseline and 2 years during an associative memory encoding paradigm as described in detail in previous studies. Subjects viewed a series of novel faces (faces unfamiliar to the participants) paired with fictional first names, and were explicitly instructed to try to remember the name associated with each face. In this study, we compared blocks of encoding of novel face-name pairs to repeated face-name pairs. After each scanning session, subjects underwent forced-choice recognition testing, in which faces were presented with the correct and an incorrect name printed underneath in a counterbalanced order. At baseline, only 14 face-name pairs were tested postscan, and at 2-year follow-up, all 84 novel face-name pairs were included in the postscan test, so longitudinal assessment of change in postscan test performance was not reliable.

fMRI data analysis. fMRI data were preprocessed and analyzed using statistical parametric mapping (SPM2; http://www.fil.ion.ucl.ac.uk/spm). Data were realigned to the standard SPM2 EPI template, and smoothed with a Gaussian kernel of 8 mm. Data were modeled with the canonical hemodynamic response function, and a high pass filter of 260 s was used to filter out low-frequency variations. T1 structural data were processed through a semiautomated anatomic reconstruction and labeling procedure producing individual anatomic regions of interest (ROI) using FreeSurfer software (http://surfer.nmr.mgh.harvard.edu). Two subjects were excluded from analyses involving anatomic ROIs created in FreeSurfer because of poor quality volumetric data.

Statistical analysis. We first examined longitudinal fMRI activity at the whole brain level, generating group-averaged activation maps for the novel vs repeated (NvR) contrast using SPM2 random effects models at baseline and follow-up. SPM2 whole brain repeated measures analyses were then used to investigate within-subject changes in function over the 2-year period. Statistical maps were thresholded at a significance level of p < 0.001 with a minimum extent threshold of 20 contiguous voxels.

We investigated activation changes within the hippocampus using 2 ROI approaches. First, we created an empirically defined functional ROI in the right hippocampus based on the CDR 0.5 group at baseline (see Results and figure 1C). Second, we utilized an unbiased, a priori defined anatomic ROI based on FreeSurfer volumetric analyses. We further constrained the anatomic ROI using a union mask based on the activated voxels at baseline and 2-year follow-up from the NvR contrast for all 51 subjects to examine hippocampal regions engaged in the task.

We extracted percent signal change estimates within ROIs using the SPM ROI Toolbox (http://marsbar.sourceforge.net/). Analysis of variance (ANOVA) and paired t tests were used to assess between-group and within-group differences in percent signal change estimates. Pearson correlations were used to investigate the relationship between hippocampal signal and clinical status. A linear regression model with covariates was used to investigate predictors of clinical decline.

Standard procedures and subject consents. All subjects provided informed consent in accordance with the Human Research Committee guidelines of the Brigham and Women’s Hospital (Boston, MA).

RESULTS Subjects. At baseline, 21 subjects were classified as CDR 0 and 30 as CDR 0.5. Subject demographics are shown in the table. CDR 0 subjects were similar to CDR 0.5 subjects in age, years of education, Mini-Mental State Examination, and postscan test performance at baseline (p > 0.05 for all comparisons). Of the 51 subjects, 17 were
Whole brain analyses based on baseline clinical status. We first examined whole-brain fMRI activation patterns in the groups divided by baseline CDR rating (0 vs 0.5) at baseline and at 2-year follow-up (figure 1). Regions with greater activation for novel compared to repeated face-name pairs included the fusiform, prefrontal cortices, thalamic, and MTL regions, in a pattern very similar to our previous reports.16,18 In the CDR 0 group, repeated-measures analyses revealed no regions with significant differences in activation over 2 years. However, the CDR 0.5 subjects demonstrated a longitudinal decrease in activation that was specific to the right hippocampus (figure 1C). There were 2 clusters within the right hippocampus: anterior peak: 33, −9, −18; posterior peak: 39, −24, −15; p = 0.001. This anterior hippocampal region from the CDR 0.5 group was then used as the functional ROI mask to extract fMRI signal response for all subjects in subsequent analyses. No regions showed greater activation at follow-up compared to baseline in either group.

