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Encoding Activity in the Medial Temporal Lobe Examined With Anatomically Constrained fMRI Analysis

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ABSTRACT: Functional neuroimaging studies have produced a sizable number of observations of increased activity in the human medial temporal lobe (MTL) during encoding of novel memories. The studies have suggested possible functional specialization within the anatomical components of the MTL (hippocampus and the entorhinal, perirhinal, and parahippocampal cortical areas). Neuroimaging studies have just begun to link anatomical regions to specific functions. To address functional specialization hypothesis, a method is described for using high-resolution structural information from magnetic resonance imaging MRI to constrain the analysis of functional magnetic resonance imaging (fMRI) data, for independent assessment of functional activity change in each component of the MTL. With this method, increased activity was detected throughout the MTL in a group of participants (n = 5) who encoded novel pictures. A separate group (n = 5) who encoded words exhibited lower-levels of evoked activity. Laterality effects were found reflecting increased right hemisphere activity during picture encoding (parahippocampal cortex) and increased left hemisphere activity during word encoding (posterior hippocampus and parahippocampal cortex). Neither condition provided evidence for greater activity in the posterior hippocampus than in the anterior hippocampus during encoding, although the greatest increases in activity were observed in the parahippocampal cortex. The anatomically driven methodology is shown to provide detailed comparison of levels of activity change across specific brain areas and to provide increased sensitivity to functional change in each region of the MTL. *Hippocampus 2002;12:363–376.* © 2002 Wiley-Liss, Inc.

KEY WORDS: fMRI; memory function; encoding

INTRODUCTION

The use of functional neuroimaging techniques has provided a great deal of insight into the neural circuitry that supports memory function in the brain (see reviews by Cabeza and Nyberg, 2000; Schacter and Wagner, 1999; Lepage et al., 1998). Recent studies using fairly high-resolution functional magnetic resonance imaging (fMRI) have indicated areas in the medial temporal and frontal lobes for which increased activity is correlated with successful encoding (Buckner et al., 1998a,b; Brewer et al., 1998; Wagner et al., 1998b; Kirchhoff et al., 2000) and material specific laterality (e.g., Kelley et al., 1998; Wagner et al., 1998a). Because neuroimaging is a correlational technique, these patterns of increased activity during encoding are best understood within the context of neuropsychological reports that have documented the critical role of the medial temporal lobe (MTL), including the hippocampus and adjacent cortical areas, in memory formation (Scoville and Milner, 1957; Squire, 1992). While the neuropsychological reports provide the critical causal link between memory formation and the MTL, neuroimaging studies can go beyond those reports to identify the networks of areas that support memory function in participants who do not have a history of neurological disorder.

Early reports of MTL activation during memory function relied on positron emission tomography (PET) as a technique (e.g., Squire et al., 1992; Buckner et al., 1995), which is limited by relatively low spatial resolution. Improvements in technique and the use of functional magnetic resonance imaging (fMRI) have increased the spatial resolution at which it is possible to detect changes in brain activity. This improvement in imaging technique has been paralleled by increasing precision in lesion studies for both experimental animals (e.g., Murray and Mishkin, 1998; Zola et al., 2000) and postmortem analysis of human amnesia (Rempel-Clower et al., 1996). However, convergence between these two areas has just begun to suggest hypotheses about functional specialization for components of the MTL memory system that can be tested with fMRI (Fernandez et al., 1998, 1999).

To address specific hypotheses about the relationship of function and anatomy, it is necessary to use a technique that uses precise anatomical information to guide

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the analysis and interpretation of data about functional activity. Several reports have suggested methods for using the anatomical boundaries of the MTL to improve the analysis of fMRI data (Fernandez et al., 1998; Small et al., 1999; Zeineh et al., 2000). This article describes a method that also uses high-resolution anatomical information to constrain the analysis of functional neuroimaging data. This technique can easily be applied to standard whole-brain imaging protocols, increases sensitivity to signal change within regions of the MTL without blurring anatomical boundaries, and permits testing of specific anatomical questions comparing activity across the components of the MTL.

A particularly effective paradigm for examining human memory with fMRI has been to examine activity associated with encoding novel memories in the MTL (Stern et al., 1996; Kirchhoff et al., 2000; Wagner et al., 1999; Fernandez et al., 1998; Brewer et al., 1998; Gabrieli et al., 1997). This task has been shown to elicit significant activations in the MTL, particularly the parahippocampal cortex and posterior hippocampus. Some investigators have suggested that the consistent finding of posterior activity indicates that the hippocampus is functionally specialized such that posterior areas are particularly involved in encoding novel memories (Fernandez et al., 1998; Gabrieli et al., 1997). Reviews of large numbers of studies of encoding have not provided systematic support for this idea. Schacter and Wagner (1999) concluded that both PET and fMRI studies tend to elicit activity throughout the MTL and also suggested that a failure to see anterior activity may be the result of artifactual loss in signal-to-noise in the anterior temporal lobe. Furthermore, Schacter et al. (1999) examined encoding and retrieval in the hippocampus using PET and found similar areas to be active in both tasks (although PET has lower spatial resolution than fMRI).

However, previous studies addressing this issue have most often used techniques based essentially on finding statistically reliable activity increases in one area and not observing reliable increases in other areas either by identifying the focus of an "active area" (Schacter et al., 1999; Schacter and Wagner, 1999) or identifying "activated pixels" (Fernandez et al., 1998; Small et al., 1999). This approach risks overinterpreting an apparent lack of activity in one region because increased activity may be obscured by high variance. In addition, active areas thus identified may extend over important anatomical boundaries, raising the question of which area is exhibiting increased activity. Using the anatomical boundaries of the MTL to guide the analysis of fMRI data and aggregating functional data within the anatomical boundaries can address comparisons between anatomical areas more directly. This approach tests targeted hypotheses about the activity of the anatomically defined regions of interest (ROI) and also provides a quantitative estimation of signal change in each ROI that can be used to compare across neuroanatomical areas.

Focusing theoretical questions about changes in activity on specific regions within the MTL also produces an increase in sensitivity to detecting these changes by reducing the problem of multiple comparisons that occurs when examining every voxel of fMRI data independently. As reported by Fernandez et al. (1998) and Small et al. (1999), using an anatomically defined region enabled detection of a small but reliable change in hippocampal activity across groups (Small et al., 1999) or performance conditions (Fernandez et al., 1998). The current technique expands on these reports by providing a systematic method for identifying the relevant anatomical boundaries and describing an analysis method that reduces spatial blurring while maintaining maximum sensitivity to signal change. This methodology has some characteristics in common with an anatomical method proposed by Zeineh et al. (2000) but differs in subsequent fMRI analysis technique and avoids the additional scanning and analysis time required to prepare an unfolded representation of the MTL memory system structures.

The anatomical ROI approach described in the present study uses the boundaries of the MTL that are identifiable on high-resolution MRI to constrain the analysis of functional activity in the MTL during memory encoding (as done in the previous reports mentioned above). The high-resolution structural MRI is typically collected immediately after the functional study and provides anatomical reference for localizing changes in activity, as well as being used to normalize the participants' data to the standard Talairach and Tournoux (1988) atlas. The high-resolution structural images can also be used to identify the borders of the components of the MTL: hippocampus (anterior and posterior) and entorhinal, perirhinal, and parahippocampal cortical areas following established methods (Amaral and Insausti, 1990; Insausti et al., 1998) (see Anatomical Methods below). These borders are used to group functional data into ROIs in order to examine changes in memory-related activity in each region. Activity changes during encoding of novel memories are compared for the anterior and posterior areas of the hippocampus and the left and right hemispheres are examined for areas of the MTL exhibiting lateralized changes in activity based on the type of stimuli being encoded.

METHODS

Subjects

A total of 10 volunteers recruited from the UCSD community (5 men, 5 women; age range 19–35). Five participants received the picture-encoding protocol and the other five received the word-encoding protocol.

