

Working Memory-Related Hippocampal Deactivation Interferes with Long-Term Memory Formation

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Previous findings indicate that the hippocampus does not only play a role in long-term memory (LTM) encoding, but is important for working memory (WM) as well, in particular when multiple items are being processed. A recent study showed that maintenance of multiple items was associated with hippocampal activation (hippocampus-dependent WM), while maintenance of individual items induced hippocampal deactivations (hippocampus-independent WM). Here, we used two complimentary approaches to investigate whether WM-related activity patterns occur also during LTM encoding of sequentially presented items and whether they are related to the primacy effect, i.e., improved LTM encoding of items presented at the beginning of the list. Intracranial electroencephalogram in epilepsy patients and functional MRI in healthy subjects were recorded during a word-list learning task. As expected, the proportion of successfully encoded items was higher at the beginning of the list than at later list positions. Items at the beginning of the list which were subsequently forgotten were associated with negative blood oxygen level dependent responses and positive direct current slopes, corresponding to hippocampal deactivations, suggesting that they were not processed in hippocampus-dependent WM. These deactivations were absent for items later in the list and for subsequently remembered items. These data show that if processing of items at the beginning of the list is accompanied by hippocampal activity patterns previously observed during hippocampus-dependent WM, these items are subsequently remembered. However, deactivations of the hippocampus as previously observed during WM maintenance of individual items predicts failure of LTM encoding.

Key words: declarative memory; working memory; long-term memory; intracranial EEG; fMRI; hippocampus

Introduction

Stimuli which are presented at the beginning or end of a sequence have a higher chance for long-term memory (LTM) encoding than items at intermediate positions. These serial position effects are known as “primacy” and “recency” effects. It has been proposed that the primacy effect is due to the fact that items at early list positions undergo more extensive rehearsal than items presented later (Rundus, 1971; Brodie and Murdock, 1977). This idea suggests a link between LTM formation and rehearsal processes, which are attributed to working memory (WM) (Baddeley, 1992). A possible locus of interaction was found in the medial temporal lobe (MTL), because this region is not only important for LTM encoding, but also supports WM maintenance for novel items (Ranganath and D’Esposito, 2001; Stern et al., 2001; Nichols et al., 2006) or associations between item features (Hannula et al., 2006; Olson et al., 2006; Piekema et al., 2006). To explore the neural signature underlying multiitem WM in the MTL, we recently conducted a Sternberg paradigm (Sternberg, 1975) with serially presented items in a combined intracranial electroen-

cephalogram (iEEG) and functional MRI (fMRI) study (Axmacher et al., 2007). We found evidence for load-dependent effects on hippocampal blood-oxygen level dependent (BOLD) activity and medial temporal direct current (DC) potentials during successive presentation of multiple items. Our data suggest that WM for individual items is related to a hippocampal deactivation (hippocampus-independent WM), and that this deactivation is reduced during WM for multiple items (hippocampus-dependent WM).

Activity changes in the MTL during maintenance of an item in WM should have an impact on LTM encoding, which depends on the same region. Indeed, several fMRI studies showed that WM maintenance facilitates encoding of these items into LTM by activating rehearsal processes in the hippocampus (Ranganath et al., 2005) or in the parahippocampal cortex (Schon et al., 2004). This facilitation of LTM encoding by WM maintenance may account for the primacy effect, because initial items in a list can be maintained with little interference until presentation of later items. However, it is still an open question which forms of WM support memory for these items. Here, we directly addressed this issue by reanalyzing iEEG data from a LTM study with lists of 12 serially presented words (Fernández et al., 1999) as well as new fMRI data using the same paradigm (Fig. 1). From our previous findings, we hypothesized that presentation of items early in the list should induce DC potentials with a positive slope and BOLD deactivations in the hippocampus, while subsequent items should be associated with increasingly negative DC slopes and

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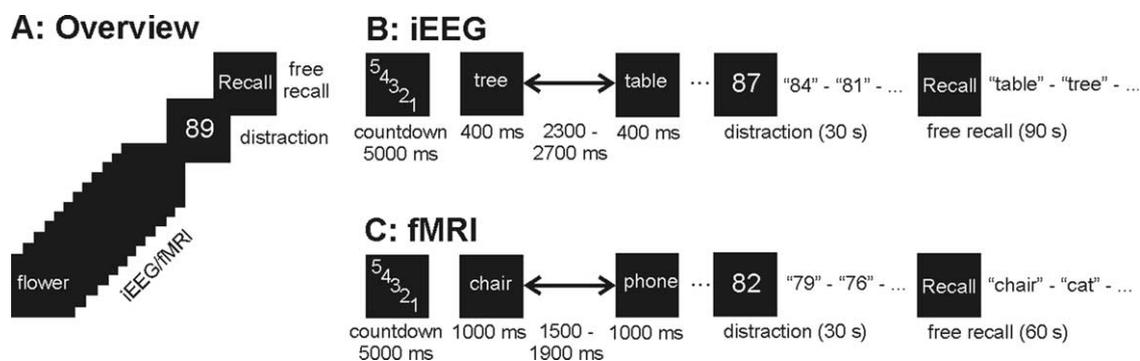


Figure 1. *A*, Overview of the paradigm. After presentation of 12 consecutive words, a number between 80 and 90 was presented and subjects had to count backward by three aloud. After 30 s of this distraction task, there was a free recall phase in which subjects loudly pronounced all words they remembered. *B*, *C*, Timing of the paradigm in the iEEG (*B*) and fMRI (*C*) version of the experiment.