Relationship of change in fMRI activity to change in clinical status. Subjects were then classified into 4 groups based on change in their clinical status over the 2-year follow-up: 1) stable CDR 0 subjects (CDR-SB 0 at baseline and follow-up, n = 15); 2) stable CDR 0.5 subjects (CDR 0.5, no change in SB, n = 12); 3) slow decliners (CDR 0/0.5 at baseline, decline in SB = 0.5, n = 11); and 4) fast decliners (CDR 0/0.5 at baseline, decline in SB ≥1, n = 13). We chose to define CDR-SB ≥1.0 as the cutoff for fast decline, as this degree of change was felt to be more clinically meaningful based on previous data predicting conversion to clinical dementia.19 Consistent with this classification, utilizing the National Alzheimer’s Coordinating Center diagnostic criteria, we found that 5/11 slow decliners progressed to meet criteria for amnestic or multidomain MCI, and 1 progressed to probable AD, whereas 4/13 of the fast decliners progressed to MCI, and 4 progressed to dem...
mentia (3 to probable AD, 1 to probable vascular/ mixed dementia).

We first focused on percent signal change estimates from the empirically defined functional ROI in the right hippocampus (figure 1C). The longitudinal change in hippocampal activation (NvR response at 2 years − NvR response at baseline) differed among the 4 groups ($F_{3,47} = 5.29, p = 0.003$; figure 2). Groups with a constant CDR-SB score had stable hippocampal activation over the 2 years, while the fast decliner group demonstrated a loss of hippocampal activation ($p < 0.001$). There was also a trend toward a difference in baseline activation among the 4 groups ($p = 0.085$), with a post hoc $t$ test revealing higher baseline activation for the fast decliner group compared to the stable CDR 0 group ($p = 0.02$). No differences were observed between baseline and 2-year scans in a calcarine cortex ROI sampled as a control region, indicating that the observed effects were regionally specific.

We also compared the longitudinal changes in hippocampal signal dividing subjects into just 2 groups, comparing those with no change in CDR-SB score ($n = 27$) and those with any worsening ($\geq 0.5$) in CDR-SB score ($n = 24$). We again observed a greater loss of hippocampal signal among the subjects experiencing any clinical decline using both the functional ROI ($p = 0.004$) and anatomic ROI ($p = 0.036$) analyses.

We calculated the difference in fMRI response between the 2-year and baseline scans, and correlated this longitudinal difference in activation with the overall change in CDR-SB score. Across all 51 subjects, greater decline in CDR-SB score was associated with greater loss of hippocampal activation ($R = −0.51; p < 0.001$). Interestingly, greater baseline hippocampal activation was associated with greater decline on CDR-SB ($R = 0.40; p = 0.003$) and greater loss of hippocampal signal ($R = 0.81; p < 0.001$, figure 3), consistent with the hypothesis that hippocampal hyperactivation precedes clinical decline and loss of hippocampal activation.

We then performed a series of analyses to determine if other demographic or volumetric factors might account for the observed results. Age was not correlated with baseline hippocampal signal ($R = 0.13, p = 0.360$), but there was a weak correlation between age and change in hippocampal signal ($R = 0.22$.)

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**Table: Subject demographics**

<table>
<thead>
<tr>
<th>Baseline CDR</th>
<th>CDR 0</th>
<th>CDR 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>Age, y, mean ± SD</td>
<td>73.3 ± 5.4</td>
<td>75.8 ± 5.2</td>
</tr>
<tr>
<td>Education, y, mean ± SD</td>
<td>15.2 ± 2.5</td>
<td>16.1 ± 2.8</td>
</tr>
<tr>
<td>M/F</td>
<td>8/13</td>
<td>14/16</td>
</tr>
<tr>
<td>MMSE at baseline, mean ± SD</td>
<td>29.7 ± 0.5</td>
<td>29.5 ± 0.7</td>
</tr>
<tr>
<td>Postscan test performance at baseline, mean ± SD</td>
<td>87.4 ± 10.8</td>
<td>83.8 ± 11.4</td>
</tr>
<tr>
<td>Median CDR-SB at baseline (range)</td>
<td>0 (0)</td>
<td>1.5 (0.5–3.5)</td>
</tr>
<tr>
<td>Median change in CDR-SB (range)</td>
<td>0 (0–2)</td>
<td>0.5 (0–2)</td>
</tr>
<tr>
<td>Stablea</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Slow declinerb</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Fast declinerc</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>APOE4 carrier</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Abbreviations: CDR = Clinical Dementia Rating; CDR-SB = Clinical Dementia Rating Sum of Boxes score; MMSE = Mini-Mental State Examination (total score).

* Stable: no change in CDR-SB over 2 years.
* Slow decliner: change in CDR-SB = 0.5 over 2 years.
* Fast decliner: change in CDR-SB score ≥ 1 over 2 years.

