

# Level of Sustained Entorhinal Activity at Study Correlates With Subsequent Cued-Recall Performance: A Functional Magnetic Resonance Imaging Study With High Acquisition Rate

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**ABSTRACT:** Functional magnetic resonance imaging (fMRI) with high acquisition rate was performed during the intentional memorizing of words to specify which medial temporal lobe structure is important in determining what words are subsequently remembered in a cued-recall test and to characterize the time course of activation in that structure. Functional images of six healthy young subjects were analyzed by two subject- and voxel-wise statistics: First, to identify brain areas transiently engaged in encoding of words, brain activity during memorizing visually presented words and watching a fixation cross was compared by a Kolmogorov-Smirnov statistic (KS-test). Second, to identify brain areas whose activity correlates with memory encoding success, a Kendall's correlation was calculated between signal intensity at study and performance in a subsequent cued-recall test. Averaged signal intensities were plotted as a function of time to depict the time course of brain activity detected by both statistical tests. The level of slowly modulated, sustained activity in Brodmann area 28 (entorhinal cortex) did not respond transiently as study words appeared, but did correlate positively with subsequent test performance. More left than right activity in Brodmann area 45 (dorso-lateral prefrontal cortex) and bilateral activity in Brodmann area 44 (premotor cortex) exhibited transient hemodynamic responses that did not show any relation to subsequent memory performance. Thus, the study identified a novel pattern of slowly modulated brain activity in human entorhinal cortex that may represent a declarative memory encoding state whose level predicts whether experiences will be remembered or forgotten. *Hippocampus* 1999;9:35–44. © 1999 Wiley-Liss, Inc.

**KEY WORDS:** declarative memory; encoding; medial temporal lobe; functional neuroimaging; human

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## INTRODUCTION

Evidence that the medial temporal lobe is essential for declarative memory came initially from the pervasive and severe amnesia that occurs in humans and animals after bilateral damage to that brain region (Scoville and Milner, 1957; Squire and Zola-Morgan, 1991). Functional neuroimaging studies have now confirmed the importance of the medial temporal lobe in normal declarative memory processing. For example, studies contrasting encoding of novel relative to familiar stimuli found consistently a medial temporal lobe activation (Tulving et al., 1994, 1996; Grady et al., 1995; Haxby et al., 1996; Stern et al., 1996; Gabrieli et al., 1997; Dolan and Fletcher, 1997; Rombouts et al., 1997). Improved spatial resolution with functional magnetic resonance imaging (fMRI) revealed that this activation is centered within the posterior parahippocampal cortex (Stern et al., 1996; Gabrieli et al., 1997; Rombouts et al., 1997). Moreover, the improved temporal resolution of single-trial fMRI allowed assessment of transient, event-related encoding activity in the same posterior parahippocampal area that predicted whether experiences are remembered or forgotten independently of item novelty (Brewer et al., 1998; Wagner et al., 1998).

Imaging studies utilizing across- or within-subject correlations have also shown medial temporal lobe activity related to declarative memory encoding. Varying magnitudes of medial temporal lobe activity were correlated positively with encoding success measured as

subsequent memory performance (Grasby et al., 1993; Cahill et al., 1996; Alkire et al., 1998; Fernández et al., 1998). However, because correlations were based on signal intensity assessed across subjects or within subjects but with poor temporal resolution (15 s/set of images), they may not be based on the same kind of transient, rapidly changing activity as detected by event-related fMRI (Brewer et al., 1998; Wagner et al., 1998) or by designs that compare blocks of alternating conditions (e.g., encoding of novel items versus encoding of familiar items) (Stern et al., 1996; Gabrieli et al., 1997; Rombouts et al., 1997).

The basic mechanisms and processes that lead to stable declarative memory traces are not well understood yet. At a minimum, memory formation is the product of an interaction between stimulus features (e.g., novelty or distinctiveness), concomitant processes (e.g., semantic or associative processing), subject states (e.g., shifting levels of attention or motivation), and subject traits (e.g., stable factors of education, vocabulary knowledge). Experimental designs using a single-trial approach like event-related fMRI (Brewer et al., 1998; Wagner et al., 1998) are optimal for relating brain activity to subsequent memory performance on a stimulus-by-stimulus basis. Across subject designs (Cahill et al., 1996; Alkire et al., 1998; Grasby et al., 1993) are optimal for relating brain activity to some combination of traits and global states that extend throughout the experiment. These designs, however, would not be able to measure variations in mnemonic states that are longer than a single stimulus period and shorter than the whole experimental session. Nor can across-subject analyses discern whether variation in activation reflects stable trait differences or shifting state differences. A slow modulation of brain activity that influences mnemonic state can best be identified by relating brain activity of single subjects to subsequent memory performance over time periods longer than a single stimulus presentation but shorter than the entire experiment. Hence, in a previous fMRI study (Fernández et al., 1998) posterior hippocampal signal intensity acquired during memorizing five sequentially presented words correlated positively with the retrievability of these five words in a subsequent free recall test. However, the actual time course and, therefore, the nature of brain activity correlating with encoding success has not been characterized yet due to poor temporal resolution or across-subject designs.

