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ABSTRACT

The ability to form associations between choice alternatives and their contingent outcomes is an important aspect of learning that may be sensitive to hippocampal dysfunction in memory disorders of aging such as amnestic mild cognitive impairment (MCIa), or early Alzheimer disease. In this preliminary study we examined brain activation using functional magnetic resonance imaging (fMRI) in 12 healthy elderly participants and nine patients with MCIa during an associative learning task. Using a high-field 3.0-Tesla MRI scanner, we examined the dynamic neural response during associative learning over trials. The slope of signal attenuation associated with learning was analyzed for differences between groups within an a priori defined hippocampal region. Results indicated dynamic signal attenuation associated with learning in the healthy elderly sample, but not in MCIa. The absence of an associative learning effect in the MCIa sample reaffirms an important link between the learning difficulties that are commonly encountered in MCIa and the mesial temporal region.

The hippocampal region, including hippocampus, entorhinal cortex, and nearby brain structures, is affected early in the course of Alzheimer's disease (AD) (Jack et al., 1998, 1999, 2000). Amnestic mild cognitive impairment (MCIa) is a putative prodromal syndrome to AD in which learning and memory related cognitive abilities are the predominant impairment, but the patient has not yet lost the functional capacity to warrant a diagnosis of

dementia (Petersen, 2004; Winblad et al., 2004). Characterizing the hippocampus in MCIa may yield important new ways to predict whether a patient will eventually develop AD (Grundman et al., 2003; Muller et al., 2007).

Quantitative structural MRI studies indicate that MCI patients exhibit volume loss in the hippocampal and other mesial temporal lobe structures (MTL) (Dickerson et al., 2001), but volumetric studies do not provide a means to make direct inferences about the functionality of this region. Functional MRI (fMRI) may be useful in this regard and has previously been used to assess the functionality of the MTL in people with suspected hippocampal compromise such as persons with MCIa (Dickerson et al., 2005; Johnson et al., 2006; Machulda et al., 2003).

A recent fMRI study with MCI has shown an adaptation response in the hippocampal region associated with stimulus repetition (Johnson et al., 2004). The effect was found for healthy elderly persons with intact learning abilities, but not in similarly aged persons who had exhibited memory decline. That study joins a growing body of functional imaging evidence suggesting that the intact hippocampus is active during encoding of new information (LePage et al., 1998; Saykin et al., 1999; Tulving & Markowitsch, 1998; Wagner et al., 1999), a function that is compromised by MCIa.

In MCI populations, some studies have found that the MTL is less responsive relative to controls (Johnson et al., 2004, 2006; Machulda et al., 2003; Petrella et al., 2006; Small et al., 1999), though other studies have shown that the MTL is paradoxically more active in MCI than controls (Dickerson et al., 2004, 2005) . Several explanations are possible for the discrepant findings including variation in the type of tasks that are used, differences in difficulty level between patient and control groups, differences in the choice of statistical analysis, and differences in the severity of deficit in the patient group. For example Celone et al. (2006) found that less impaired MCI subjects showed greater than normal magnitude of activation, while more impaired MCI subjects exhibited smaller signal change compared to controls. More work is needed to clarify the cerebral activation issues in MCI if fMRI is ever to be useful as a clinical tool in this population.

To date, the majority of fMRI studies examining MCI have utilized declarative memory tasks to measure MTL signal change over discrete trials of episodic stimuli (e.g. novel and previously learned items). However, a broad body of literature in animals and humans employing tasks of associative learning has implicated the mesial temporal lobe in forming relationships between choices and their contingent outcomes. For example, non-demented elderly with hippocampal atrophy can learn a series of object discriminations as well as non-atrophied peers, but they are seriously impaired on a subsequent generalization test in which familiar information is presented in novel recombinations or novel contexts (Myers et al., 2002, 2003). Myers et al. (2002) implemented an eight-pair concurrent visual discrimination task. On