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**Figure 2** Hippocampal signal at baseline and follow-up

Percent signal change values extracted from functionally defined region of interest (ROI) in the right hippocampus. The ROI analyses confirmed that subjects who declined by ≥1 in Clinical Dementia Rating Sum of Boxes (CDR-SB) (fast decliners) showed the greatest decrease in right hippocampal activation ($p < 0.001$), and demonstrated increased activation in the region at baseline compared to those with no change in CDR-SB ($p = 0.02$).
There were no significant differences in baseline fMRI or change in fMRI activation between APOE ε4 carriers and noncarriers, using either functionally or anatomically defined ROI.

To further investigate whether the observed longitudinal changes in activation were explained by changes in hippocampal volume, we examined fMRI activity within an anatomically defined hippocampal ROI determined by FreeSurfer. Subjects with stable CDR-SB scores showed no change in hippocampal activation within the anatomically defined ROI between baseline and 2-year scans, while the greatest loss of activation over the 2 years was observed in the fast decliner group (p = 0.011). The prespecified 2-group comparison between the stable CDR 0 subjects and fast decliners indicated that fast decliners experienced greater decline in test performance (p = 0.01). This effect was due to both slight improvement in neuropsychological memory performance over the 2 years in the stable CDR 0 subjects (p = 0.057), suggestive of a practice effect commonly observed in cognitively normal adults, and a trend toward worsening neuropsychological performance among the fast decliners (p = 0.211).

DISCUSSION This longitudinal fMRI study demonstrates that clinical decline in older individuals is accompanied by significant loss of hippocampal activation over time. Consistent with our previous findings, subjects with the highest activation at baseline demonstrated the greatest clinical decline, supporting the hypothesis that there is a period of hyperactivation in the earliest stages of prodromal AD. These subjects also demonstrated the greatest loss of hippocampal activation over 2 years, consistent with the hypothesis that hyperactivity may be an indicator of impending hippocampal failure. These longitudinal fMRI findings were not explained by age, APOE status, or hippocampal volume loss. Our results suggest that longitudinal fMRI is capable of detecting early brain dysfunction predictive of subsequent decline, and tracking clinically meaningful changes over the course of early cognitive impairment.

Previous work from our group using a scene-encoding task in a separate group of older subjects found that fMRI activation in the posterior MTL was predictive of subsequent cognitive decline, but these studies did not include longitudinal imaging. The current study replicates our finding that increased baseline MTL activation is associated with greater subsequent clinical decline, in a separate cohort of subjects and using a different paradigm. The face-name paradigm engages more anterior hippocampal regions at baseline and was associated with loss of anterior hippocampal activation over time.
suggesting that the hyperactivity predictive of decline may be regionally specific to the demands of the cognitive task. It is possible that this finding represents a regression to the mean phenomenon; however, we did not observe any longitudinal differences in the cognitively stable normal older individuals, and the loss of hippocampal activation paralleled clinical decline on both the CDR and neuropsychological measures of episodic memory.

In addition to our own studies that have reported evidence of increased hippocampal activation in the earliest stages of MCI, relative to normal controls,2,3,20,21 several other groups have reported hyperactivation in MCI, in particular for successful memory formation.8,9,22 This paradoxical increase in activation has also been observed in carriers of the APOE ε4 allele,11,12 even among young carriers,13 and in asymptomatic offspring of individuals with confirmed familial late-onset AD.23 We did not observe a significant effect of APOE carrier status in this study, perhaps because of the more advanced age of our subjects. We have previously speculated that hyperactivity might reflect an increase in neuronal recruitment to maintain memory performance in the setting of early AD pathology,16 which is supported by the finding of regionally specific hyperactivity according to memory task demands. The finding that increased activity is a predictor of impending hippocampal failure, however, suggests that the hyperactivity may be an indicator of neuronal stress or be directly related to the pathophysiologic process of AD. Hyperactivity could be related to aberrant sprouting of cholinergic fibers,24 excitotoxicity from the excessive stimulation of NMDA receptors,25 or stress from Aβ-induced neuronal alterations (e.g., excitatory activity triggers compensatory inhibitory mechanisms, which jointly contribute to network dysfunction).26

It should be noted that blood oxygen level dependent (BOLD) is an indirect measure of neuronal activity, and may reflect other physiologic properties, including cerebrovascular changes.27 The BOLD signal may depend on baseline perfusion, cerebral blood volume, vascular compliance, and interactions between these variables.28 The increase in BOLD activation observed in our fast decliner group could represent a decreased resting oxygenation state, increased perfusion, or some other disruption in neurovascular coupling.