Thus, the aim of the present study was to determine which medial temporal lobe structure is important in encoding words for a subsequent cued-recall test and to analyze the temporal dynamics of medial temporal lobe activity correlating with declarative memory encoding. Functional MRI with high acquisition rate was performed on young healthy subjects as they saw and intentionally studied individually presented words arranged in small sets of five words each. Two voxel- and subject-wise statistics were used to analyze the imaging data acquired at study. To identify brain areas transiently engaged in the encoding of words, the activity of an assumed active phase (encoding of five words) and an assumed baseline (watching a fixation cross during a short interval between the sets of five words) were compared. To detect areas correlating with encoding success, the mean signal during encoding of sets of five words was correlated with later memory for each set in a subsequent cued-recall test which occurred

following an intervening distraction task. Finally, to reveal the time course of brain activity detected by these two statistical tests, time series of averaged signal intensities operationalized by encoding success were plotted in a way similar to that done in event-related fMRI.

## MATERIALS AND METHODS

### Participants

Six healthy volunteers from the Stanford community (three females, three males, mean age: 24.2 years, range: 21–29) participated for pay. Each gave written informed consent, were right-handed, had normal vision, and had English as their first language.

### Materials

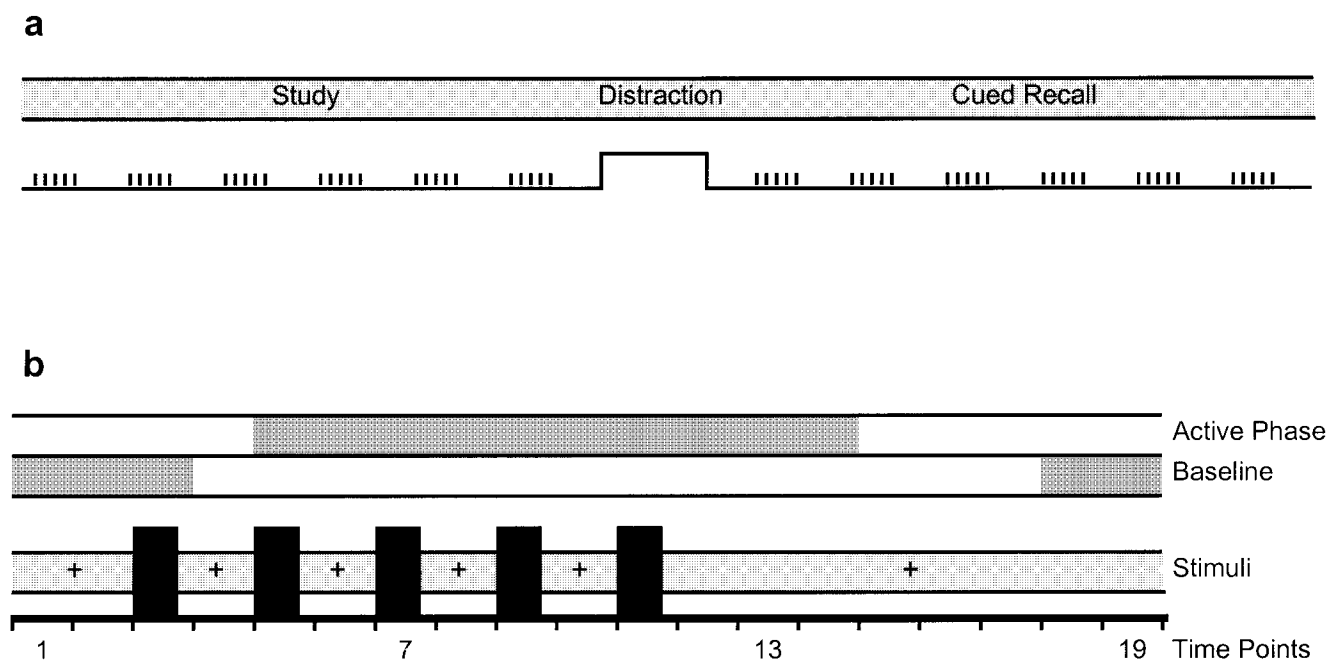
The study stimuli were 240 words with a mean frequency of 51 occurrences per million (Kucera and Francis, 1967), and they ranged from five to 11 letters in length. The test stimuli were three-letter word stems that were the first three letters of the study words. They were selected such that each stem was unique and could be completed by at least five different words. The study words were never the highest-frequency completion of the stem. Words were divided pseudorandomly into eight study lists of 30 words. Across study lists, word length was equated. The order of lists and of words across and within lists was counterbalanced across participants.