Procedure

Picture encoding: Participants (n = 5) were told they would be shown a series of pictures and instructed to memorize all the pictures. Full-color magazine-quality photographs were shown to participants in the scanner by back projection onto a screen visible through a mirror placed over the participant's eyes in the scanner. Each picture was shown for 2.5 s each with a 500-ms ISI and participants were asked to press a button whenever a picture appeared on the screen (to ensure that they were maintaining attention to the stimuli). Encoding demands were manipulated in alternating 36-s blocks. During low-memory demands blocks, the same picture was shown 12 times in succession. The same picture was used during all low-memory demand blocks and thus, quickly

became extremely familiar. During the high-memory encoding condition, 12 novel pictures were shown in each block. Each scanning run began and ended with a low-memory-demand block and contained 7 total blocks (3 high-memory demand). Three scanning runs were administered to each participant (132 pictures total).

Word encoding: Participants (n = 5; a separate group from picture encoding) were told that they would be shown a series of words and instructed to memorize all the words. The design paralleled the picture-encoding task with words presented for 2.5 s each with a 500-ms ISI. Low-memory-demand blocks contained 12 repetitions of a single word and each high-memory-demand blocks contained 12 novel words. Words varied in length from 5–13 letters (mean = 7.03, SD =1.83) with mean frequency of 20.1 (SD 9.1) occurrences per million (range 6–40). As with the picture-encoding task, three scanning runs were administered to each participant (132 words total). Owing to time constraints, no subsequent behavioral test of memory encoding success was given. However, similar paradigms have reliably reported significant memory for stimuli presented in this manner (Stern et al., 1996; Wagner et al., 1998b; Brewer et al., 1998; Kirchhoff et al. 2000).

Imaging: During the encoding task, T2* gradient-recalled echoplanar BOLD images were collected in 24 6-mm sagittal slices covering the brain (FOV, 24 cm; flip angle, 90°; TE, 40 ms) on a GE SIGNA 1.5-tesla (T) scanner fitted with a high-performance local gradient coil and whole-brain radiofrequency (RF) coil (Wong et al., 1992a, b). Images were collected in the sagittal plane so that (1) the long axis of each voxel (6 mm through plane) would be in the left/right dimension and cross fewer critical anatomical boundaries in the MTL, and (2) geometric distortion in the superior/inferior direction (due to artifact from the ear canal or frontal sinus) would result in signal compression, rather than signal loss. All EPI images were corrected for distortion due to magnetic inhomogeneity (Reber et al., 1998b) to attempt to correct for this artifact compression. The TR was 3.6 s, with 10 repetitions in each 36-s block and 70 total repetitions for each scanning run. After completion of the three functional runs, a whole-brain high-resolution structural image was collected using a 3D MPRAGE pulse sequence (flip angle = 10°, FOV = 24 cm, 0.9375 × 0.9375 × 1.2 mm).

Anatomical Analysis of the MTL

The medial temporal lobe (MTL) is composed of the hippocampus proper (here defined as the pyramidal cell fields CA1, CA3, dentate gyrus, and subicular complex) and the cortical areas on the adjacent parahippocampal gyrus. The cortex of the parahippocampal gyrus is composed of three cortical areas: the entorhinal cortex, perirhinal cortex, and parahippocampal cortex. This study describes a method of segmenting the MTL into 10 distinct regions of interest (ROI): the anterior hippocampus, posterior hippocampus, entorhinal cortex, perirhinal cortex, and parahippocampal cortex (each bilaterally). The key anatomical reference points are the hippocampus proper, the cortical areas of the parahippocampal gyrus (PHG), the amygdala, temporal pole, and calcarine sulcus. The current use of these boundaries is to improve the analysis of fMRI data collected during memory tasks. The boundaries are identified on high-resolution (1-mm^3) structural images that have been normalized to the standard atlas and projected onto functional data at lower resolution (2.5 × 2.5 × 2.5-mm postnormalization re-sampled from the original 3.75 × 3.75 × 6-mm EPI images). The change in resolution will necessarily introduce some partial voluming effects in the low-resolution functional data. The current approach is to classify each voxel as belonging to exactly one area for later aggregation. In addition, because of the relatively low resolution of the EPI images, and since functional changes arise from BOLD signals that are potentially a few millimeters from the cortical areas exhibiting the neural activity change, the white matter of the PHG is classified as part of the adjacent cortical area. This heuristic creates ROIs that have enough voxels to capture the benefit of averaging out uncorrelated noise across voxels and cover the entire cortical area. Although this means that the resulting ROI designations on the high-resolution structural are not completely accurate measurements of cortical volume, the ROI sizes should still be related to cortical size and may be useful in studies of cortical atrophy in memory-disordered patients. It should also be noted that because EPI images have a differential susceptibility to dropout than the structural MRI, tissue may be visible on the structural image and included in an ROI for which no reliable data were collected functionally. This effect is accounted for in the subsequent analysis and aggregation of data across the anatomical ROI.

The following description lists the ROI defined and the guidelines used to identify the boundaries of each area along the medial/lateral axis, the inferior/superior axis, and the anterior/posterior axis. The majority of the anatomical landmarks used to identify boundaries are most easily observed in the coronal plane. Many of the boundaries are standard and fairly obvious. The critical distinction between the anterior/posterior hippocampus (based on the uncal apex or gyrus intralimbicus) is taken from Insausti et al. (1998), where it is used to define the end of the perirhinal cortex in their volumetric measurements of entorhinal and perirhinal cortical areas. The most posterior extent of the parahippocampal cortex is taken to be the appearance of the calcarine sulcus on the normalized coronal image as this seems to be associated with moving into brain areas closely associated with visual processing. The anterior boundaries of the perirhinal/entorhinal areas are also taken from Insausti et al. (1998) but are unlikely to be critical for the analysis of fMRI data, as the temporal pole region is not normally imageable with EPI because of the proximity of the artifact induced by the frontal sinus:

Hippocampus, anterior (Hf-Ant): The medial/lateral boundaries of the hippocampus are easily found, since the hippocampus is bounded by white matter laterally and CSF medially. Superior to the Hf-Ant is the amygdala in the most anterior portion and white matter posterior to the amygdala. The inferior edge of the Hf-Ant is bounded by the white matter of the parahippocampal gyrus (PHG; which is included in the cortical ROI; see below). The anterior boundary is at the amygdala. The amygdala/hippocampus boundary is often difficult to ascertain on coronal images and is more easily visible on a sagittal image through this area. The posterior boundary of the Hf-Ant is defined as 2 mm past the uncal

apex (gyrus intralimbicus; Insausti et al., 1998) and is seen most clearly on sagittal slices.

Hippocampus, posterior (Hf-Post): The medial and lateral boundaries are easily visible as cerebrospinal fluid (CSF) medially and white matter and the CSF of the third ventricle laterally. Superior to the Hf-Post is white matter in the anterior portion and CSF of the third ventricle in the posterior portion. Inferior to the Hf-Post is the white matter of the PHG. The anterior boundary is defined by the posterior edge of the Hf-Ant (see above). The posterior boundary is found at the posterior edge of the hippocampus bounded by the third ventricle and white matter.

Entorhinal cortex (ERC): The entorhinal cortex is the part of the anterior portion of the PHG on the medial wall of the gyrus. The medial boundary of the ERC is visible as CSF between the PHG and the brainstem. The definition of the lateral boundary of the ERC depends on whether the collateral sulcus appears in the usual form (a single sulcus) or appears as a bifurcation of the sulcus (a double sulcus). In the usual form of the collateral sulcus, the medial boundary is defined as the inflection point between the edge of the PHG and the collateral sulcus. When a double sulcus is evident, the lateral boundary of the ERC is defined as the depth of the medial portion of the collateral sulcus. For the purposes of regional analysis of fMRI data, the superior boundary of the ERC is defined as the gray matter of the anterior hippocampus, thus including the medial white matter of the PHG into the ERC region. The inferior boundary of the ERC is the ventral edge of the PHG visible as distinct from neighboring CSF. The anterior boundary of the ERC is defined as occurring at the anterior edge of the amygdala (following Insausti et al., 1998). The posterior boundary of the ERC is defined as being a projection from the posterior edge of the Hf-Ant inferior (on a coronal plane) through the PHG. Thus the ERC is inferior to the Hf-Ant (and the PHC is inferior to the Hf-Post; see below).