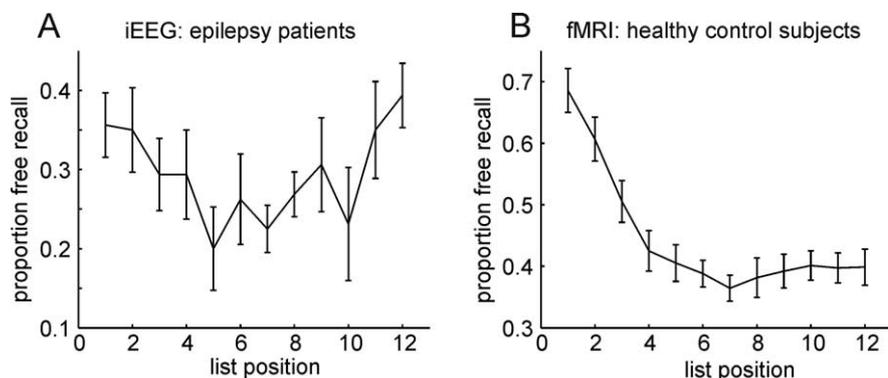


Figure 2. Recall performance as a function of list position. *A*, Primacy effect in the group of healthy control subjects which were investigated with fMRI. *B*, Primacy and recency effects in the group of epilepsy patients with intracranial EEG electrodes.

increased BOLD activity. We wondered whether these changes of DC potentials depended on subsequent memory as well, so that multiitem (hippocampus-dependent) WM may explain differential processing of subsequently remembered and forgotten items.

Materials and Methods

Subjects

iEEG. Eight epilepsy patients (5 women; mean age \pm SD: 34.1 \pm 8.3 years) participated in the iEEG study. In all patients, unilateral hippocampal sclerosis was confirmed histologically. Recordings were performed at the Department of Epileptology, University of Bonn, Germany. All patients had bilateral hippocampal depth electrodes. No seizure occurred within 24 h before the experiment. Only data from the hemisphere contralateral to the seizure onset zone were considered for analysis.

fMRI. Nineteen healthy subjects (5 women; mean age \pm SD: 25.3 \pm 3.2 years) participated in the fMRI study. They were recruited from the University of Bonn as well as via newspaper.

The study was approved by the local medical ethics committee, and all subjects and patients gave written informed consent.

Experimental paradigm

We will first describe the paradigm in the iEEG version and then mention the differences in the fMRI version. In the iEEG version (Fig. 1*B*), 20 blocks with 12 words in each block were presented. Each block started with a countdown of 5000 ms length, during which the numbers “5” to “1” were presented (each for 1 s). Afterward, words were presented in uppercase letters for a duration of 400 ms. Interstimulus intervals between all words were randomized and ranged from 2.3 s to 2.7 s (mean 2.5 s). During the intertrial interval, a fixation cross was presented. Word length ranged from 4 to 11 (mean 6) and word frequency ranged from 15 to 175 per million (mean 75 per million). Words were selected consecutively from the CELEX database of German words (Baayen et al., 1993).

While the same words were used for all subjects through the experiment, both the order of lists (containing 12 words) and the order of words within each list was completely randomized in each subject. All subjects were required to use a rote strategy to memorize each word avoiding memory aids such as making rows, sentences, stories or pictures. In other words, this strategy only involved subvocal repetitions of the words. This was explained thoroughly to all subjects; during debriefing, all subjects assured not to have used any elaborate strategy. During the distraction task they were instructed to count backward by threes, starting at a number between 80 and 90 displayed on screen. Recall was initiated directly after the distraction phase by presenting the German word “Abruf” (retrieval). Upon presentation of this word, subjects were instructed to stop counting immediately and say loudly, in arbitrary order, all words from the previous phase that they could recall. The duration of the recall phase was 90 s. Each word which was recalled by the subject was marked by the experimenter on a prepared form.

In the fMRI version (Fig. 1*C*), 40 blocks (also with 12 words in each block) were presented. Stimuli were presented for 1000 ms using goggles (which required longer presentation times). Interstimulus intervals ranged from 1.5 s to 1.9 s (mean 1.7 s). The duration of the recall phase was 60 s. This period was thus 30 s shorter than for the iEEG subjects to limit the overall length of the paradigm. Despite this shorter recall phase, the number of words recalled by the fMRI subjects was significantly higher than the number of words recalled by the patients (Fig. 2). An overview of the paradigm in the two versions is provided in Figure 1.

Recordings and analyses

Statistical analyses were performed using SPSS (SPSS), and *p* values in the ANOVAs were Huynh–Feldt corrected for inhomogeneities of covariance when necessary (Huynh and Feldt, 1976). For the *post hoc t* tests in the behavioral data, values of *p* < 0.05 were considered significant. We tested both effects of position and linear effects of position. Effects of position are effects of ANOVAs with the different positions entered as repeated measures, whereas linear effects of positions are calculated as follows: For each subject, we fitted a linear regression line on the DC slopes (or on BOLD responses, respectively) for consecutive positions. The slopes of these linear regression lines were then compared with 0 using two-tailed paired *t* tests. The results are identical to the “test of within subject contrast” in SPSS and indicate whether across the group of subjects, linear effects across consecutive positions went in the same direction (i.e., were either all positive or negative). In other words, effects of position tested the null-hypothesis that slopes of DC potentials (or BOLD responses, respectively) from a number of different positions are identical (i.e., if the first three positions entered the ANOVA, that there is

no difference between positions 1, 2 and 3). In contrast, linear effects of positions tested the null-hypothesis that slopes of DC potentials (or BOLD responses, respectively) at consecutive positions do not show a consistent linear change (i.e., that they change in the positive direction for some subjects and in a negative direction for other subjects). In other words, the test for linear effects of position makes the a priori assumption that if there are effects across positions, these should be linear.

iEEG. Multicontact depth electrodes were inserted for diagnostic purposes using a computed tomography-based stereotactic insertion technique (Van Roost et al., 1998). The location of electrode contacts was ascertained by postimplantation MRI in each patient. Depth EEG was referenced to linked mastoids, recorded at a sampling rate of 173 Hz (12-bit analog-digital conversion), and bandpass-filtered (0.03 to 85 Hz, 6 dB/octave). EEG trials were visually inspected for artifacts (e.g., epileptiform spikes), and 4.9% of all trials were excluded from analysis. From the contralateral (nonfocal) electrode in each patient, we took into account the hippocampal electrode contact with the maximum late positive component (same contacts as in Fernández et al., 1999). Long-lasting (DC) shifts of the EEG were analyzed by calculating the slope of the linear regression line to the event-related potentials (from 0 to 2000 ms) averaged across trials. Data were analyzed using the EEGLAB package created by Delorme and Makeig (2004) running with MATLAB (Mathworks) as well as by our own MATLAB routines.