### Procedure

The experiment consisted of eight runs with each run comprising study, distraction, and cued-recall (Fig. 1a). During study, words appeared on screen for 0.88 s with a 2-s interstimulus interval (ISI). After the presentation of five words, a 12.96-s ISI occurred during which a central fixation cross was presented (Fig. 1b). Subjects were instructed to memorize the words without rehearsing or using elaborative strategies. After presentation of 30 words (one run), subjects were instructed to count backward by seven, starting at a number between 81 and 99 displayed on screen for 30 s. Following this distraction task, 30 three-letter word-stems of studied words were presented with identical duration and ISIs as during the study phase, but in a new random order. Subjects were required to complete aloud the word stems with studied words, or to say “pass” to those stems they could not complete with a studied word. Responses were recorded on audio tape using a pneumatic intercom.

### Image Acquisition

Eight contiguous slices (field of view: 20 cm, 7-mm slice thickness) were imaged perpendicular to the longitudinal axis of the hippocampus (Fig. 2c). The first slice was centered 7 mm anterior to the pes hippocampus. Such sets of eight functional



**FIGURE 1.** Experimental design. **a:** Depiction of one of eight experimental runs consisting of a study, distraction, and cued-recall task. Each bar represents one word (study) or one three-letter word stem (cued-recall). **b:** Zoomed in on one set of five words representing one trial. Each black bar represents one word. The light gray

horizontal bar represents the interstimulus interval during which a fixation cross was displayed. The dark gray horizontal bars depict the assumed active phase and the assumed baseline as used for statistical analysis. A set of eight images was acquired every 1.44 s, and 19 sets of these images were acquired for each trial.

images were acquired every 1.44 s using a T2\*-weighted gradient-echo spiral pulse sequence on a GE Signa 1.5-Tesla magnet (TR: 720 ms; TE: 40, flip angle: 80°, spatial resolution:  $2.41 \times 2.41 \times 7$  mm, two spiral interleaves, quadrature receive-only birdcage head coil) (Glover and Lai, 1998). Head motion was minimized using a fixed bite-bar formed to the subject's upper incisors, allowing the subject to respond orally. Residual motion was corrected using an automated registration algorithm (Woods et al., 1992).

### Image Analysis

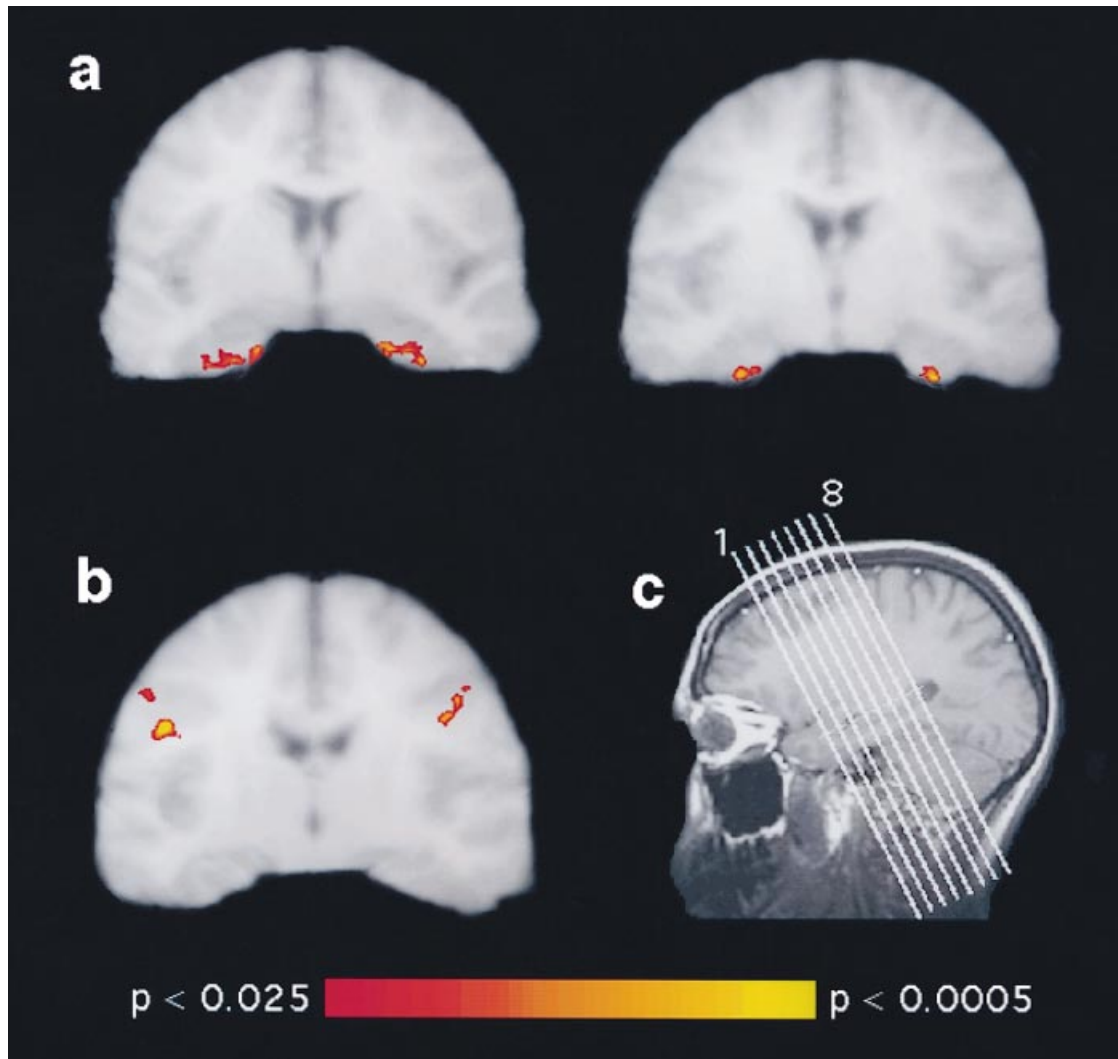
The data were normalized for each subject to correct for global changes in signal intensity by scaling the mean whole brain signal intensity of the baseline (see below) to a fixed value of 1,000. Linear drifts of the overall signal intensity occurring during a run were also removed. A variability coefficient filter (standard deviation/mean signal intensity = 0.1) was used to remove voxels at risk to be contaminated by artifacts.