Perirhinal cortex (PRC): The perirhinal cortex is the lateral cortical area on the anterior portion of the PHG. The medial boundary of the PRC is the ERC. The lateral boundary of the PRC is defined as the depth of the collateral sulcus, thus including primarily the medial bank of the collateral sulcus (although some of the lateral bank is often included due to unavoidable partial voluming arising from the relatively low resolution of EPI). As with the ERC, the superior boundary of the PRC is the hippocampus, thus including the lateral portion of the white matter of the PHG into the PRC region. The inferior boundary of the PRC is the ventral edge of the PHG visible as distinct from the neighboring CSF. The anterior edge of the perirhinal cortex is defined as the temporal pole (following Insausti et al., 1998, although it should be noted that fMRI generally provides little or no sensitivity in areas anterior to the anterior edge of the amygdala). The posterior edge of the PRC is identical to the posterior edge of the ERC and is the boundary defined between the anterior and posterior hippocampus regions.

Parahippocampal cortex (PHC): The parahippocampal cortex is the posterior cortical area within the parahippocampal gyrus. Whereas the anterior portion of the PHG is divided into the ERC and PRC, the posterior PHG is considered to be a single cortical area. The PHC is bounded medially by CSF separating the temporal lobe from the brain stem and bounded laterally as the depth of the collateral sulcus, including primarily the lateral bank of the collateral sulcus in addition to the cortex of the PHG. The superior boundary of the PHC is the posterior hippocampus and the CSF of the third ventricle, thus including the white matter of the PHG. The inferior boundary of the PHC is the ventral edge of the temporal lobe. The anterior boundary of the PHC is the posterior edge of the PRC and ERC; that is, it is at the same level as the boundary that divides the hippocampus into anterior and posterior portions. The posterior boundary of the PHC is defined as the first coronal slice on which the calcarine sulcus is visible.

RESULTS

Voxel-Based Analysis

Each of three scanning runs for each subject was preprocessed by motion correction through time (AFNI), elimination of voxels exhibiting signal change >10% in a single TR (indicating a nonhemodynamic event) and spatial smoothing within each sagittal slice (7.5-mm FWHM). Each run was then normalized to the Talairach and Tournoux (1988) atlas using an automatic transformation algorithm (MNI_Autoreg; Collins et al., 1994) and averaged together within-subject. To identify areas of consistent activity across each group, a one-sample *t*-test was performed on the observed signal change for each voxel across the participants in the group. Areas of significant activity were those which exhibited a significant change by this random-effects analysis ($t(4) > 5.59$, $P < 0.005$ uncorrected) across a cluster of at least 350 mm^3 in volume. Monte Carlo simulations with noise data have shown that this statistical threshold provides adequate control for a random effects analysis. Figure 1 presents areas of significantly increased activity for both conditions.

Encoding pictures: Using standard voxel-based analysis, 16 areas were identified that exhibited increased activity when memory demands were high during encoding (i.e., when novel pictures were shown instead of a repeated familiar picture). However, it should be noted that this includes a cluster of 19,078 mm^3 (1,267 voxels) on the right side that includes posterior visual areas, extensive ventral temporal lobe activity, extensive MTL activity, and activity in the thalamus and cerebellum. A similar pattern of activity observed on the right side although it was identified as two clusters (8,406 mm^3 and 4,609 mm^3 in extent) that cover a similar area. Table 1 lists the foci within these clusters to indicate the span of brain regions that exhibited increased activity. Increased activity in 32 brain regions during memory encoding is summarized in Table 1. When a series of novel pictures are shown to participants with instructions to memorize, increased visual processing is demanded and increased attention is likely paid to these novel stimuli. Increased activity in memory-related areas, such as the hippocampus, parahippocampal cortex, and thalamus, reflects the memorization of the stimuli. Two areas in prefrontal cortex exhibit increased activity as well that have been associated with memory-encoding

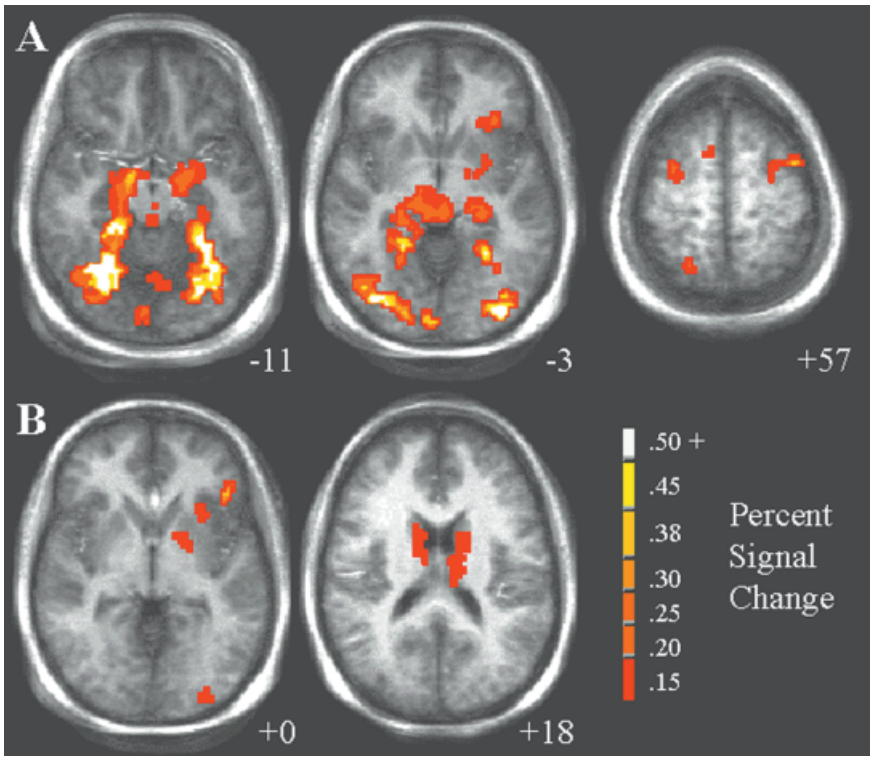

FIGURE 1. Areas of increased activity during encoding identified using a traditional cluster-based analysis ($t > 5.59$, clusters > 350 mm^3 in volume). A: Areas exhibiting increased activity during picture encoding. B: Areas exhibiting increased activity during word encoding. All images are shown on axial images from averaged, normalized structural magnetic resonance imaging and are oriented according to the radiologic convention (the left side of the brain is on the right side of the image). Numbers at the lower right corner of each image denote the axial level within the standard atlas. For a list of areas exhibiting increased activity by coordinates, see Table 1.

processes (e.g., Kirchhoff et al., 2000). Although this analysis demonstrated increased activity in the MTL, this voxel-based technique is not sufficient to provide a detailed comparison of components of the MTL. With a standard analysis, it is not possible to compare directly the level of activity evoked in anterior and posterior areas or across the hemispheres. To compare two areas directly, we need definitions of the boundaries of these areas, as will be provided in the anatomical ROI analysis below.

Encoding words: In contrast to the extensive increased activity associated with encoding novel pictures, relatively little activity was detected during word encoding. Six areas of increased activity were identified during memorizing words (Table 2), including regions of the basal ganglia (predominantly on the left), left prefrontal cortex, and left occipital cortex. The predominantly left-sided activity is consistent with left lateralization of language in right-handed participants. The relative lack of increased activity during word encoding probably reflects the low power of the particular protocol used, rather than providing evidence that the MTL is not activated in this task. The question of whether the MTL exhibited increased activity is specifically addressed in the anatomical ROI analysis below.