One other potential explanation for apparent decreases in functional activation over time could be loss of hippocampal volume. Controlling for volume both within ROI analyses and in a multivariate model, we still observed loss of hippocampal activation. We did not observe significant hippocampal atrophy over the 2 years using automated volumetric analyses, perhaps because many of our subjects were still at relatively early stages of prodromal AD, prior to significant neuronal loss. It has been postulated that hippocampal atrophy may occur later in the pathophysiologic sequence of AD.29,30 We hypothesize that those subjects who demonstrated the greatest decrease in function will subsequently manifest hippocampal atrophy, and are actively pursuing this hypothesis in ongoing longitudinal studies.

Over the course of the study, we modified our postscan memory test to include more test items at the follow-up scan. Unfortunately, this limited our ability to determine if loss of hippocampal signal accompanies worsening performance on postscan memory testing. Longitudinal fMRI studies now underway in our laboratory include more detailed postscan testing, and we will be able to analyze successful vs failed encoding using event-related analyses.

If fMRI is to be useful as a tool for evaluating longitudinal change in brain function or the efficacy of pharmacologic interventions, it is essential to

![Figure 4 Change in neuropsychological test scores and hippocampal signal](https://example.com/figure4.png)
demonstrate the stability of fMRI measures over extended periods of time in the absence of clinical change. Two recent studies suggest that fMRI reproducibility is reasonable over short time frames in older populations.31,32 Our finding of reproducible patterns of activation over 2 years in clinically stable normal subjects is encouraging that fMRI can provide a reliable estimate of hippocampal activation over time, and thus may prove a useful tool for detecting clinically meaningful change in longitudinal pharmacologic studies in early prodromal AD.

Our results in subjects with very mild impairment demonstrate that clinical decline is related to the loss of hippocampal activation, and support previous findings that paradoxically increased activation may be predictive of impending clinical decline. These findings suggest that fMRI may prove valuable in tracking very early progression of brain dysfunction, prior to the point of irreversible neuronal loss, and at a time when disease-modifying therapies are likely to be most efficacious.

AUTHOR CONTRIBUTIONS
Statistical analysis was conducted by Jacqueline O’Brien and Reisa Sperling.

ACKNOWLEDGMENT
The authors thank Mary Foley and the Arthiouna A. Martins Center MRI Core for assistance with MRI; Jeanette Gunther, the Gerontology Research Unit staff, and the Massachusetts Alzheimer’s Disease Research Center (ADRC) for assistance with subject recruitment and evaluation; and Akram Bakkour, Rebecca Berensky, Maija Pihlajamaki, Ali Arri, and Francine Grodstein for assistance with analyses and comments.

DISCLOSURE
Ms. O’Brien has received research support from the NIH (T32 MH017119 [trainee]). Ms. O’Keefe, Mr. LaViolette, and Ms. DeLuca report no disclosures. Dr. Blacker serves on the editorial boards of the American Journal of Geriatric Psychiatry and Experimental Gerontology; and receives research support from the NIH (4 R37 MH060009-09 [Co-PI], 5 P01 AG05134-25 [Co-PI of Core], 2 R01 AG07370-15 [PI of subcontract], 1R01AG029411-01 [Co-PI], R01NS062028 [PI], and U01 AG032984 [PI of subcontract], the Fidelity Foundation, and the Alzheimer’s Association. Dr. Dickerson receives research support from the NIH (NIA R01 AG29411 [PI]). Dr. Sperling has served on a scientific advisory board for Archimedes Pharma Ltd (formerly Link Pharmaceuticals); serves on the editorial board of Alzheimer’s Disease and Associated Disorders; has received speaker honoraria from Pfizer Inc.; has served as a consultant for Elan Corporation, Wyeth, Brinol-Myers Squibb, and Bayer; her husband has served as a consultant for GE Healthcare. She receives research support from Elan Corporation, Wyeth, Brinol-Myers Squibb, the NIH (NIA R01AG207435 [PI]; NIA R01 AG29411 [Co-I]; 5P01 AG05134-25 [PI-Project 1]), and from the Alzheimer’s Association.

Received August 4, 2009. Accepted in final form February 4, 2010.

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