each trial, subjects saw a pair of objects that differed in color or shape but not both (e.g. red square versus red triangle, or blue diamond versus green diamond). The left–right ordering of the two objects was random; one member of each pair was arbitrarily designated as rewarding. Over repeated trial blocks, subjects learned to choose the rewarded member of each pair. Nondemented elderly with and without hippocampal atrophy could learn this at equivalent speeds. This learning phase was followed by an unsignaled transfer phase, in which relevant stimulus dimensions remained the same, but irrelevant features were altered (e.g. yellow square versus yellow triangle, or blue circle versus green circle). Individuals with no hippocampal atrophy transferred with near-ceiling accuracy, continuing to choose the previously-rewarded stimulus feature (e.g. square beats triangle, regardless of color). Individuals with atrophy performed significantly worse on transfer. Data from a subsequent study indicate that these atrophied subjects had not simply forgotten the earlier information, but were specifically impaired at generalizing that learning to new contexts (Myers et al., 2003). The results of these studies suggest that the hippocampal region may not be required for simple stimulus-response learning, but it normally participates during this learning, helping to establish stimulus representations that are sufficient to support subsequent generalization when task demands change (Gluck & Myers, 1993, 2001). Individuals with hippocampal atrophy are therefore able to learn but, without full benefit of hippocampal region mediation during that learning, are impaired at subsequent transfer.

The objective of this fMRI study was to build upon the prior results of Myers et al. (2002, 2003) by examining MTL signal changes during associative learning over trials in a concurrent discrimination task adapted to fMRI that was similar to the learning phase of Myers et al. (2002). Given the results of the aforementioned fMRI studies, the right hippocampus was predicted to exhibit blood oxygen level-dependent (BOLD) signal attenuation associated with learning the correct response in this associative learning paradigm. Furthermore, the slope of attenuation was expected to differentiate MCIa patients from healthy controls.

METHODS

Participants

Twenty-one participants were studied and are described in Table 1.

MCI

Nine participants met criteria for MCI diagnosis. Our diagnostic criteria for MCI were consistent with those proposed by Winblad et al. (2004), and they included: (a) presence of memory complaints by participant and/or

TABLE 1. Demographic and Neuropsychological Data

	MCI		Controls	
	Avg	SD	Avg	SD
Age	76.1	5.7	71.8	5.5
Education	15.6	3.1	17.6	2.0
Gender f/m	5/4	–	8/4	–
MMSE	26.25	3.1*	29.5	1.0
WRAT-III reading	111.6	9.5	114.8	3.9
Boston Naming Test	52.9	7.2	57.3	1.7
Controlled Oral Word	33.6	10.4*	45.8	8.4
RAVLT total	28.0	5.2**	47.0	7.8
RAVLT delayed recall	2.7	1.6**	8.7	2.4
BVMT-R total	13.3	4.6**	26.3	5.2
BVMT-R delay	4.3	2.2**	9.9	1.9
Trail Making B	90.0	14.8*	69.4	17.1

$*p < .05$; $**p < .001$ statistical significance.

MCI=Mild Cognitive Impairment; MMSE=Mini Mental State Exam; WRAT-III=Wide Range Achievement Test – 3rd Edition; RAVLT=Rey Auditory Verbal Learning Test; BVMT-R=Brief Visuospatial Memory Test-Revised.

Note: All cognitive scores are raw scores except WRAT-III standard score.

informant, (b) cognitive deficits on memory testing together with subjective report of decline over time by participant and/or information, (c) intact functional status, (d) cognitive and functional status not consistent with a diagnosis of dementia. All MCI participants were classified as amnestic on the basis of objective cognitive testing, though some showed deficits in other domains as well. The patients were referred from the several memory disorders clinics at a large university-based medical center. These subjects, as well as the controls, received a battery of neuropsychological tests as part of this study to verify and document the extent of any selective memory impairment. In addition, T2-weighted images acquired as part of this study were required to be rated as normal by a neuroradiologist for final inclusion of the participants.

Prior to inclusion in this study, the MCI patients were presented to a diagnostic consensus panel (consisting of experienced physicians and neuropsychologists in dementia research and clinical practice) for support of the diagnosis. Clinical information presented to the panel included medical history, laboratory tests, brain imaging findings, neurological examination and neuropsychological test results. Exclusion criteria for this study consisted of Hachinski score (Hachinski, 1990) greater than 4, abnormal brain imaging findings, prior neurological disease or neurosurgery, prior major psychiatric disorder, chronic major medical conditions such as poorly controlled diabetes, poorly controlled hypertension, or cardiac disease.