fMRI. Thirty-six axial slices were collected at 3T (Trio, Siemens). We collected 18 T2*-weighted, gradient-echo echo planar imaging scans (slice thickness: 2 mm; interslice gap: 1.0 mm; matrix size: 128 × 128; field of view: 230 mm; echo time: 33 ms; repetition time: 2700 ms). Preprocessing was done using FSL software (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) and the following steps were performed: (1) Realignment with three-dimensional motion correction. (2) Normalization onto the MNI-atlas (Montreal Neurological Institute). (3) Spatial smoothing with an 8 mm Gaussian kernel (full-width at half-maximum). (4) Modeling of the expected hemodynamic responses (box-car regressor in a general linear model) and convolution of the regressors with a canonical hemodynamic response function to represent brain physiology. We used regressors of 1 s length triggered to the presentation of each word. The following set of regressors was used: Three regressors for the first, second, and third subsequently remembered word; three regressors for the first, second, and third subsequently forgotten word; one common regressor for all words later in the list which were subsequently remembered; and one common regressor for all words later in the list which were subsequently forgotten (resulting in a total of eight regressors). Parameter estimates from a general linear model are invalid if regressors are collinear, i.e., if there is a strong degree of correlation between adjacent vectors. Because we used a rapid event-related design, it may be suspected that the precondition of regressor orthogonality is violated in our case. We calculated the correlation between the model regressors for first versus second; first versus third; and second versus third item, both for subsequently remembered and subsequently forgotten items. We found that all collinearity values (measured as the cosine of the difference between the angles of the vector pairs) were smaller than 0.19 (with 0 indicating orthogonality and 1 indicating collinearity), corresponding to angles larger than 79° (with 90° corresponding to orthogonality and 0° corresponding to collinearity). These low collinearity values strongly suggest that our general linear model is valid. In addition, we calculated a second general linear model where the first seven remembered and forgotten items were modeled with separate regressors, while the remaining five remembered and forgotten items were modeled with two further regressors. This model contained 16 regressors: seven regressors for the first to seventh remembered item, seven regressors for the first to seventh forgotten item, one common regressor for subsequently remembered items presented later in the list, and one common regressor for subsequently forgotten items presented later in the list. (5) Temporal filtering of the acquired time-series to reduce high- and low-frequency noise attributable to scanner drifts and physiological noise. The subsequent steps were conducted using SPM2 (www.fil.ion.ucl.ac.uk/spm/); (6) Calculation of parameter estimates for each condition covariate from the least mean squares fit of the model to the data. (7) Random-effects group analyses with subject as the random factor were performed with

SPM2 on each regressor by entering the t-contrast images of each subject corresponding to a particular regressor into a second-level one-sample *t* test. (8) Definition of contrasts. We investigated serial position effects separately for subsequently remembered and forgotten words by using the following contrasts: (1) increasing activation for subsequently remembered words: $\text{word\#1}_{\text{rem}} = -1/\text{word\#3}_{\text{rem}} = +1$; (2) increasing activation for subsequently forgotten words: $\text{word\#1}_{\text{forg}} = -1/\text{word\#3}_{\text{forg}} = +1$. All figures with fMRI results are displayed using neurological convention (left hemisphere on the left side of the figure). To identify significant activations, we used an uncorrected threshold of $p < 0.001$ and a minimum cluster size of 5 contiguous voxels. Time courses for each subject were extracted for anatomically selected regions in the left and right hippocampus (selected via PickAtlas software) (Maldjian et al., 2003) using the Marsbar extension of SPM (Brett et al., 2002) and event-locked peristimulus time histograms were constructed.

Results

Behavioral data

First, we calculated whether long-term memory depended significantly on serial position (Fig. 2). A two-way ANOVA with “position” as repeated measure and “group” (epilepsy patient or fMRI subject) as independent variable revealed significant main effects of position ($F_{(11,275)} = 11.652$; $p < 10^{-12}$; $\epsilon = 0.710$) and group ($F_{(1,25)} = 14.309$; $p < 0.001$) and a significant position × group interaction ($F_{(11,275)} = 4.818$; $p < 10^{-4}$; $\epsilon = 0.710$). We thus conducted separate analyses for the epilepsy patients and the fMRI subjects.

In the group of epilepsy patients, a one-way ANOVA with position as repeated measure revealed a significant effect (Fig. 2A) ($F_{(11,77)} = 2.839$; $p < 0.01$). *Post hoc* comparisons showed that recognition performance of the first item was indeed superior to that of items at the fifth, sixth, and seventh position (primacy effect). Recognition performance of the second and third items differed only significantly from recognition of the fifth item. At the end of the list, there was a significant difference in recognition of the 11th and fifth item and between the 12th and fifth, sixth, seventh, and eighth item (recency effect). In the group of fMRI subjects, a one-way ANOVA with position as repeated measure indicated a significant effect as well (Fig. 2B) ($F_{(11,198)} = 21.156$; $p < 10^{-16}$; $\epsilon = 0.588$). *Post hoc* comparisons showed that recognition performance of the first three items was superior to recognition performance of items at all other positions (primacy effect). No recency effects at the end of the list became apparent.