Data were analyzed using averaged five-word trials. Each trial represented 19 time points beginning 2.88 s (2 time points) before the onset of the first word and lasting until 13.52 s after the offset of the fifth word of each five-word set (Fig. 1b). Forty-eight of these five-word trials were averaged for each subject. Two activation maps based on voxel-wise statistics were constructed separately for each subject. 1) A non-parametric Kolmogorov-Smirnov statistics (KS test) was performed by assigning individual time points in each trial as part of the "active phase" or "baseline." The first three and the last two time points of each trial were considered as the baseline, and the points 5 to 14 as the active

phase (Fig. 1b). (2) A voxel- and subject-wise Kendall's correlation was performed between the number of successfully encoded words in each particular set of five words, measured as the subsequent cued-recall performance, and the mean T2\*-signal intensity during the assumed active phase. To test the significance, the correlation coefficients were transformed into Z scores (Siegel and Castellan, 1988). Each voxel in the functional map whose *P* value exceeded the 95% level of significance ( $P < .05$ ) was coded.

The resulting matrices were processed with a median filter (spatial width: two voxels) and overlaid on corresponding anatomical scans. The anatomical localization of significant voxel clusters was determined using anatomical landmarks as described by Amaral and Insausti (1990), Insausti and colleagues (1998), and Jackson and Duncan (1996). To depict results most reliable across subjects, composite maps were made by normalizing each slice separately (Fig. 2). The Kolmogorov-Smirnov statistics composite map was generated by summing the chi-square distribution, and the Kendall's correlation composite map was calculated by averaging the Z scores.

To depict the time course of encoding related fMRI signal, the data were averaged across voxels within regions of interest (ROIs). To initially define the anatomical localization, the posterior part of the inferior frontal gyrus, the premotor cortex, and the entorhinal cortex were delineated separately for each subject according to anatomical landmarks (Amaral and Insausti, 1990; Jackson and Duncan, 1996; Insausti et al., 1998). Within each of these areas, all significant ( $P < .05$ ), median-filtered voxels of the Kolmogorov-Smirnov statistics (Brodmann area 44 and 45) or the Kendall's



**FIGURE 2.** Statistical composite maps ( $n = 6$ ). **a:** Maps (slice 3 and 4) based on the Kendall's correlation between the fMRI signal intensity and the number of subsequently recalled words. Reliable correlations occur bilaterally in the entorhinal cortex (slice 3 and 4) and lateral primary motor cortex (slice 7 and 8, not shown). **b:** Map (slice 5) based on the Kolmogorov-Smirnov statistics comparing the

fMRI signal during memorizing words and watching a fixation cross. Significant activations occur bilaterally in the Brodmann area 44 (premotor cortex) (slice 5) and Brodmann area 45 (posterior part of the inferior frontal gyrus) (slice 2, not shown). **c:** Depiction of the approximate slice position.

correlation (entorhinal cortex) were defined as belonging to respective ROIs. The graphs (Fig. 3) depict the average signal intensity sorted according to the subsequent memory performance.