Anatomical ROI

Regions of interest were identified for 10 areas within the MTL memory system according to the landmarks identifiable from structural MRI described above (see Figure 2). The 10 ROIs were identified individually on each of the 10 participants. The mean sizes of each region are listed in Table 3. Note that the volumes of the cortical areas are not necessarily comparable to those reported elsewhere (e.g., Insausti et al., 1998; Juottonen et al., 1999) because the brain volumes are normalized to the Talairach and Tournoux atlas (1988) before identification of the anatomical ROI and also because adjacent white matter is included in the ROI in anticipation of partial voluming in the functional MRI data (see Anatomical Methods, above). Reported ROI volumes reflect the total number of voxels classified as belonging to each ROI (each voxel is $1 \times 1 \times 1$ mm^3). From these designations, a functional voxels are partitioned into the ROI for subsequent analysis of this lower resolution data (each voxel is $2.5 \times 2.5 \times 2.5$ mm^3). Analysis of significant signal change in each ROI is carried out using the classification of the functional voxels (see below). To identify areas where signal dropout and artifact might have a greater effect on assessing functional activity, the number of voxels in each region having low signal (defined as voxels where the measured average fMRI signal was <20% of the rest of the combined ROIs) is reported together with the percentage of ROI volume this accounted for (Table 3).

Although the anatomical volumes were constructed after the structural images were transformed to the standard atlas space, considerable variability exists in the size and location of the hip-

TABLE 1.

Coordinates of Areas Exhibiting Increased Activity During Picture Encoding

	x	*Y*	*z*	*Region*
Occipital cortex	27	−76	26	R superior occipital gyrus BA 19[a]
	39	−78	15	R medial occipital gyrus BA 18/19[a]
	31	−78	−15	R inferior occipital gyrus BA 18[a]
	6	−89	−5	R BA 17/18
	−28	−77	29	L superior occipital lobe BA 19[b]
	−28	−88	10	L medial occipital gyrus BA 18[b]
	−36	−87	−2	L inferior occipital gyrus BA 18[b]
	−41	−79	−7	L inferior occipital gyrus BA 19[b]
Fusiform gyrus	42	−70	−5	R fusiform gyrus BA 19/37[a]
	27	−67	−12	R fusiform gyrus BA 19/27[a]
	−31	−70	−11	L fusiform gyrus BA 19/37[b]
Medial temporal lobe	21	−30	−4	R posterior Hf[a]
	21	−45	−10	R parahippocampal gyrus BA 36/37[a]
	15	−16	−10	R anterior hippocampus[a]
	−30	−45	−10	L parahippocampal gyrus BA 36/37[b]
	−26	−31	−4	L posterior hippocampus[c]
	−22	−12	−12	L anterior hippocampus[a]
Parietal cortex	21	−60	52	R precuneus (BA 7)
	14	−76	33	R precuneus (BA 7)
	18	−57	16	R occipitoparietal sulcus (BA 31)
Prefrontal cortex	43	13	28	R dorsolateral prefrontal cortex BA 9/44
	−27	22	2	L inferior frontal lobe BA 47 (insula)
	29	−4	58	R BA 6
	9	7	61	R BA 6
	−39	2	54	L BA 6
	20	2	32	R anterior cingulate
Subcortical structures	5	−14	8	R thalamus[a]
	−14	−15	11	L thalamus[c]
	−2	19	9	L caudate
	−18	−1	12	L putamen
Cerebellum	−7	−68	−17	Cerebellum
	18	−30	−18	R anterior cerebellum[a]

[a],[b], and [c] were regions contained within three large clusters spanning several brain areas. For these regions, reported coordinates are for subclusters identified to communicate the span of the larger region.

pocampus across participants (Fig. 3; see also the standard deviations on region size in Table 3). If the standardization routines were completely accurate, the hippocampus would be the same size and shape after normalization across all participants, but this is not the case. This variability across participants reinforces the need for a method based on individual anatomical boundaries to most carefully assess changes in hippocampal activity across a group of participants.

To identify the level of increased activity in the MTL ROI during encoding, the average observed signal for every voxel in the ROI was summed to create a single time series for each region. This summation was done with data that were not spatially smoothed (unlike the voxel-based analysis described above), since the anatomical identification of the areas and the averaging across functionally related voxels accomplishes the two principal goals of spatial smoothing: (1) improving alignment across intersubject anatomical variability, and (2) increasing signal-to-noise by averaging out uncorrelated noise across voxels. Each ROI for each subject was then analyzed by regression (general linear model) for fit to an idealized hemodynamic response (assuming a base delay of 6s from onset to peak signal response, but fitting for variability in delay across areas + 2s for each area for each participant individually). This provides a direct estimate of the size of the change in activity during encoding for each ROI for each subject. Average time series for the activity within the left and right hippocampal regions during picture encoding are shown in Figure 4.

Although sensitivity to signal change may vary across the anatomical ROI due to differential susceptibility to imaging artifacts, the summation technique effectively weights voxels such that areas

TABLE 2.

Coordinates of Areas Exhibiting Increased Activity During Word Encoding

	x	y	z	*Region*
Basal ganglia	−9	−4	14	Left caudate (head extending to tail) and left thalamus
	10	0	14	Right caudate (head extending to tail)
	−19	1	−2	L putamen
Left PFC	−44	29	2	L inferior prefrontal cortex BA 44/47
	−33	19	−5	L inferior prefrontal cortex BA 47
Visual	−33	−88	6	L medial occipital gyrus BA 18

with low levels of signal contribute less than more robustly imaged areas. While this method probably reduces the effect of artifact-contaminated voxels, it cannot account for loss of signal in voxels that might have exhibited a task-dependent change in level of activity. Thus, the estimates of increased signal change during memory encoding in the anterior cortical areas (i.e., entorhinal and perirhinal cortex) might underestimate the signal change occurring in these areas.

The average change in activity in each ROI of the MTL during picture encoding is shown in Figure 5. Significantly increased activity was observed in the anterior and posterior hippocampus and the parahippocampal cortex bilaterally ($t(4) > 3.38$, $P < 0.05$). In the anterior cortical areas, the right perirhinal exhibited a significant increase in signal ($t(4) = 2.86$, $P < 0.05$) while the increase in activity in the entorhinal cortex was marginal bilaterally ($t(4) > 2.25$, $P < 0.10$). The left perirhinal cortex did not exhibit a significant increase in activity ($t(4) = 1.52$, $P > 0.20$). As shown in Table 3, the perirhinal cortex typically exhibits the greatest level of signal dropout and is the area where sensitivity to increased signal change is likely to be lowest.

Less overall areas of increased activity were observed during the word-encoding task (Fig. 6) across the ROIs defined in the MTL. In this sample of 5 participants, significant increases were only observed in the left posterior hippocampus and left parahippocampal cortex. The level of observed signal change was small across the group, but the ROI method provides some opportunity to identify slight, but reliable, increases in activity across a small group of participants.

While the ROI analyses were first examined using regions identified in the normalized transformations of the structural data, a separate analysis was also performed examining the original EPI data. The advantages of the use of normalized anatomical images are that they highlight the anatomical variability across participants (even when data were transformed to the standard atlas) and the smaller voxel size reduces the number of voxels that cross anatomical boundaries. Because the voxels are smaller, they are more likely to be entirely contained within a single anatomical area. However, the resampling of data to the higher resolution necessarily creates some spatial blurring that could potentially affect the results of the ROI analyses. For comparison, the results of using the

TABLE 3.

*Anatomical Regions of Interest**

Region	Region-of-interest volume (mm^3)	No. of fMRI voxels included (15.6 mm^3)	Signal dropout	
			Voxels with low signal	% of region
Left anterior hippocampus	2434 (158)	152.8 (10.1)	7.7 (18)	1.2
Right anterior hippocampus	2578 (213)	164.2 (13.9)	3.5 (8.1)	0.4
Left posterior hippocampus	1556 (83)	96.2 (5.8)	0 (0)	0
Right posterior hippocampus	1548 (71)	94.1 (4.1)	0 (0)	0
Left entorhinal cortex	2084 (214)	127.5 (11.7)	42.0 (30)	30.4
Right entorhinal cortex	2087 (180)	126.3 (13.0)	20.0 (19)	17.1
Left perirhinal cortex	1735 (118)	107.6 (8.4)	37.2 (25)	31.6
Right perirhinal cortex	1635 (114)	99.5 (10.6)	23.4 (24.6)	21.8
Left parahippocampal cortex	2504 (216)	153.1 (14.2)	1.8 (2.9)	0.9
Right parahippocampal cortex	2455 (123)	152.4 (8.8)	1.4 (2.7)	0.8

*Numbers in parentheses indicate the standard deviation across the 10 participants. Voxel size (15.6 mm^3) refers to the voxel size of the normalized data, resampled to 2.5 × 2.5 × 2.5 mm. Voxel size within the original data was 84.4 mm^3.