Next, we wondered whether trials are remembered and forgotten in sequences, i.e., whether successful encoding of items at the beginning of the list predicts encoding of consecutive words. We compared the conditional probability that the second item was successfully encoded given that the first item was successfully encoded with the unconditioned probability that the second item was successfully encoded. In the same way, we compared the conditional probability that the third item was successfully encoded given that the first and second item were successfully encoded with the unconditioned probability that the third item was successfully encoded. For the iEEG subjects, encoding success at the second position was indeed slightly higher if the first item was successfully encoded: While overall probability for LTM encoding at the second list position was 0.35, it increased to 0.51 if only trials where the first word was already successfully encoded were taken into account (conditional probability of encoding the second item given that the first item was encoded). For the third item, the overall encoding probability of 0.29 increased to 0.37 if only trials were taken into account where the first item was successfully encoded. It further increased to 0.41 if only trials where the first and second item were successfully encoded were taken

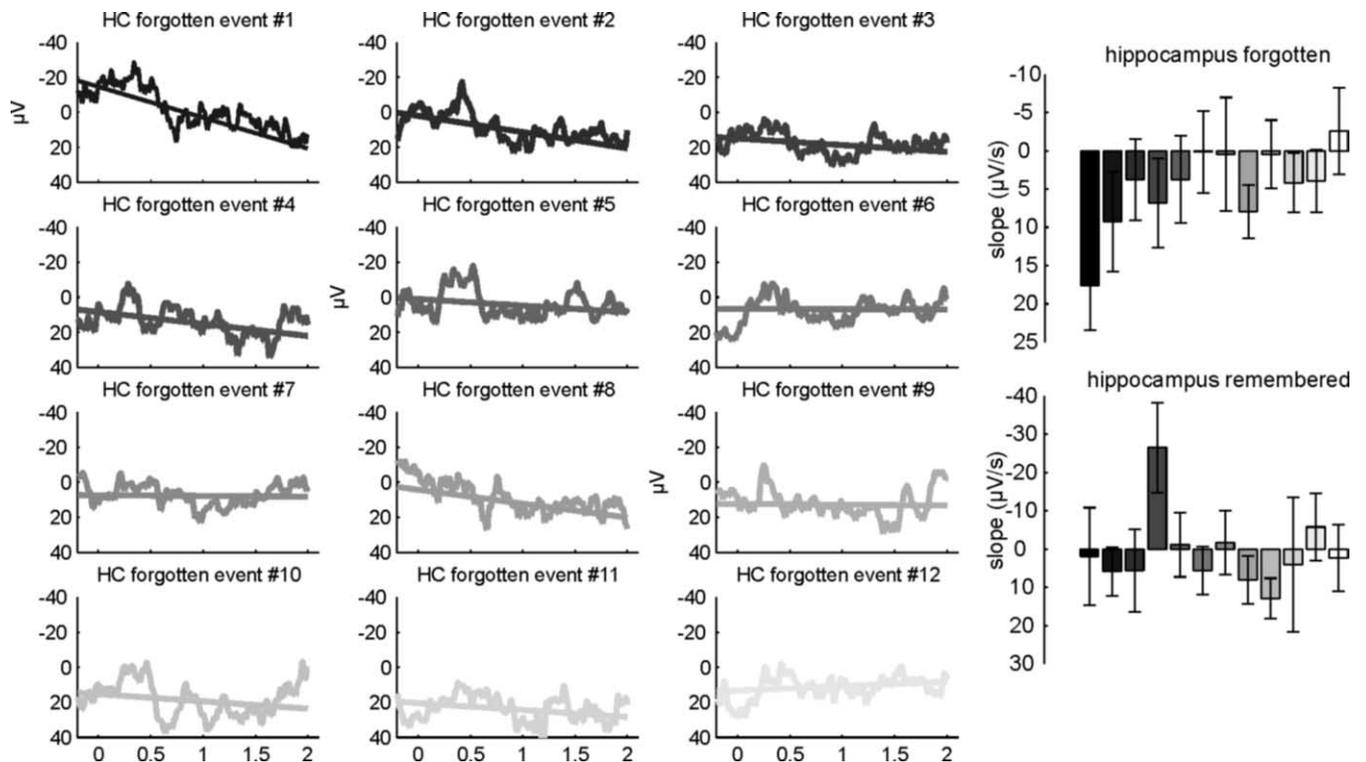


Figure 3. Position-dependent shifts of DC potentials. The slope of the DC potential in the hippocampus depends on list position for subsequently forgotten items, but not for subsequently remembered items.

into account. Similar results were obtained for the fMRI subjects: Overall probability for LTM encoding at the second list position was 0.61 and increased to 0.70 if only trials with correct encoding of the first word were taken into account. For the third item, the overall encoding probability of 0.51 increased to 0.56 if only trials were taken into account where the first item was successfully encoded. It further increased to 0.60 if only trials where both first and second item were successfully encoded were taken into account. Together, these results suggest that in successful trials, items at the beginning of the list were processed in a sequence.

Intracranial EEG data

We calculated the slopes of the DC potentials as a function of the items' list positions (Fig. 3). Based on our previous findings using a Sternberg paradigm with serial presentation of items (Axmacher et al., 2007), we predicted that the DC potentials during presentations of successive words would turn to more negative values at later list positions. More specifically, we expected positive DC slopes for initial items (hippocampus-independent WM) and more negative slopes for successive items (hippocampus-dependent WM). We compared the slopes of the DC potentials across positions with the zero-hypothesis of no significant shift of the slopes by calculating the linear regression of the slopes across list positions. Linear regressions of DC slopes across position 1–5 were calculated, where a monotonic decrease in recognition performance was apparent in our behavioral analysis (Fig. 2A). A two-way ANOVA with “memory” and position as repeated measures revealed a significant interaction between the factor memory and a linear effect of position ($F_{(1,7)} = 10.464$; $p < 0.05$). We thus performed separate one-way ANOVAs of subsequently remembered and forgotten items and found a significant linear effect of position on the DC slopes of subsequently forgotten items ($F_{(1,7)} = 8.130$; $p < 0.05$), but not of subsequently remembered items ($F_{(1,7)} = 1.170$; $p > 0.3$). For subsequently forgotten

items, DC potentials had a positive slope at early list positions, which was reduced later in the list. To test whether the positive slopes observed at early list positions actually corresponded to deviations from zero, we calculated two-tailed t tests between the slopes at each list position and zero for later forgotten items. This analysis revealed a significant difference at the first position ($t_{(7)} = 3.026$; $p < 0.05$), but not at any other position.