## RESULTS

### Behavioral Results

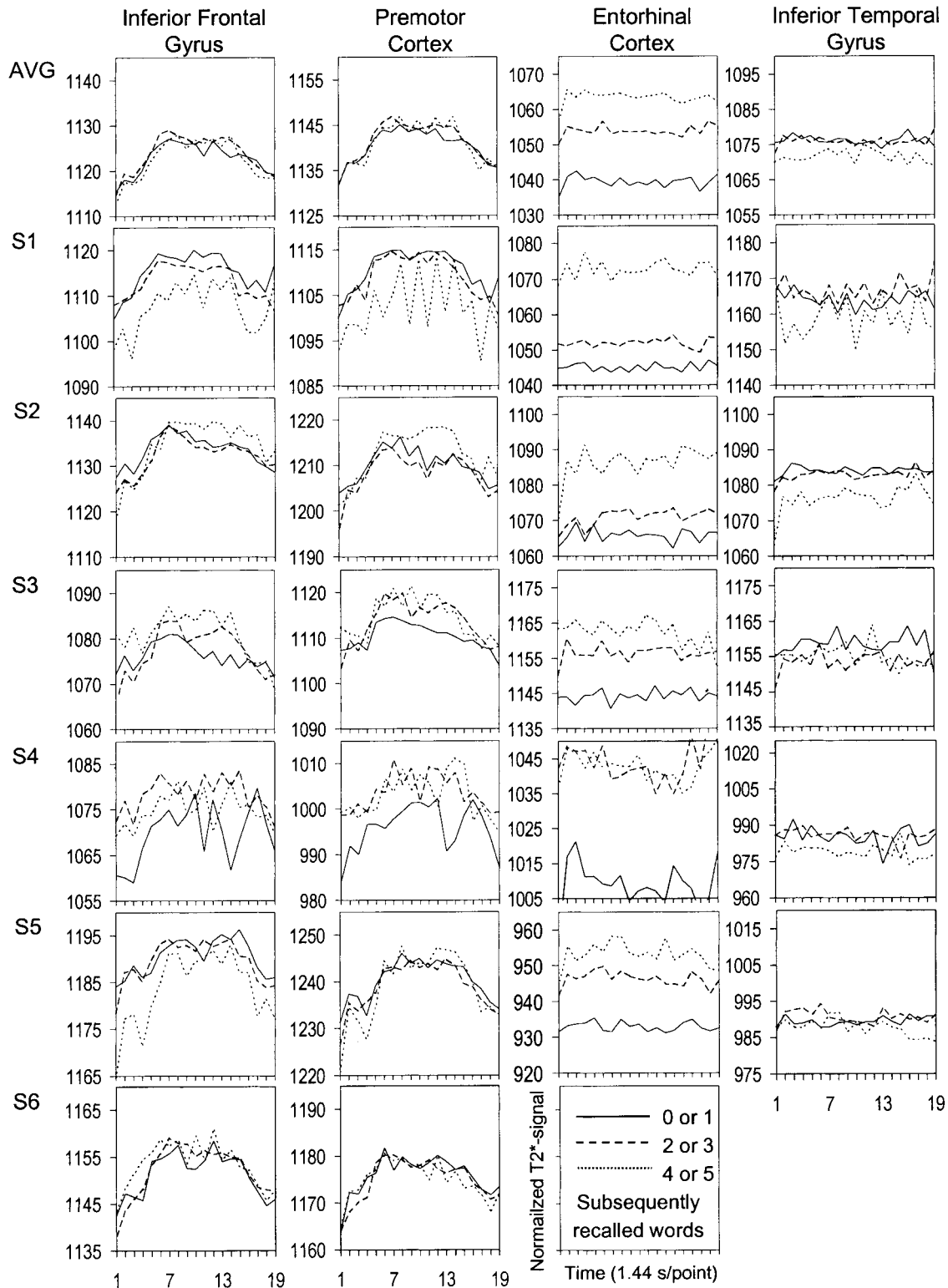
The mean rate of correctly completed stems was 45.8% (range: 36.7–62.5%). Mean recall rates were similar across runs

(range: 41.7–50.7%), and they did not vary significantly from the beginning to the end of the sessions ( $F(7, 35) < 1$ ).

### Imaging Results

#### *Kolmogorov-Smirnov statistic (KS test)*

Brain activity during memorizing visually presented words and watching a fixation cross (active phase versus baseline) was compared using a voxel- and subject-wise KS test. This comparison revealed in all subjects greater activity during word-study than fixation within the posterior part of the inferior frontal gyrus (left > right; Brodmann area 45) and within bilateral areas



**FIGURE 3.** Time course of fMRI signal intensity. Averaged time courses of the normalized T2\*-signal intensity within regions of interest and conditionalized according to the subsequent recall rates. Each column represents data from one anatomical region. The upper most row (AVG) depicts grand averages across subjects. The following

rows depict the data of each subject (S1-S6). Subject S6 did not exhibit an entorhinal correlation; thus, no plots of data from the temporal lobe are shown for this subject, and the respective grand averages are based on the data of the remaining five subjects (S1-S5).

anterior to the precentral gyrus representing basal aspects of the premotor cortex (Brodmann area 44) (Fig. 2b).