Twelve controls were also studied. The same exclusion criteria applied. Additionally, the controls were required to demonstrate neuropsychological

evidence of normal cognitive function at the time of the study (no cognitive score could be more than one standard deviation below the normative mean for the subject's age). The demographic characteristics and neuropsychological data for the controls as well as the MCIs are presented in Table 1. The controls were recruited by advertisement, physician referral, and by selected mailings to a local registry of healthy elderly who had previously expressed an interest in participating in research.

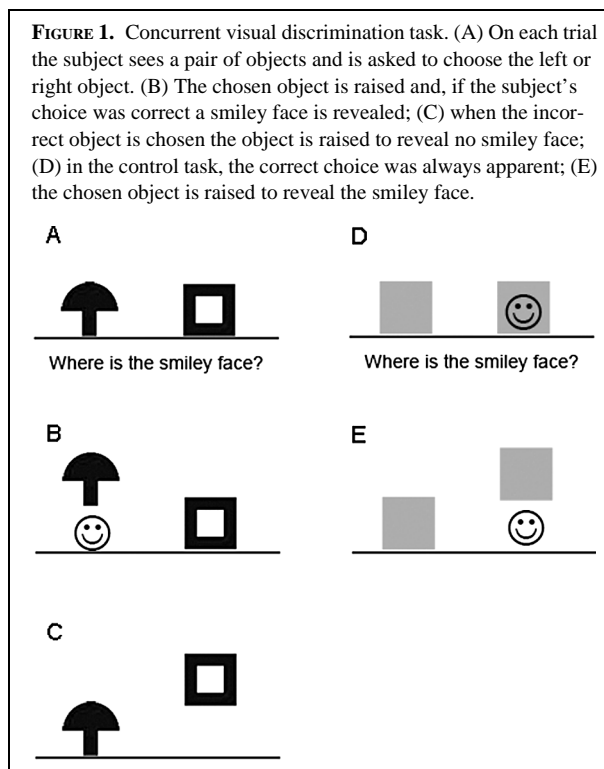## Procedures

Neuropsychology

All participants received a cognitive battery including the Mini Mental State Exam (Folstein et al., 1975), Brief Visuospatial Memory Test-Revised (Benedict, 1997), Rey Auditory Verbal Learning Test (Spreen & Strauss, 1998), Boston Naming Test (Goodglass & Kaplan, 1987), Controlled Oral Word Association Test, Trail Making Test (Reitan & Wolfson, 1993), and an estimate of verbal intellectual ability—The Wide Range Achievement Test – 3rd Edition, Reading Recognition Subtest (Jastak Associates, 1993).

Functional MRI Setup

Participants were provided with instruction on the fMRI task and underwent practice prior to scanning. They were then situated on the bed of a GE long bore 3.0-Tesla MRI scanner, equipped with a two-button MR-compatible response device, and fitted with a high-resolution goggle system containing vision correction and screen resolution set at 800×600 from Resonance Technology (Northridge, CA, USA). Head motion was constrained by foam padding. The software Presentation (http://www.neurobs.com) was used to deliver visual stimuli and response feedback from a PC computer via the goggle system and also record the responses through the button-box connected to the serial port of the stimulus-delivery computer. A BNC cable connecting the scanner to the computer enabled Presentation to detect each and every slice acquisition from the scanner, thereby enabling precise synchrony between slice acquisition and event-related stimulus delivery.

fMRI Task

We have adapted the concurrent discrimination task described by Myers et al. (2002) for event-related fMRI. Subjects were presented with six individually presented pairs of abstract shapes that repeated six times over the course of the scan. In addition to the object pairs, a control condition was presented in which subjects saw two gray squares side by side; one of the squares was clearly marked as the correct choice; the subject's task was merely to choose that square. Feedback was provided for both the object and control trials, as shown in Figure 1. As in Myers et al. (2002), if the subject's

FIGURE 1. Concurrent visual discrimination task. (A) On each trial the subject sees a pair of objects and is asked to choose the left or right object. (B) The chosen object is raised and, if the subject's choice was correct a smiley face is revealed; (C) when the incorrect object is chosen the object is raised to reveal no smiley face; (D) in the control task, the correct choice was always apparent; (E) the chosen object is raised to reveal the smiley face.

response was correct, the chosen object was raised to reveal a smiley face. If incorrect, the chosen object was raised but no smiley face appeared. Target locations were counterbalanced.