Finally, we compared DC shifts during encoding of subsequently remembered and subsequently forgotten items separately for each list position. There was a significant difference only at the fourth list position ($t_{(7)} = 2.55$; $p < 0.05$), but not at any other position (all $t < 1.51$, all $p > 0.17$). Possibly, this lack of an effect is related to the reduced statistical power for position-specific analyses of subsequent memory effects.

Functional MRI data

Based on our predictions of serial position effects in the hippocampus during presentation of items early in the list, we compared time courses of BOLD activity in anatomically defined regions of interest in bilateral hippocampus for different list positions (Fig. 4). These regions were taken from PickAtlas (Maldjian et al., 2003) and are centered at the MNI coordinates 30/–15/–18 (right) and –30/–15/–18 (left) (Fig. 4A). For subsequently forgotten items, we observed a deactivation during presentation of the first item which was reduced during presentation of items presented later in the list (Fig. 4B). These results are consistent with our iEEG findings because positive DC shifts likely correspond to deactivations (Birbaumer et al., 1990; Rösler et al., 1997; Speckmann and Elger, 1999; Axmacher et al., 2007). For subsequently remembered items, this deactivation was not apparent (Fig. 4C), again in line with our iEEG results. To quantify these results, we conducted two-way ANOVAs with memory and position (first three items, according to the behavioral data) as re-

peated measures at the peak of the BOLD responses (averaged values at 6 and 7 s).

The analysis of BOLD responses in the left hippocampus revealed results similar to our iEEG data: We observed a trend for a memory \times position interaction ($F_{(2,36)} = 3.169$; $p = 0.054$) and a significant interaction between memory and a linear effect of position ($F_{(1,18)} = 5.785$; $p < 0.05$). We thus analyzed subsequently remembered and forgotten items separately. For subsequently remembered items, there was no effect of position ($F_{(2,36)} = 0.331$; $p = 0.682$; $\epsilon = 0.833$). For subsequently forgotten items, however, we found a trend for an effect of position ($F_{(2,36)} = 2.943$; $p = 0.070$; $\epsilon = 0.922$) and for a linear effect of position ($F_{(1,18)} = 4.249$; $p = 0.054$), again consistent with our iEEG data. For the right hippocampus, a three-way ANOVA did not reveal an effect of, or interaction with, the factor position (all $p > 0.3$), but a significant main effect of memory ($F_{(1,18)} = 8.898$; $p < 0.01$).

Finally, we investigated on a whole-brain level regions showing increased activation during the first three list positions (Fig. 5). We found that for both subsequently remembered and subsequently forgotten items, activity was most pronounced in the anterior cingulate cortex (remembered: MNI $-3/39/15$; forgotten: MNI $-3/36/15$), a region commonly related to conflict processing. These effects did not survive correction for multiple comparisons, but did have a large extension of 60 voxels for the serial position contrast of subsequently forgotten words and even 204 voxels for the serial position contrast of subsequently remembered items. Because of this large extension, activations were significant at the cluster level ($p < 0.05$ for subsequently forgotten items and $p < 0.001$ for subsequently remembered items). In addition, activation was found in bilateral frontal regions and in the bilateral parahippocampal gyrus; a full list of all significantly activated regions is provided in Table 1.

The fMRI results reported so far are from a general linear model where only the first three subsequently remembered and forgotten items were modeled with separate regressors, based on the behavioral position effects at the beginning of the list. Although collinearity between these regressors was low (see Materials and Methods), it might be argued that BOLD activity during presentation of these items is difficult to tear apart in fMRI because of the relatively rapid item presentation. Therefore, we calculated a second general linear model which allowed to compare activity at the first and seventh list position (in this model, activities during presentation of the first seven subsequently remembered and forgotten items were modeled with separate regressors). Figure 6 depicts position effects as a function of subsequent memory. The results are very similar to the results from the previous general linear model (where only the first three positions were taken into account): In the same anatomically defined region of interest in the left hippocampus used