### **Kendall's Correlation**

A second voxel- and subject-wise analysis of the same data set, a Kendall's correlation between the mean fMRI signal intensity at study (active phase) and the number of subsequently recalled words, revealed in five out of six subjects clusters of voxels with a significant ( $P < .05$ ) positive correlation. This activation occurred bilaterally within the medial temporal lobe, in an area covering the entorhinal cortex (Fig. 2a; Brodmann area 28). These correlations were most pronounced in the posterior part of the entorhinal cortex, just anterior to the lateral geniculate nucleus. Three subjects exhibited additional voxel clusters with significant correlations in the entorhinal cortex at the level of and anterior to the amygdala. Thus, varying levels of entorhinal activity within individuals during study of specific sets of words predicted later memory for those words. Three out of six subjects exhibited additionally significant correlations bilaterally in the lateral and basal aspect of the primary motor cortex (Brodmann area 4), suggesting different amounts of subvocal rehearsal (Awh et al., 1996) for subsequently remembered and not remembered words in some subjects (Fernández et al., 1998).

### **Temporal Analyses**

To characterize the dynamic pattern of fMRI signal, time courses within significantly active voxels were plotted in relation to subsequent memory performance for each set of five words: low study success (zero or one word recalled), intermediate study success (two or three words recalled), and high study success (four or five words recalled). The hemodynamic response in the frontal structures (posterior part of the inferior frontal gyrus and inferior part of the premotor cortex) seemed to show an initial signal rise prior to the stimulus onset (Fig. 3, first and second column). However, these signal rises did not reach significance ( $P > .3$ , paired-sample *t*-tests). About 3 seconds following the first stimulus onset, an event-related signal rise occurred. This transient, event-related signal rise exhibited in some subjects individual responses to each single word (e.g., Fig. 3, subject S1, premotor cortex). The grand averages, however, showed in both frontal areas single hemodynamic responses to the whole set of five words, probably due to a latency jitter of the hemodynamic response across subjects. At the end of each word set, signal returned to baseline before the next event-related signal rise.

Activation in entorhinal cortex exhibited also an insignificant increase of signal intensity prior to stimulus presentation ( $P > .3$ , paired-sample *t*-tests). Thereafter, however, there was sustained activity in entorhinal cortex (Fig. 3, third column). The mean amplitudes of this sustained activity correlated positively with subsequent memory performance ( $F(2, 8) = 32.268$ ,  $P < .01$ ). As a control, an area of similar size was randomly selected in the inferior temporal gyrus near to the entorhinal area. This control area showed neither an event-related hemodynamic response pattern nor a correlation with study success (Fig. 3, fourth column).

## DISCUSSION

The level of sustained entorhinal activity at study correlated positively with later cued-recall for words. The entorhinal cortex is a key component of the medial temporal lobe memory system (e.g., Squire and Zola-Morgan, 1991; Mishkin et al., 1997). Input from distributed polymodal association areas and many paralimbic regions such as the parahippocampal or perirhinal cortex converge on the entorhinal cortex (e.g., Insausti et al., 1987; Amaral and Insausti, 1990; Suzuki and Amaral, 1994). The entorhinal cortex, in turn, provides the strongest direct cortical projection to the hippocampus via the perforant path terminating on the granule cells of the dentate gyrus (e.g., Insausti et al., 1987; Amaral and Insausti, 1990; Suzuki and Amaral, 1994). Despite its critical position within the medial temporal lobe memory system, no previous imaging study has reported specific entorhinal activations in relation to declarative memory encoding. Entorhinal ablations in rats and monkeys, however, lead to impairments in declarative memory performance almost as severe as ablations of the whole medial temporal lobe (Murray and Mishkin, 1986; Suzuki et al., 1993; Zola-Morgan et al., 1993; Meunier et al., 1993, 1996; Leonard et al., 1995). Furthermore, patients with Alzheimer's dementia show, at the beginning of their disease, a declarative memory impairment and a pronounced neuronal cell loss in entorhinal cortex (La Rue, 1992; Gomez-Isla et al., 1996; Fox et al., 1998; Juottonen et al., 1998). The lesion evidence, therefore, is in accord with the present result which indicates that entorhinal cortex plays an important role in human declarative memory formation. The result further suggests that entorhinal lesions impair declarative memory formation not only by disconnecting the hippocampus (Hyman et al., 1984; De Lacoste and White, 1993), but also by impairing intrinsic entorhinal processing.