Trials were presented as discrete events. The stimulus onset asynchrony ranged from 5 to 18 s (mean of 7 s). The trials were jittered around the TR (±0, .15 or .5 TR). The concurrent discrimination (choice) trials were a maximum of 4 s and ended with response selection. The feedback trial immediately ensued indicating whether the response was correct and lasted for one second duration. In the rare event a choice was not made in the 4-s time frame, the feedback trial consisted of a reminder to respond quickly. Subjects were provided with instructions and practice prior to scanning to ensure they understood the task.

Imaging Protocol

MR imaging was performed using a General Electric 3.0-Tesla SIGNA (Waukesha, WI) MRI system. A T2* gradient-echo, echo-planar imaging (EPI) pulse sequence was used to obtain BOLD fMRI data. The homogeneity of the static magnetic field (B0) in the brain was optimized using higher order shims prior to the functional trials. The BOLD EPI parameters were as follows: echo time (TE) = 30 ms; repetition time (TR) = 2000 ms; flip angle = 90°; acquisition matrix = 64 × 64 voxels; field of view (FOV) = 240 mm.

Thirty sagittal slices of brain were acquired within each TR. Voxel resolution was $3.75 \times 3.75 \times 4$ mm, with a 1-mm skip between slices. One hundred and fifty-two temporal volume images were collected, of which the initial three image volumes acquired during the first 6 s were discarded.

Anatomic Imaging

In the same session as the functional scans, T1 and T2 weighted anatomic images were acquired and later viewed by a neuroradiologist for structural abnormalities including gliosis and ischemic disease that might have warranted exclusion and/or clinical follow-up.

The T2 weighted scan sequence was a 2D axially prescribed fast recovery fast spin echo pulse sequence with the following parameters: slice thickness 1.7 mm with .3 mm skip; TE=90.8; TR=9000; NEX=2; FOV=240 mm; matrix=256×128. Image acquisition time was approximately 5 min.

A 3D IR-prepped fast gradient echo pulse sequence was employed to obtain high-resolution T1-weighted structural images. In order to obtain whole-brain coverage, the imaging parameters were as follows: inversion time=600 ms, fast gradient echo read-out with TR/TE/flip=9 ms/1.8 ms/20°; acquisition matrix=$256 \times 192 \times 124$ (axial 256×192 in-plane, interpolated to 256×256); FOV=240 mm; slice thickness=1.2 mm (124 slices); ±16 kHz receiver bandwidth; acquisition time 7.5 min.

Field Mapping

Even after high-order shimming, there are residual magnetic field (B0) inhomogeneities across the brain that cause regional image distortions in echo planar images such as in the inferior prefrontal regions near the mesial temporal lobe and in the frontal and ethmoid sinuses. Image distortions were corrected by measuring 3D field maps across the brain (co-planar with the fMRI slices). This was accomplished by measuring the phase of non-EPI gradient echo images at two echo times (7 and 10 ms). The phase difference between the two echo images is proportional to the static field inhomogeneity (Jezzard & Balaban, 1995). The warp correction was performed using FSL3.2 (Jenkinson, 2003).

Image Processing

Following echo-planar image reconstruction the files were converted to Analyze7.5 file format and reoriented to the axial plane. The 4D image time-series was motion-corrected to overcome minor head movement during the scan. The field map from each subject (described above) was then applied to each image in the time series to correct for echo-planar related distortions in image space. The images were then normalized into a standard atlas space (using the T2* weighted template provided through SPM2), and then smoothed with an 8 mm full-width at half-maximum Gaussian kernel.