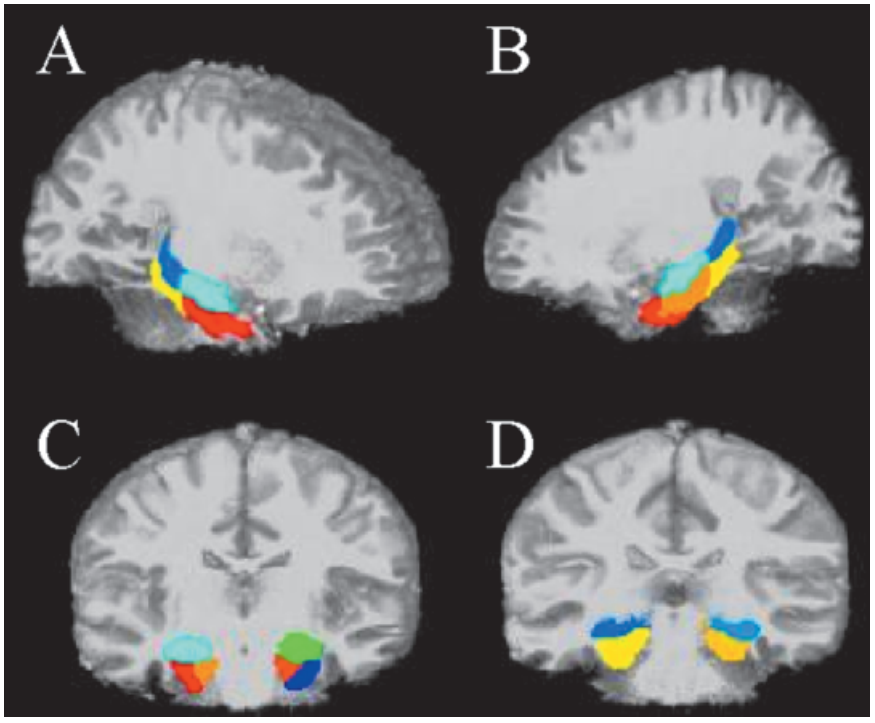

FIGURE 2. Anatomical regions of interest shown for a representative participant. A: Lateral view of the right medial temporal lobe (MTL) shown from the right side. B: Medial view of the right MTL. C: Coronal view of the anterior MTL bilaterally. D: Coronal view of the posterior MTL bilaterally. Components of the MTL are designated by the following colors: anterior hippocampus: light blue (L), green (R); posterior hippocampus: dark blue (L and R); entorhinal cortex: orange (L), orange-red (R); perirhinal cortex: red (L), purple (R); parahippocampal cortex: yellow (L), yellow-orange (R).

original (non-normalized) anatomical and EPI data are shown in Table 4. The results of this analysis are highly consistent with the results shown in Figures 5 and 6. The consistency between the two approaches suggests that the two variations on this approach are generally similar and provide similar accounts of the increases in observed activity in these regions.

In addition to identifying the areas that exhibited significantly increased activity, it is also possible to directly compare the levels of evoked activity across components of the MTL. To address the hypothesis suggested by Gabrieli et al. (1997), that the posterior hippocampus is specialized for encoding, the levels of increased activity were compared across the anterior and posterior portions of the hippocampus within each hemisphere as well as bilaterally for both the picture-encoding and word-encoding groups (by paired *t*-tests). No evidence for more activity in the posterior hippocampus was observed in any contrast ($ts < 1.00$), suggesting that the overall level of activity in the posterior and anterior portions of the hippocampus were essentially similar during memory encoding. There was a trend within the cortical areas for increased activity in the parahippocampal cortex compared with the anterior cortical areas within each of the groups ($t(4) = 2.18$, $P < 0.10$ for picture encoding; $t(4) = 2.54$, $P < 0.07$ for word encoding). Although this contrast must be considered carefully in light of the increased signal loss and imaging artifacts occurring in the anterior cortical areas, there may be some anterior-posterior organization of the MTL during memory encoding in the cortical areas of the parahippocampal gyrus, rather than the hippocampus itself.

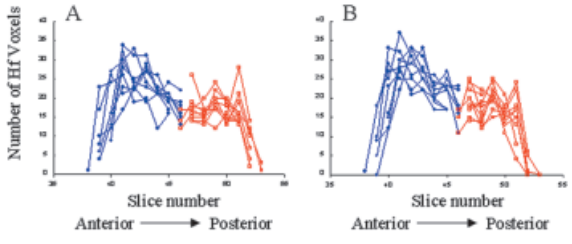

FIGURE 3. Variability in hippocampal size across participants. The number of voxels included in the hippocampal regions of interest (ROI) are shown for each coronal slice for the left hippocampus (A) and right hippocampus (B). Blue lines indicate voxels in the anterior hippocampus, red lines indicate voxels in the posterior hippocampus. Although the ROI were identified in the normalized space of the Talairach and Tournoux (1998) atlas, considerable variability is apparent across the 10 participants in the size of the hippocampus on each slice. Hf, hippocampal formation.

Laterality effects within the MTL can also be examined by comparing levels of increased activity in ROIs in the left and right hemisphere. Similar to other reports of lateralized effects during encoding (e.g., Kelley et al., 1998), increased activity was observed in the right PHC compared with the left PHC ($t(4) = 3.40$, $P < 0.05$) during picture encoding. There was an unusual trend for more activity in the left anterior hippocampus ($t(4) = 2.19$, $P < 0.10$), although the effect of laterality comparing the entire hippocampus was not reliable ($t(4) = 1.31$). During word encoding, increased activity was observed in the left posterior hippocampus