The localization of the correlation within the medial temporal lobe, however, differs from findings in other neuroimaging studies correlating brain activity at study with subsequent memory performance (Alkire et al., 1998; Cahill et al., 1996; Fernández et al., 1998). The activity of the amygdala was correlated with encoding success of emotional information (Cahill et al., 1996), but there is evidence that the amygdala and hippocampal region participate in dissociable aspects of learning and memory (e.g., Bechara et al., 1995; Cahill et al., 1995). Activity of the posterior hippocampus proper has been correlated with later free recall performance of words (Alkire et al., 1998; Fernández et al., 1998). The reason for these different locations of encoding activation in closely interconnected structures (Mishkin et al., 1997, 1998) is uncertain. There is evidence that different aspects of declarative memory may depend dissociably on the hippocampus or parahippocampal structures (Eichenbaum et al., 1994, 1996; Mishkin et al., 1997, 1998; Vargha-Khadem et al., 1997; Tulving and Markowitsch, 1998). It is, however, hazardous to relate those dissociations to the differences between the present study and the Fernández et al. (1998) study. First, there is no clear-cut difference between the declarative memory processes subserving free and

cued recall (e.g., Paller, 1990). Second, the fMRI dissociation rests on two null findings—no hippocampal activation in the present study and no entorhinal activation in the previous study. Lack of activation is a particularly weak source of evidence in cognitive fMRI studies in which the signal-to-noise ratio is very small. This may be particularly true in the medial temporal lobe where many imaging studies have failed to observe reliable activations during declarative memory performance (e.g., Shallice et al., 1994).

Tracking the time course of activations provides new insights into dynamics of memory processing. The transitory, time-locked hemodynamic response to word presentation in prefrontal and premotor cortices is in agreement with previous studies (e.g., Kelley et al., 1998) and indicates the temporary involvement of these areas during encoding of sequentially presented words. Sets of words, independent of their subsequent retrievability, seem to have been processed to a similar degree and in the same frontal areas. The transient activation of the Brodmann area 45 (dorso-lateral prefrontal cortex), which was more pronounced on the left side, may indicate semantic working memory processes that participate during intentional encoding (Gabrieli et al., 1996). The bilateral activation of Brodmann area 44 (premotor cortex) may represent activity correlated with motor programming in subvocal rehearsal (Awh et al., 1996) or activity in the frontal eye field (Bodis-Wollner et al., 1997), because more saccades can be assumed during word reading than during fixation.

In contrast to the frontal activations, entorhinal activity did not respond transiently as study words were presented. The sustained level of activation suggests that the entorhinal contribution to later successful memory was based on an encoding state that was independent of word properties. This encoding state, as measured by fMRI, appeared to be determined prior to the presentation of the first word in each study set and maintained throughout the five-word study set. Such an event-unrelated pattern of cortical activity may partially explain previous failures to activate the medial temporal lobe in functional neuroimaging studies comparing experimental conditions known to induce high and low subsequent recall rates, such as contrasting semantic and perceptual encoding or verb generation and reading (Petersen et al., 1988; Frith et al., 1991; Démonet et al., 1992; Kapur et al., 1994; Raichle et al., 1994; Fletcher et al., 1995; but see Wagner et al., 1998). This slowly modulated encoding state may also explain within- as well as across-subject correlations between medial temporal lobe activity and encoding success found previously (Grasby et al., 1993; Cahill et al., 1996; Fernández et al., 1998). In particular, the fact that the correlation found by Fernández et al. (1998) was almost insensitive to shifts of the assignment between study word lists and acquired signal by 0, 3, or 6 seconds clearly indicates that those correlations were also based on slowly modulated shifts of brain activity and not on rapidly changing, transient activations.

In contrast to studies utilizing event-related fMRI (Brewer et al., 1998; Wagner et al., 1998), we identified neither transient responses in the medial temporal lobe nor differential prefrontal activations for subsequently well- and less-well-remembered word sets. Although we detected transient hemodynamic responses in frontal areas, the present paradigm was probably not powerful

enough to detect transient medial temporal or frontal activity in relation to subsequent memory due to a much smaller number of trials (e.g., 480 single-word epochs in the study of Wagner et al. (1998) versus 48 five-word epochs in the present study). Moreover, averaging across sets of words may dilute single stimulus effects. That is, the cost of measuring longer time periods is a loss of stimulus-specific effects. Nevertheless, Kelley and collaborators (1998) were able to find a posterior parahippocampal activation in an fMRI study comparing experimental blocks of memorizing visually presented words and watching a fixation cross. This activation, however, seems to be more posteriorly located than that revealed by Wagner and colleagues (1998), thus that area might not be covered by the imaging planes used here.