Functional Image Analysis

All image analyses were performed in SPM2 (www.fil.ion.ucl.ac.uk/
spm). Analyses of the time-series data were performed on individual partici-
pants using a boxcar model convolved with the canonical hemodynamic
response function. The statistical model included high frequency signal fil-
tering (high pass filter=128 s) and the AR1 method of accounting for tempo-
ral autocorrelation (Kiebel & Holmes, 2004). The time series data were
statistically analyzed using the general linear model (Friston et al., 1995).
Each participant's performance accuracy scores were used in a subject-spe-
cific regressor (parametric modulation) according to the following rule. Pre-
sentation of the initial pair (regardless of whether or not the answer was
guessed correctly) and all incorrect responses, were weighted with a score of
1 (meaning not learned; the initial items were always model as not learned
because the subject had not yet received feedback by which learning could
occur). The first two subsequent correct responses were weighted with a
score of 0.5. All other subsequent and consecutive correct responses were
weighted with a score of 0 (denoting the correct item in the pair had been
learned). This rule explicitly equates learning with gradual signal attenuation
and was used to feature brain locations where pair specific adaptation or sig-
nal attenuation occurs as learning takes place over trials. First-level contrast
images, characterizing individual participant's average slope of signal
change associated with learning were then entered into a second-level ran-
dom effects analysis.

Slope differences between groups were tested using a two-sample *t*-test
model. For this analysis, an anatomical mask was selected based on prior
results with other memory encoding tasks (Johnson et al., 2004, 2006;
Trivedi et al., 2006) implicating the right side for nonverbal encoding tasks.
The a priori ROI was defined once only, on a template brain in standard atlas
space, and included the right hippocampus and subiculum from the
amygdala to the tail. The parahippocampal and fusiform gyri were not
included. The results of the two sample *t*-tests were thus only assessed
within the mask region. The purpose of the mask was to impose a more strin-
gent hypothesis driven restriction on the number of simultaneous compari-
sons based on our prior work. Because of the restricted region of interest
(ROI) analysis, uncorrected *p* values were used ($p < .01$).

Voxel-based Morphometry

The T1 volume was subsequently used for voxel-based morphometry
(VBM) to determine whether there were volumetric differences between
groups that might account for any observed fMRI differences. The VBM
procedure utilized a standard approach (Good et al., 2001) that included
optimized normalization to standard atlas space (and resampled at 2-mm

isotropic voxels), modulation of the normalized image by the Jacobian determinants in order to preserve volume information, followed by spatial smoothing to 12 mm. The Gaussian smoothing function differed from that of the functional images because the intrinsic smoothness of the high resolution structural scans was less than that of the functional scans. A comparable two sample *t*-test was performed between groups within the previously defined search region to determine if atrophy was accounting for the differences in the fMRI data.

RESULTS

Neuropsychological Results

The cognitive data are presented in Table 1 for the MCI and control groups. Age, education and Wide Range Achievement Test Reading Standard Score (an estimate of maximal verbal IQ) were not statistically different. Group differences on the Boston Naming Test raw scores approach significance ($p=.052$). The learning and delayed recall components of the Rey Auditory Verbal Learning Test and the Brief Visuospatial Memory Test-Revised were all significantly different between groups, with the MCI participants performing more poorly ($p < .001$). Similarly, MCI subjects were significantly different on the MMSE, COWAT, and Trailmaking Test B ($p < .05$).

Behavioral Results

Plots of reaction time and performance accuracy are shown in Figure 2 and Table 2. The plots reveal that both groups responded progressively quicker over trials and with greater accuracy. Repeated-measures ANOVAs were implemented to determine whether performance differed by group in magnitude or slope over trials. For reaction time (RT), the effect of group was non-significant ($F[1,19]=1.78$, $p=.19$), though qualitatively the controls exhibited shorter RTs. The effect of trials was significant ($F[1,19]=13.12$, $p=.002$), indicating a learning effect. There was no trial by group interaction ($F[1,19]=1.55$, $p=.182$). With regard to the accuracy data, there was a significant effect of group ($F[1,19]=11.41$, $p=.003$), with controls being more accurate. There was also a significant effect of trials ($F[1,19]=21.27$, $p < .001$), indicating that accuracy improved with repetition and feedback. The trial by group interaction was again not significant ($F[1,19]=1.51$, $p=.195$).