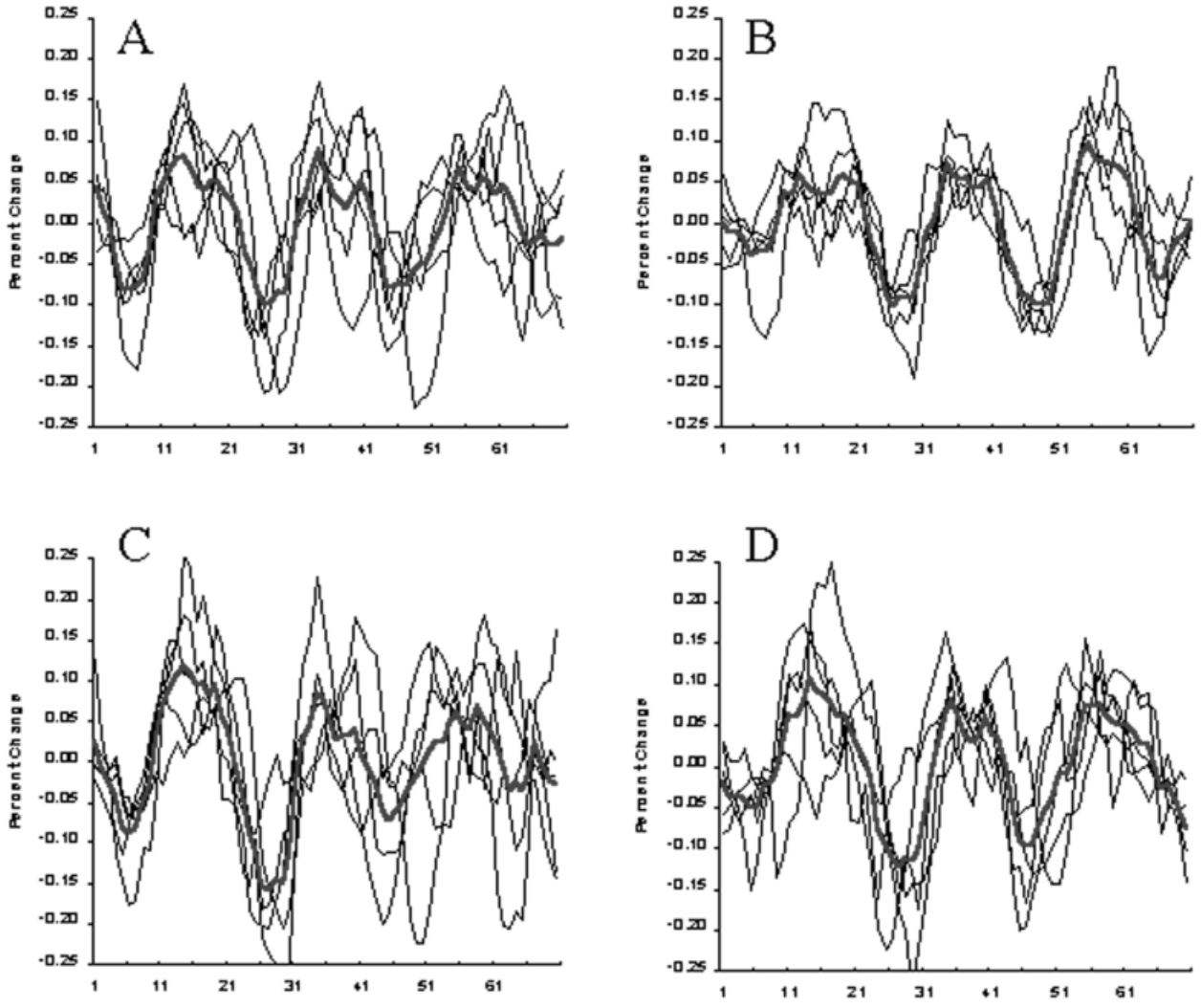

FIGURE 4. Average time series during picture encoding for right anterior (A) and posterior (B) hippocampal regions and the left anterior (C) and posterior (D) hippocampal regions. Although there is considerable variability across individual participants (thin lines) the increase in average activity for the group (thick lines) is clear for all four regions.

($t(4) = 2.83$, $P < 0.05$) and the parahippocampal gyrus ($t(4) = 4.01$, $P < 0.02$), consistent with previous reports (Wagner et al., 1998b). No other laterality effects were observed, $ts < 1.30$

GENERAL DISCUSSION

For the picture-encoding task, the memory demands of encoding novel pictures results in an extensive increase in activity throughout the visual system and MTL areas. Increased activity is easily detectable with a random effects analysis, in spite of only having five participants in this condition, using a traditional cluster-oriented analysis. The large number of brain areas exhibiting increased activity during picture encoding reflects the broad nature of the experimental contrast used. Repeated viewing of the same picture with instructions to memorize likely results in habituation and reduced activity in visual and attention-oriented areas compared with the instruction to memorize pictures when a series of novel pictures are presented. The traditional analysis identifies a large number of areas in which increases in activity surpass the statistical threshold but doesn't afford comparison of changes in activity across components of the MTL memory system. The technique of separately assessing activity in each component of the MTL memory system, i.e., the hippocampus (anterior and posterior), and entorhinal, perirhinal, and parahippocampal cortical areas, allows for a more detailed analysis of functional change in the MTL.

The ROI-based methodology confirms that during picture encoding, reliable increases in activity are specifically observed in the hippocampus (anterior and posterior), the parahippocampal cortex, and the right entorhinal cortex with trends for increased activity observed in the perirhinal and left entorhinal cortex. The level of increased signal change in each area was quantitatively assessed

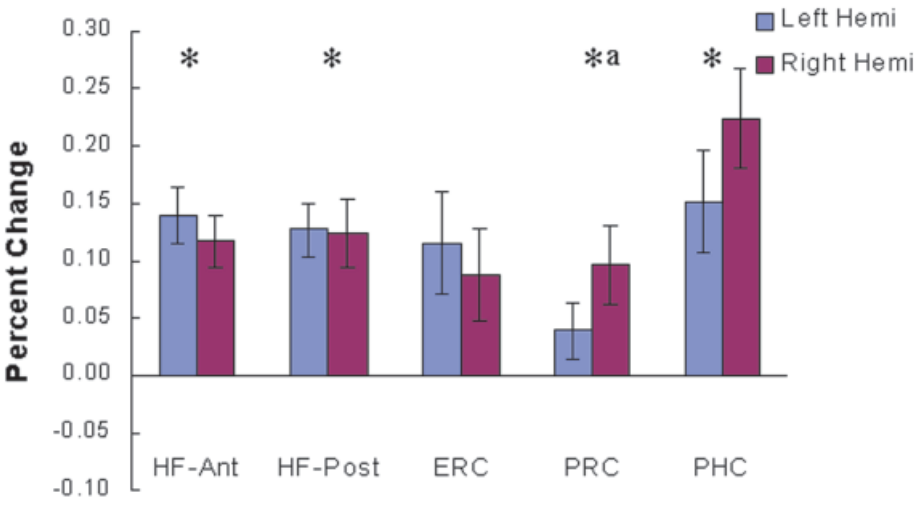

FIGURE 5. Activity in the medial temporal lobe (MTL) during picture encoding by region of interest (ROI). The change in activity in each area is assessed separated for each participant and mean levels of increased activity are shown (n = 5). *$P < 0.05$ bilaterally; *a, $P < 0.05$, right hemisphere only. Hf-Ant, anterior hippocampus; Hf-post, posterior hippocampus; ERC, entorhinal cortex; PRC, perirhinal cortex; PHC, parahippocampal cortex. Error bars indicate the standard error of the mean.

and compared. The area that exhibited the largest increase in activity during encoding was the parahippocampal cortex, an area reported very frequently as the focus of increased activity during encoding (Stern et al., 1996; Wagner et al., 1999). It has been suggested that, within the hippocampus (Fernandez et al. 1998), the posterior portion of the hippocampus is selectively involved in encoding novel memories. By segmenting the hippocampus into anterior and posterior ROI, the levels of increased activity were found to be similar in both portions of the hippocampus. In addition, a laterality effect was observed in the parahippocampal cortex. Consistent with previous reports (Kelley et al., 1998), increased activity was observed in the right parahippocampal cortex compared with the left.

The observed levels of evoked activity were much lower during encoding of words for a paradigm that matched the presentation rate and protocol structure. Very little activity was detected using a standard whole-brain, clustering analysis for this task. This may have been due to the fact that the words are not truly novel or that memorizing the words has different demand characteristics than memorizing pictures. It may also be difficult to detect these smaller changes in activity in a small sample (5 participants). Kirchhoff et al. (2000) reported generally lower levels of increased activity during word encoding compared with picture encoding, similar to our results. Other reports have found greater regions of increased activity during word encoding in the MTL (e.g., Wagner et al., 1998b; Kopelman et al., 1998; Dolan and Fletcher, 1997; Martin

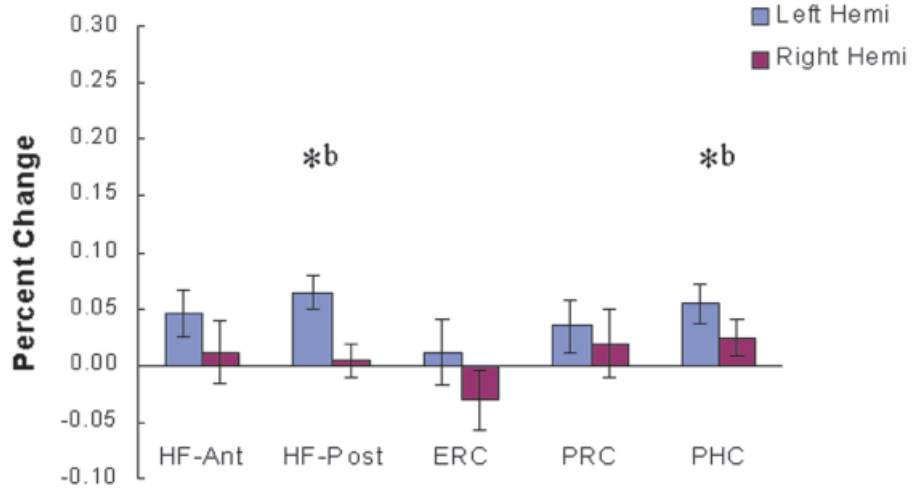

FIGURE 6. Activity in the medial temporal lobe (MTL) during word encoding by region of interest (ROI). The change in activity in each area is assessed separated for each participant and mean levels of increased activity are shown (n = 5). *b, $P < 0.05$, left hemisphere only. Hf-Ant, anterior hippocampus; Hf-post, posterior hippocampus; ERC, entorhinal cortex; PRC, perirhinal cortex; PHC, parahippocampal cortex. Error bars indicate the standard error of the mean.