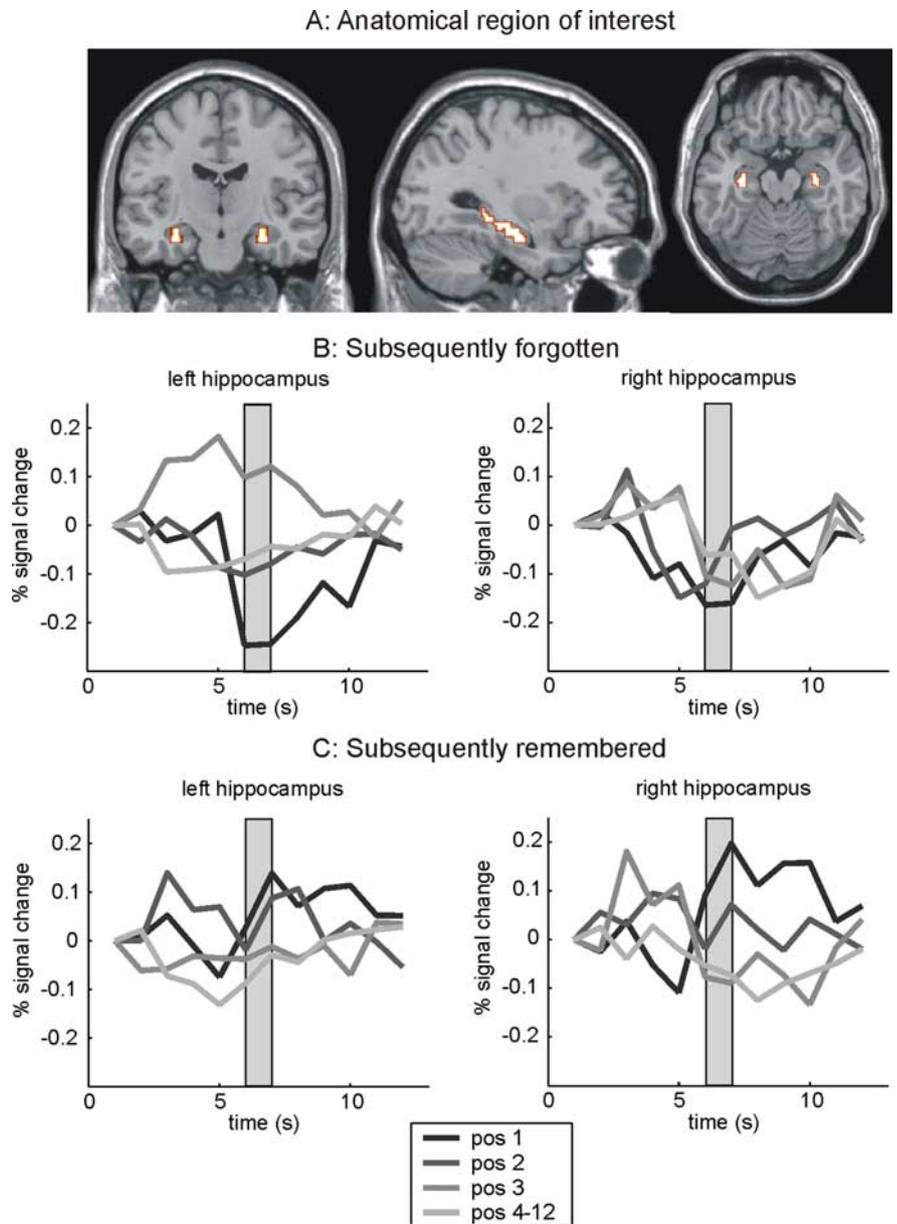


Figure 4. Position-dependent BOLD responses in the hippocampus. Time course of BOLD activity in anatomically defined regions of interest in the bilateral hippocampus (**A**). **B**, For subsequently forgotten items, there was a deactivation during presentation of the first item, which was reduced for later list positions. **C**, For subsequently remembered items, no deactivation was apparent. The time period selected for statistical analysis is marked in light gray.

in the first general linear model, we observed a negative BOLD response for subsequently forgotten items presented at the first position, which was absent for items presented at the seventh position (Fig. 6A) ($F_{(1,18)} = 4.746$; $p < 0.05$). In contrast, for subsequently remembered items, no deactivations were visible neither at the first nor at the seventh position, and BOLD responses did not differ (Fig. 6B) ($F_{(1,18)} = 0.109$; $p = 0.746$). No effects of position were observed in the right hippocampus, in contrast (subsequently forgotten items: $F_{(1,18)} = 0.696$; $p = 0.415$; subsequently remembered items: $F_{(1,18)} = 0.186$; $p = 0.671$). On the whole-brain level, results were also similar to the findings from the previous general linear model. The contrast between activity at the seventh position and activity at the first position yielded significant activation in the anterior cingulate cortex both for subsequently remembered (MNI $-6/30/24$) (Fig. 6B) and subsequently forgotten items (MNI $-3/33/15$) (Fig. 6A). These

position effects (position 3 > position 1)

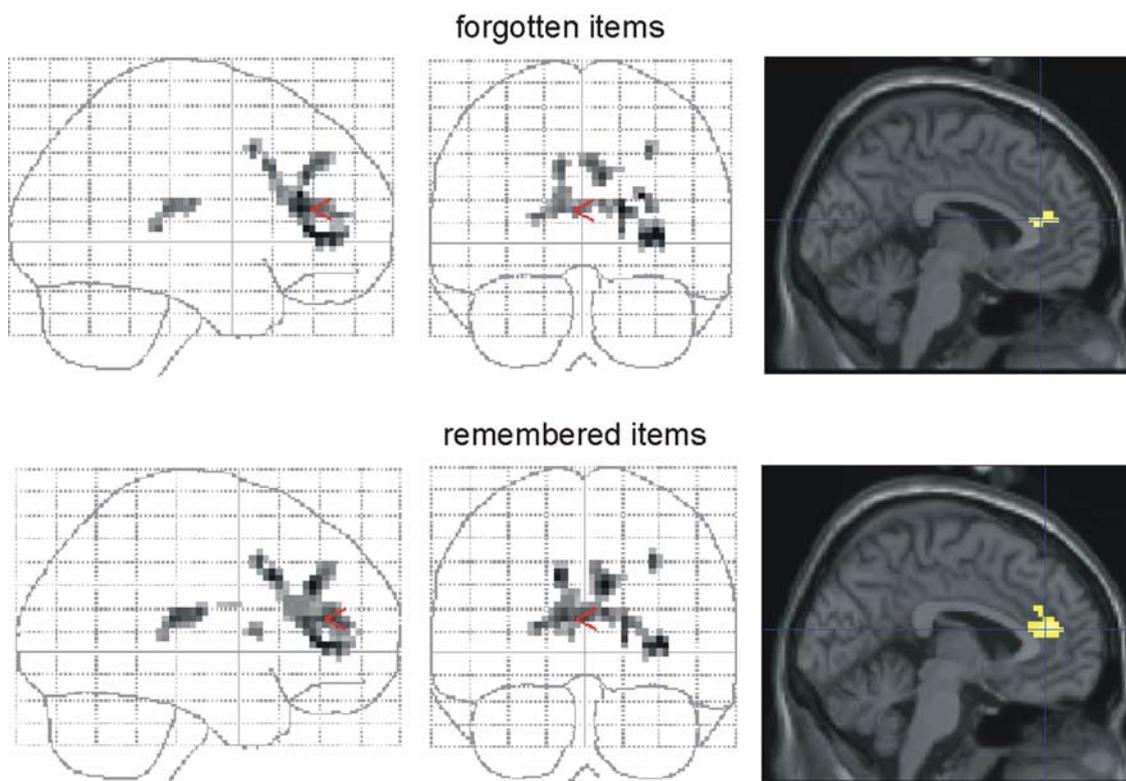


Figure 5. Position effects in the anterior cingulate cortex. For both subsequently forgotten and remembered items, we observed an increased activation of the anterior cingulate cortex (remembered: MNI $-3/39/15$; forgotten: MNI $-3/36/15$).