Imaging Results

The group analysis within the hippocampal ROI indicated significant differences in the slope of learning related BOLD signal over trials. The
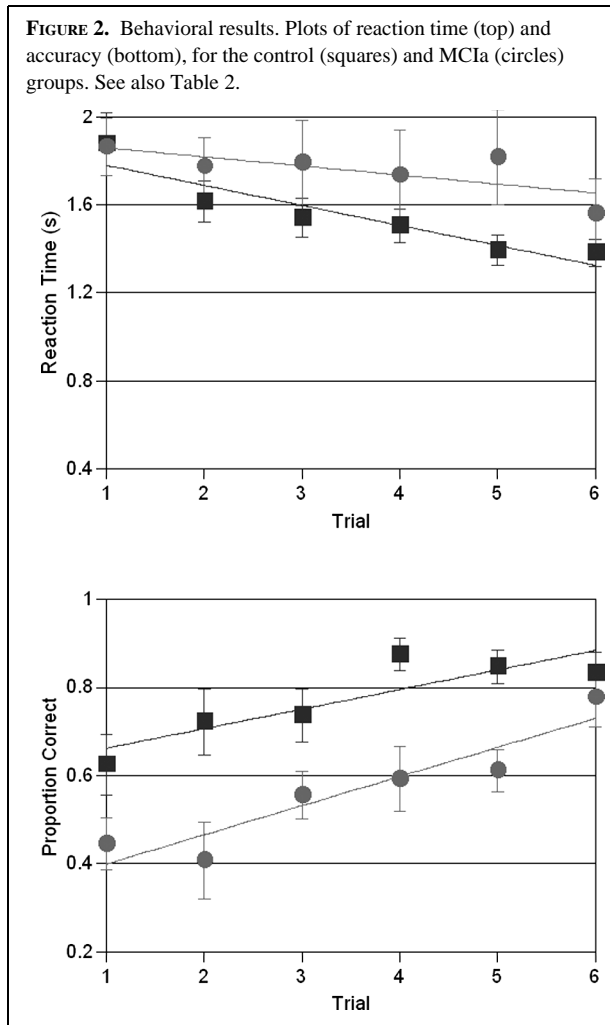
FIGURE 2. Behavioral results. Plots of reaction time (top) and accuracy (bottom), for the control (squares) and MCIa (circles) groups. See also Table 2.

TABLE 2. Task accuracy over trials

Trial	Controls		MCI	
	Mean	SD	Mean	SD
1	0.63	0.24	0.44	0.20
2	0.72	0.26	0.41	0.30
3	0.74	0.21	0.56	0.19
4	0.88	0.13	0.59	0.25
5	0.85	0.13	0.61	0.17
6	0.83	0.16	0.78	0.24

Note: scores represent mean proportion correct.

controls exhibited a steeper negative slope than did the MCI participants. This effect was observed in two clusters located in the hippocampal body (x,y,z: 30, –28, –8; $t=3.29$, $p_{unc}=.002$) and anterior hippocampus (x,y,z: 36, –8, –26; $t=3.27$, $p_{unc}=.002$). These results are shown in Figure 3. This figure depicts the statistical parametric map indicating the two clusters within the MTL search region where the control group exhibited a steeper slope associated with learning over trials than the MCI patients. Figure 4 illustrates the shape of the average hemodynamic response early in the task and again upon the final trial for a single subject. The hemodynamic response is observed to decrease as a function of trial in the hippocampal region.

In order to determine whether the group results were due to atrophy in the region of interest we conducted a voxel-based morphometry analysis with the same apriori ROI and extracted the VBM atrophy values underlying the activation coordinates. This was accomplished by extracting the principal component of a small sphere (2 mm radius) at the maxima in the two activation clusters and then extracting the atrophy signal from the VBM data at the same locations in the same fashion. A small homogenous ROI was selected because we were only concerned with gray matter at the site of peak activity differences. This was a conservative approach for determining gray matter values at peak activation maxima; a larger ROI may have obfuscated volumetric findings under the fMRI maxima. The extracted ROI values were then entered into a correlation analysis. In the anterior cluster there was a relationship between fMRI signal and gray matter volume (GMV; $r=.55$, $p=.009$). The posterior hippocampal cluster did not show this relationship ($r=.05$, $p=.86$), suggesting that fMRI signal and GMV were not related at this location in these data.