TABLE 4.

Signal Change Estimates from ROI Defined on Original Data

Region	Hemi-sphere	Signal change	SE	*t*-value	*P*
Picture encoding					
Anterior hippocampus	Left	0.156	0.034	4.555	0.010*
	Right	0.126	0.023	5.468	0.005*
Posterior hippocampus	Left	0.125	0.025	5.031	0.007*
	Right	0.130	0.042	3.100	0.036*
Perirhinal cortex	Left	0.064	0.041	1.555	0.195
	Right	0.116	0.032	3.598	0.023*
Entorhinal cortex	Left	0.088	0.037	2.345	0.079
	Right	0.115	0.026	4.410	0.012*
Parahippocampal cortex	Left	0.222	0.058	3.841	0.018*
	Right	0.313	0.046	6.829	0.002*
Word encoding					
Anterior hippocampus	Left	0.053	0.027	1.979	0.119
	Right	0.020	0.029	0.685	0.531
Posterior hippocampus	Left	0.052	0.017	3.170	0.034*
	Right	0.002	0.019	0.123	0.908
Perirhinal cortex	Left	0.002	0.035	0.066	0.950
	Right	0.019	0.036	0.530	0.624
Entorhinal cortex	Left	0.018	0.022	0.823	0.456
	Right	−0.056	0.032	−1.743	0.156
Parahippocampal cortex	Left	0.055	0.020	2.686	0.055
	Right	0.009	0.026	0.360	0.737

ROI, region of interest.

*, regions exhibiting reliable increases in activity during encoding.

et al., 1997). The current experimental protocol probably suffers from a lack of sensitivity due to the small number of participants and/or the slow pacing of the presentation of the words (3 s each). The results of activity observed during word encoding presented here serve to demonstrate the increased sensitivity of the anatomical ROI methodology. Even using a protocol that appears to have low power, specific anatomical hypotheses (e.g., about laterality) can be tested with an anatomically constrained technique.

The anatomical ROI analysis indicated that although overall levels of signal change in the MTL were low across this set of five participants who performed the word-encoding task, the increases were reliably above chance in the left posterior hippocampus and left parahippocampal cortex. While change in activity in the anterior hippocampus wasn't reliably greater than chance, it was also not reliably lower than the level of activity observed in the posterior hippocampus. Similar to the picture-encoding group, there was no evidence for differential activation of the hippocampus along an anterior-posterior axis. The laterality effects exhibited during word encoding were robust in both the posterior hippocampus and parahippocampal cortex, with greater increases in activity being observed in the left hemisphere. The finding of greater left-sided activity in the MTL during word encoding has been frequently reported (Kelley et al., 1998; Wagner et al., 1998a; Martin et al., 1997; Dolan and Fletcher, 1997).

Comparison of the anterior and posterior hippocampus for both the picture-encoding and word-encoding conditions indicates that increased activity during encoding was not different in the anterior and posterior portions of the hippocampus. Although some reports have suggested that increases in the posterior hippocampus are larger or more reliable than the anterior hippocampus (e.g., Stern et al., 1996; Gabrieli et al., 1998; see also Schacter and Wagner, 1999), the fact that the posterior hippocampus is adjacent to the parahippocampal cortex may have affected these previous studies. Commonly used spatial smoothing techniques may cause signal changes from the PHC to affect the posterior hippocampus and lead to higher estimates of increased activity. In addition, traditional clustering techniques might tend to group these two adjacent areas together. A similarly sized signal change in the anterior hippocampus could be missed (type II error) because there is less likely to be a large adjacent increase in cortical activity in the anterior cortical areas (possibly due to signal artifact in this area of the brain). The current ROI approach avoids this problem by not smoothing across anatomical boundaries and by grouping activity within anatomically defined clusters rather than functionally defined ones that may cross important anatomical boundaries. Note that the current technique maintains the advantages of these techniques by smoothing and grouping activity within the anatomical boundaries. In general, in order to investigate hypotheses that adjacent parts of the MTL may have distinct functional characteristics, it will be necessary to use anatomical landmarks to define the areas in question rather than risk blurring the boundaries between areas by spatial smoothing or clustering.

Although no evidence was found for more posterior activity in the hippocampus during encoding, the experiment presented here does not address the hypothesis that retrieval based activity preferentially activates the anterior hippocampus (Gabrieli et al. 1997; Stark and Squire, 2000). Although these reports have reported clusters of activated voxels in the anterior MTL during memory retrieval, these reports have not used the technique described here of separately identifying the anterior and posterior portions of the hippocampus that would allow direct testing of this hypothesis.

The anatomical method used in the present study allows for systematic aggregation across a set of functional voxels (sampling points) constrained by anatomical landmarks individually identified on high-resolution images of each participant. This method provides increases in sensitivity associated with spatial smoothing by enabling uncorrelated noise across voxels to be reduced by averaging within an ROI. Potential problems in aligning specific anatomical areas that can occur even after normalizing to the Talairach and Tournoux (1988) atlas are also avoided as each participant's ROI are individually determined. Finally, the aggregation across voxels within an ROI allows for estimation of the overall change in activity in that ROI and affords direct comparisons to other ROI to test hypotheses about functional organization, e.g., along an anteroposterior dimension or across hemispheres (laterality).

As seen in the analysis of the word-encoding results, the anatomical ROI methodology provides much greater sensitivity to small increases in signal change than a traditional analysis. The increase in sensitivity arises from two features of the method. First, aggregation across all the voxels in an anatomical region can extract a consistent, but weak signal that might be missed by a threshold-based analysis. Second, each anatomical ROI instantiates a specific hypothesis that there will be a change in activity in that area, effectively eliminating the problem of multiple comparisons that frequently complicate the analysis of whole-brain fMRI data. In the most conservative case, the hypothesis could be considered of whether any of the ROI exhibit changes in activity, in which case the number of parallel comparisons would equal the number of ROI (10 in the current experiment). However, in the current experiment, there are 10 individual hypotheses that each area exhibits an independent change in activity, meaning that it is not necessary to correct for parallel comparisons. Thus, even with samples as small as 5 participants, a correct, random-effects analysis can indicate that small changes are reliable (and thus likely to replicate).

The power of the method used here derives partly from controlling the number of parallel tests by focusing on ROIs instead of individual voxels and partly from the technique of examining estimates of average signal change across the activated region. Fernandez et al. (1998) and Small et al. (1999) also identified specific ROIs within the MTL, but used the number of voxels passing a statistical threshold as an indicator of increased signal. That method will be less sensitive when small signal changes occur consistently within a region across a set of participants; it is also unclear whether the null hypothesis (that there is no change) predicts zero voxels activated for selected thresholds. Estimates of signal change within a region should be normally distributed around zero under the null hypothesis (i.e., if there is no increase in activity) and comparing observed signal change across a group of participants will meet the assumptions of traditional statistical models. The average correlation technique described by Zeineh et al. (2000) will be similar in principle to the technique used here. In that report, the authors also extract information across every region of the MTL. However, the cortical rendering technique carries a requirement of additional resampling (into high-resolution voxels), extra image collection time (to extract enough information to calculate the unfolding), and extra analysis time to unfold the cortex over and above the time required to identify the ROIs. In the case of presenting reports of increased activity within the MTL, it is not clear that the flattened representation is more effective than the signal change by area graphs presented here (Figs. 3, 4). In retinotopically organized cortex, neighbor relationships among voxels reflect representations of neighboring areas of perceptual space. However, given the compact formation of the hippocampus, it is not clear that precise spatial relationships within MTL regions can be interpreted as reflecting contributions of separate elements (e.g., pyramidal cell fields, dentate gyrus) of the hippocampal formation.