Table 1. Increased BOLD activity at early list positions

	L/R	t value	MNI coordinates		
			x	y	z
Incorrect 3 > 1					
Anterior cingulate cortex	R	4.231	18	30	15
Medial frontal gyrus	R	4.205	27	30	21
Inferior frontal gyrus	R	4.186	33	39	3
Cingulate gyrus	R	3.901	9	33	30
Middle frontal gyrus	R	3.772	30	9	42
Cingulate gyrus	L	3.749	-9	18	33
Parahippocampal gyrus	R	3.665	30	-33	6
Medial frontal gyrus	L	3.664	-21	48	12
Thalamus	L	3.656	-9	-30	15
Thalamus	R	3.632	9	-18	18
Correct 3 > 1					
Cingulate gyrus	R	4.314	9	33	30
Inferior frontal gyrus	R	4.241	33	39	3
Thalamus	L	3.981	-9	-30	15
Thalamus	R	3.912	9	-18	18
Middle frontal gyrus	R	3.885	30	9	42
Anterior cingulate cortex	L	3.866	-21	45	12
Caudate nucleus	L	3.616	-6	9	9
Parahippocampal gyrus	R	3.478	30	-33	6
Caudate nucleus	L	3.271	-9	-3	21

All regions showing significantly increased activation for presentation at the third as compared to the first position are indicated. L, Left; R, right.

clusters of activation even survived correction for multiple comparisons (false discovery rate $p < 0.05$).

Discussion

We used a word list learning paradigm to study whether neural activity patterns previously observed during a WM task occurred

during a LTM paradigm with successive presentation of items. Our iEEG and fMRI data showed deactivation during presentation of the first and incremental activation during presentation of consecutive words if these words were subsequently forgotten, but no serial position effects for remembered items. The anterior cingulate cortex was increasingly activated during presentation of subsequent items at the beginning of the list.

Neural activity related to WM during an LTM-encoding task

We analyzed the slope of DC potentials during presentation of words as a function of subsequent memory. Previously, we had used a Sternberg paradigm with trial-unique unknown faces as stimuli to investigate WM processes in the MTL (Axmacher et al., 2007). We observed a positive DC slope during maintenance of a single face and during presentation of the first face in trials with four faces, and a reversal

toward a negative shift during maintenance of multiple faces or during presentation of subsequent faces in trials with four faces. Importantly, this pattern was only observed in correct Sternberg trials, suggesting that it was indeed relevant for WM maintenance. These results indicated that the MTL acts as a multiitem WM buffer for the transient storage of novel information, con-

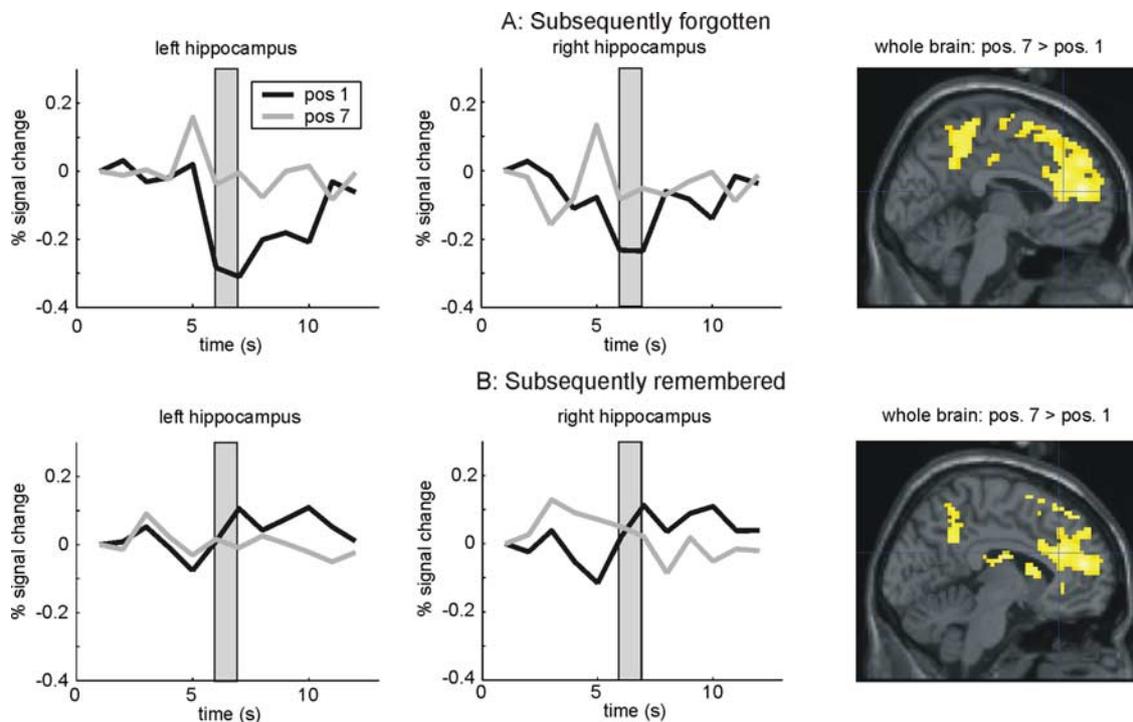


Figure 6. Alternative model: position effects in the hippocampus and cingulate cortex. Contrast of activity during presentation of the seventh and first item in anatomically selected regions of interests in bilateral hippocampus (left and middle column) and anterior cingulate cortex (right column). For subsequently forgotten items presented at the first list position, there was a negative BOLD response in the left hippocampus, which was absent at the seventh list position (**A**, left column). No negative BOLD responses occurred in the right hippocampus (**A**, middle column) and for subsequently remembered items (**B**, left and middle column). On the whole-brain level, there was an increased activation in the anterior cingulate cortex at the seventh as compared with the first list position regardless of subsequent memory (right column).