DISCUSSION

In this report we have examined the dynamic neural response during associative learning in a group of MCI patients and controls. Overall, the MCI patients expectedly performed worse than controls on a number of neuropsychological measures of encoding and recall abilities, as well as on the concurrent discrimination fMRI task itself. The imaging results were consistent with our initial hypotheses indicating a difference in learning-related BOLD attenuation in the right hippocampus. The controls exhibited a significant learning effect while the MCI patients exhibited very little learning effect. The results are consistent with prior work using repeating trials paradigms (Johnson et al., 2004; Rand-Giovannetti et al., 2006) in elderly adults. Furthermore, these findings fit well with other studies of dynamic hippocampal adaptation over successive trials during learning acquisition (Dolan & Fletcher, 1999; Henson et al., 2003; Henson & Rugg, 2003; Strange et al., 1999).

FIGURE 3. Statistical parametric map of the two-sample *t*-test delineating group differences between Controls and MCIa. The result is overlaid on an atlas brain in MCI space. The analysis was constrained to the right hippocampus based on prior studies. The ROI search region is in white on the images, and encompasses the statistical results (hot colors). The analysis resulted in two clusters in the body and head of the right hippocampus. See the text for coordinates and statistics. The right side of the brain is on the right side of the image.
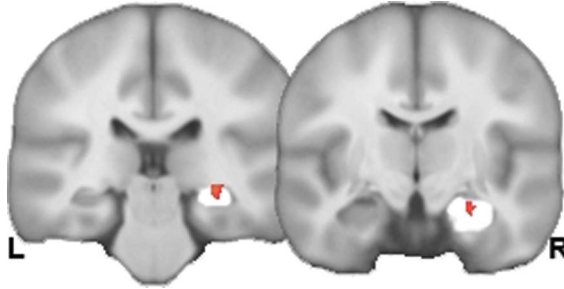

FIGURE 4. Peristimulus plots indicating a decrease in the hemodynamic response as a function of trial in the mesial temporal lobe for a healthy control subject. The mean time-course of the second trial (the first trial after feedback) and final trial are shown with solid lines. The 90% confidence intervals are represented by dashed lines. The coronal image inlay shows the subject's SPM of learning over trials overlaid on the T1 volume. The color scale is the magnitude of the *t*-statistic.
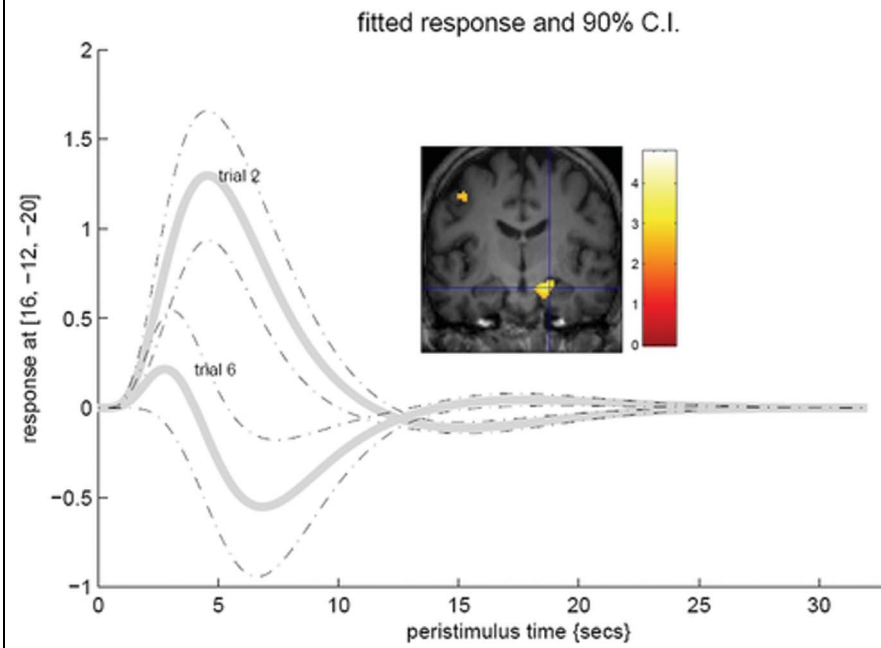
The earlier study by Myers et al. (2002) found that individuals with hippocampal atrophy, a risk factor for subsequent cognitive decline, performed worse than non-atrophied controls on the transfer portion of the concurrent discrimination task. The authors attributed impairment to reduced hippocampal region processing during the initial learning stages. Since hippocampal atrophy is a risk factor for cognitive decline, including MCI, we expected that MCI patients would show reduced learning-related hippocampal signal change. Our results confirmed this prediction. The findings are consistent with the idea that the hippocampal region is normally involved in stimulus-response learning, even if such simple learning is possible without hippocampal involvement. Similarly, in animals, classical conditioning may be spared following hippocampal lesion (e.g. Schmaltz & Theios, 1972), but electrophysiological recordings show that, when present, the hippocampus is active during this simple stimulus-response learning (Berger et al., 1983). Our results are also consistent with the idea that hippocampal region activation during this simple learning task may facilitate subsequent transfer when task demands change; this is consistent with the idea that learning in animals and humans with hippocampal region damage is often "inflexible" or "hyperspecific" (Eichenbaum et al., 1994; Gluck & Myers, 1993, 2001; Schacter, 1985).