Zeineh et al. (2000) also report spatial smoothing of their data (3–6 mm), which might blur the activation boundaries across regions. Simply averaging voxels across a region in the method reported here should provide a similar increase in signal-to-noise without as much blurring. Given the size of commonly used imaging parameters ($\sim 3 \times 3 \times 5$ or greater), some blurring near the boundaries of adjacent regions is unavoidable, but additional smoothing can allow areas such as the parahippocampal cortex to affect estimates of signal change in the posterior hippocampus. In the current study, imaging parameters were chosen to attempt to minimize cross-boundary blurring (i.e., sagittal slices so that the long axis of the voxel is less likely to cross the major boundaries). Some spatial precision may also be lost due to the method for identifying ROI being deliberately generous in the inclusion of white matter near the cortical areas of interest. A more conservative approach could be used that eliminates voxels that even partially overlap across regions or overlap significantly with adjacent white matter. The current approach is preferred in order to (1) capture blood flow effects that occur close to the ROIs but that may be displaced by 3–5 mm and (2) provide enough voxels within each region that averaging will increase signal-to-noise as smoothing normally does (by causing uncorrelated white noise across voxels to be attenuated). Crossing white matter boundaries slightly is unlikely to affect estimates of signal change within the ROIs data is not being aggregated from another functionally active region and uncorrelated signal should have little effect on average.

The principal drawback of the anatomical technique is that it can only be applied to systems in which there are a priori hypotheses about the anatomical boundaries of functionally separable areas, as in the MTL. It should also be noted that the identification of boundaries on the high-resolution structural images is somewhat time consuming. However, with some experience in human neuroanatomy, we have found that the labeling technique described above (see Anatomical Methods) can be executed in a few hours of work when using the high resolution anatomical images as the basis for the ROI (5–10 h per participant) or even more rapidly (30–60 min) when using the structural MRI as a reference to classify voxels from the low-resolution EPI images. Since there are no robust alternate techniques for specifically addressing certain questions about functional anatomy (e.g., the relative involvement of the anterior and posterior hippocampus in encoding), the investment of time is warranted.

In the present experiment, the hypothesis of functional specialization within the hippocampus for encoding was considered. The technique used to explore this hypothesis could be applied to a number of questions that have arisen from increasingly detailed studies of human memory function after neurological damage (particularly those that use post-mortem histology, e.g., Rempel-Clower et al., 1996) and studies of the effects of small, targeted lesions in experimental animals (e.g., Murray and Mishkin, 1998; Zola et al., 2000). These studies have raised important questions about the role of the components of the MTL. For example, Murray and Mishkin (1998) reported no deficits in delayed matching-to-sample after ibotenic acid (fiber-sparing) lesions to the hippocampus and suggested that the perirhinal cortex may play a more important role in supporting memory for this task. Zola et al. (2000) reported that deficits in memory function do occur after ibotenic acid lesions to the hippocampus. Further complicating the findings are the results from post-mortem histological analysis of human amnesic patients who exhibit debilitating memory dysfunction after lesions selective to the hippocampus (Rempel-

Clower et al., 1996; Zola-Morgan et al., 1986). In addition, theories of functional specialization for memory within the MTL need also to consider whether deficits in perception or performance are masking memory deficits; for example, if the perirhinal cortex plays a critical role in memory for objects, the potential role of this area in object perception needs to be addressed (Buffalo et al., 1998).

While neuropsychological (lesion) studies provide the most reliable information on whether the structures of the MTL are necessary for various kinds of declarative memory function, patients with small, selective lesions are relatively few in number. Functional neuroimaging provides an opportunity to examine larger groups of individuals who allow observation of the operation of the intact memory system in the MTL. Although fMRI is a correlational technique, that is, increased activity in an area does not permit the conclusion that this area is necessary, the ability to extract activity patterns that correlate with successful memory performance (Wagner et al., 1998b; Brewer et al., 1998; Fernandez et al., 1999) can provide a strong association between evoked activity and the anatomical basis of memory. The anatomical ROI technique described here should greatly facilitate addressing further detailed neuroanatomical questions about the components of the MTL due to the ability to detect small increases in activity in each component of the MTL and the ability to quantitatively compare the levels of evoked activity in each area.

Although one of the benefits of using the anatomical method is the ability to compare levels of evoked activity across brain regions, this should be done cautiously. Since fMRI relies on a BOLD-dependent activity signal derived from blood flow, estimates of levels of activity may be affected by characteristics of the vasculature that vary across brain regions. Because of the potential differences in sensitivity across areas due to vasculature (noncognitive) effects, comparisons across ROI should attempt to focus on areas in which there are not expected to major changes in the neural response/BOLD signal coupling. For example, comparisons across hemispheres may be considered to be relatively unaffected by these concerns. Likewise, comparisons between the anterior and posterior parts of a structure like the hippocampus should be robust. However, while it is interesting to note the larger increases in activity in the PHC compared with the hippocampus, there could be vascular or neurophysiological differences that affect the interpretation of BOLD changes. It should not be concluded the PHC is the most important area in picture encoding, as there is no way to rule out the hypothesis that the small increase in the hippocampus is absolutely critical for successful memory formation. Comparisons of activity evoked in the parahippocampal cortex across studies (e.g., comparing picture and word encoding or deep and shallow or successful and unsuccessful encoding) are more promising as methods to elucidate the specific role of the PHC in the encoding of novel memories.

Other methods of comparing activity across anatomical areas are subject to the same concerns about physiological (noncognitive) contributions to the BOLD response. In addition, it should be noted that the method used here of estimating signal change within an ROI is likely to be more accurate than the technique of counting "activated voxels" in an ROI (e.g., Fernandez et al., 1998). Although the results of both methods will likely be highly correlated, the definition of an "activated voxel" depends critically on setting an activation threshold and might underestimate the levels of activity in an ROI containing many weakly activated voxels. The technique used here aggregates across all voxels in an ROI and will capture the overall activation pattern of an anatomically defined area. Using anatomical landmarks to define the area is an improvement as well over using functionally defined ROI as the anatomical landmarks are not drawn from the functional data and thus provide no complications relating to statistical inter-dependency between defining the ROI and assessing activity within the ROI.

CONCLUSIONS

Using high-resolution anatomical images, regions of interest were defined within the MTL to provide a quantitative estimate of the level of increased activity in each anatomical component of the MTL during picture and word encoding. During picture encoding, increased activity was observed throughout the MTL, particularly the hippocampus and parahippocampal cortex. Less increased MTL activity was found for the word-encoding task, although the left posterior hippocampus and left parahippocampal cortex exhibited reliably increased activity. Laterality effects depending on the modality of the stimuli were found to be consistent with previous reports, with right-sided activity greater than left in parahippocampal cortex during picture encoding and left-sided activity being greater than right in posterior hippocampus and parahippocampal cortex during word encoding. The methodology permitted specific assessment of the relative response of the anterior and posterior hippocampus during encoding and no evidence was found to indicate specialization of the posterior hippocampus during encoding. The data do suggest a possible anterior-posterior distinction within the cortical areas adjacent to the hippocampus as the parahippocampal cortex exhibits a robust encoding response. These results demonstrate the use and value of a highly anatomically constrained approach to the analysis of fMRI data that will likely be invaluable in further studies of functional specialization within the MTL memory system.

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