Although we failed to find transient medial temporal lobe responses, we were able to detect reliably a modulation of sustained activity that powerfully correlated with subsequent cued-recall performance. The relationship, however, between slowly modulated T2\* signal shifts, neuronal activity, and potential artifacts is currently not well defined (Biswal et al., 1995; Purdon and Weisskoff, 1998). An artifact as a main cause for the correlation identified here, however, seems unlikely, because we found a correlation between brain activity and encoding success consistently across subjects (Fig. 3), and almost exclusively within an anatomically well-defined structure of the medial temporal lobe memory system (Fig. 2). Furthermore, we found this correlation after correction for global changes in signal intensity (normalization), removing linear drifts of the overall signal intensity, excluding pixels at higher risk to be contaminated by artifacts (standard deviation filter), and emphasizing spatially coherent patterns of activity (median filter). Thus, two different patterns of medial temporal lobe activity correlating with encoding success may coexist: 1) a transient, event-related activity detectable only by powerful averaging procedures (Brewer et al., 1998; Wagner et al., 1998); and 2) a slowly modulated, sustained activity detectable only by paradigms analyzing shifts of activity over tens of seconds or even minutes. Event-related fMRI studies would not have detected such long-lasting shifts of brain activity.

There is a discrepancy between PET and fMRI findings regarding encoding-related medial temporal lobe activations. Whereas PET studies show anterior and posterior medial temporal activations, fMRI studies reveal predominantly posterior medial temporal activations during encoding (for metaanalyses, see Lepage et al., 1998; Schacter and Wagner, 1999). No good explanation has been found yet for this discrepancy. Susceptibility artifacts in fMRI acquired in anterior medial temporal structures are unlikely to account for the failure to detect anterior medial temporal activations by fMRI (Schacter and Wagner, 1999). Different task demands, such as relational and non-relational encoding, may account for the difference in anterior versus posterior encoding activity (Schacter and Wagner, 1999). Our results may indicate an additional explanation for the discrepancy between PET and fMRI findings. Temporal resolution in PET is generally poorer than in fMRI. Longer scans with poorer temporal resolution may be more liable to measure slowly modulated rather than event-related activity.

Sustained hippocampal activity was previously found in an fMRI study of verbal working memory (Kato et al., 1998). In contrast to our findings, hippocampal activity rose initially with stimulus onset and returned to baseline several seconds after the end of a working memory task. Similar to the fMRI finding by Kato and colleagues (1998), a pattern of sustained entorhinal activity responding to stimulus onset (sometimes with a delay) and correlating with duration of maintained working memory was found in single-cell recordings in monkeys (Suzuki et al., 1997) and rats (Eichenbaum et al., 1996; Young et al., 1997). The present results may, therefore, reflect working memory or attentional influences at study that determine subsequent memory.

A plausible neuronal mechanism for such sustained, attentionally driven, entorhinal activity is cholinergic modulation from the basal forebrain. Cholinergic afferents constitute the single most substantial regulatory pathway of the cerebral cortex (Mesulam et al., 1992). Furthermore, the hippocampus, the amygdala, and the entorhinal cortex have the highest density of cholinergic innervation within the human brain, suggesting that cholinergic neurotransmission is likely to have a particularly strong influence upon these structures (Mesulam et al., 1992; De Lacalle et al., 1994). Pharmacological and lesion studies have shown that disruption of cholinergic function interferes with declarative memory formation in animals and humans (Ridley et al., 1986; Nissen et al., 1987; Mizumori et al., 1992; Hasselmo et al., 1996; Tang et al., 1997; Abe et al., 1998).

The vast majority of cholinergic afferents of the medial temporal lobe arise from the nucleus basalis of Meynert in the basal forebrain which is a telencephalic extension of the brainstem reticular formation regulating attention, arousal, and sleep (Lewis and Shute, 1967; Mesulam et al., 1992; De Lacalle et al., 1994). In the entorhinal cortex, the cholinergic afferents terminate primarily in those neuronal layers that gate the main hippocampal input and output (Dickson and Alonso, 1997). The major effect of acetylcholine on the neuronal level is to cause a reduction of potassium conductance so as to make cholinergic neurons more susceptible to other excitatory inputs (McCormick, 1990; Metherate et al., 1992; Mrzljak et al., 1993; Albuquerque et al., 1997). Importantly, neuromodulatory effects of acetylcholine are slow acting (Fehlau et al., 1998; Hasselmo and Linster, in press), and thus are well suited to control such slowly modulated, sustained activity as identified here.

In conclusion, we identified a pattern of human brain activity previously not delineated by functional neuroimaging techniques. It is a slowly modulated, sustained activity at study correlating positively with performance in a subsequent cued-recall test. This result verifies the importance of the entorhinal cortex in human declarative memory formation and indicates that a slowly modulated encoding state in entorhinal cortex predicts whether experiences will be remembered or forgotten.

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