Others have examined the hippocampal response during transitive inference—after initial learning of stimulus response associations have taken place. Heckers et al. (2004) found the anterior hippocampal region was active while making relational or transitive inferences on information already in declarative memory (see also Preston et al., 2004). A more recent study had extended that paradigm to schizophrenia and determined that transitive inference, or transfer, was impaired in patients compared to controls (Ongur et al., 2006). These studies all conducted fMRI after acquisition of information had already occurred, whereas the current study imaged the brain during the dynamic temporal course of acquisition over repetition and feedback.

Our results show that hippocampal attenuation during concurrent discrimination learning was detected in the right MTL for this visuospatial associative encoding task. We expected effects in the right-hemisphere due to the visuospatial modality of item presentation (Golby et al., 2001). Further, Henson, et al. (2003) reported a series of studies on repeating stimuli and concluded that across a variety of stimulus materials, the right MTL was more sensitive to stimulus repetitions than the left. Strange et al. (1999) also reported specific right hippocampal attenuation in healthy young adults during learning of visuospatial material.

Functional MRI studies in patient groups are prone to a number of potentially confounding factors. The coupling between neural activity and the hemodynamic response may be variably reduced in the patient group such that the hemodynamic response is no longer an accurate measure of

neural activity. Another possible issue is that patients with cognitive impairment may approach the task with different strategies than controls. Examination of Figure 2 indicates that the MCI subjects exhibited a similar learning slope to controls, but indeed their accuracy was significantly lower. In this study we attempted to deal with this possible issue by incorporating the learning slope directly into the analysis. The input contrast images into the group analysis represented learning-associated signal change where learning of the correct choice in each pair was associated with signal reduction in the MTL. Another possible approach (but only useful at the group level) would have been to use the behavioral learning data as a covariate in the statistical analysis.

The samples in this preliminary study were small and thus raise the possibility of vulnerability to outliers, and simultaneously, low statistical power to detect true effects. The small number of subjects prevented more elaborate regression analyses modeling other demographic or general cognitive status in the fMRI analysis. Another limitation may have been incurred by the relatively small number of fMRI acquisitions from which event averages were calculated. More acquisition time points would likely improve detection of subtle BOLD adaptation over trials. Finally, others have pointed out that the accuracy of spatial normalization of the hippocampus to standard MNI space may be diminished in MCI and AD (Dickerson et al., 2005; Krishnan et al., 2006) and this might have introduced spatial variability, affecting the final result.

Despite these limitations, the findings presented in this study are consistent with the hypothesis that hippocampal signal decreases with acquisition of stimulus-response associations derived through feedback. Although prior learning-based fMRI studies in MCI have modeled repetition (Johnson et al., 2004; Rand-Giovannetti et al., 2006), none have modeled feedback-based learning over trials as was done in the present report. Whether the fMRI-measured hippocampal encoding response can predict subsequent successful transfer is still an unanswered question that will be addressed by our laboratories. Additionally, further studies with MCI patients are needed to differentiate the role of the MTL during encoding of specific stimulus-response associations from its role in transfer of relevant information to new items.

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