sistent with its role during associative LTM encoding (Cohen and Eichenbaum, 1993). Speculatively, these findings may be related to changes of persistent activity described in animals both *in vitro* (Egorov et al., 2002) and *in vivo* (Suzuki et al., 1997; Young et al., 1997): Using electrophysiological recordings in rat brain slices from the entorhinal cortex, Egorov et al. (2002) found persistent activity in individual neurons after stimulation. This persistent activity resembles the activity patterns during WM tasks in that region (Suzuki et al., 1997; Young et al., 1997) and may also underlie the DC potentials described in our previous study (Axmacher et al., 2007) as well as in the current study. Therefore, the increase of persistent activity after consecutive stimulations *in vitro* is functionally similar to the activity during presentation of a list of items in a WM task. It should be noted, though, that this analogy between electrophysiological data *in vitro* and the neural patterns during WM maintenance must remain speculative at the current stage.

The results presented here suggest that LTM encoding of items at the beginning of a list is also affected by these WM-related activity patterns. In particular, we observed negative BOLD responses and positive slopes of DC potentials in the hippocampus for subsequently forgotten items at the initial positions (Figs. 3, 4B). This pattern is similar to our previous findings during WM encoding and maintenance of single items (Axmacher et al., 2007). It is likely that it represents a form of WM which is independent of the hippocampus, because several studies have shown that single-item WM does not rely on the hippocampus. For instance, patients with medial temporal lesions are typically unimpaired in delayed matching to sample tasks with single items (Cave and Squire, 1992). Our present findings demonstrate that hippocampus-independent WM at early list-positions is not beneficial for

LTM and results in subsequent forgetting. In contrast, subsequently remembered items at the initial positions were associated with more negative DC potentials and with a reduction of negative BOLD responses, which resembles the activity pattern observed during WM maintenance of multiple items in our previous study. This activity pattern likely reflects hippocampal activation corresponding to a hippocampus-dependent form of WM (Axmacher et al., 2007). Recruitment of hippocampus-dependent WM at early list positions thus actually supports LTM formation. However, also subsequently forgotten items at later list positions exhibited more negative DC potentials and a reduction of negative BOLD responses indicating hippocampus-dependent WM. Based on the model of Atkinson and Shiffrin (1968), we suggest that items later in the list are not rehearsed as much as the items earlier in the list (explaining the primacy effect) and that later items interfere with previous words. Thus, items later in the list do not receive the benefit of hippocampus-dependent activity during WM and in this case the related activity pattern is not predictive for LTM recall.

Relationship to previous studies on WM–LTM interactions

Recently, Khader et al. (2007) analyzed slow waves (similar to our DC potentials) in scalp EEG recordings to investigate the interaction of WM and LTM formation. Material-specific negative slow waves were observed during maintenance, which were more pronounced for subsequently remembered as opposed to forgotten items. These results suggest that activation of item representations by WM maintenance facilitates LTM encoding; importantly, only individual items were investigated in that study. We are not aware of any studies on slow waves/DC potentials in the hippocampus during memory pro-

cessing (apart from our own findings in Axmacher et al., 2007). Most likely, however, negative potentials in the hippocampus also reflect activation, and positive potentials reflect deactivation (Birbaumer et al., 1990; Rösler et al., 1997; Speckmann and Elger, 1999). The deactivation associated with processing of subsequently forgotten items at early list positions (Fig. 3) might therefore correspond to a condition which is unfavorable for LTM encoding.

Apart from these results on DC potentials, a facilitation of LTM encoding by WM maintenance was also observed in MEG and fMRI studies. Using MEG, Jokisch and Jensen (2007) observed material-specific increases of gamma band activity in visual association areas during WM maintenance; this activity was predictive of subsequent memory for these items (Osipova et al., 2006). Several fMRI studies found that activation of the hippocampus (Ranganath et al., 2005) and parahippocampal cortex (Schon et al., 2004) during WM maintenance was predictive of subsequent memory. These studies used relatively simple delayed-matching to sample paradigms in which subjects reached ceiling performance. Therefore, the effect of unsuccessful attempts to execute WM processes on LTM encoding could not be investigated. Using a WM task with a complex item manipulation, we recently found that parahippocampal activity predicted LTM formation only if the WM manipulation was executed successfully, whereas it was detrimental for LTM formation in incorrectly solved WM trials (Axmacher et al., 2008). This indicates that medial temporal regions may also be activated by unsuccessful attempts to perform a WM task, but that this activity actually deteriorates LTM formation. Similarly, the results of the current study show that activity related to WM processing interferes with LTM encoding if it is associated with hippocampal deactivation.

It should be noted that there is no behavioral monitoring of WM performance in the current experiment. Therefore, we do not know for sure how LTM is actually affected by the WM performance for specific items, i.e., it is unknown which items are actually maintained in WM. This is a problem which can only be addressed in tasks with both a WM and a LTM test (Schon et al., 2004; Ranganath et al., 2005; Axmacher et al., 2008). It would be interesting to extend these studies to lists with several items, although this would require a complex experimental design: To identify subsequent memory effects, items need to be presented individually. However, the investigation of the beneficial effect of longer rehearsal times of items at the beginning of the list on LTM encoding would require to test maintenance of all previously presented items repeatedly. In the current study, strictly speaking we can only refer to “iEEG activity patterns similar to those described during a WM task.” However, it is likely that WM processes actually did occur during the word-list learning task: The dependence of subsequent memory on DC potentials at early list positions probably reflects the primacy effect. The best-established account of the primacy effect is that it actually reflects WM processes (Atkinson and Shiffrin, 1968). This is consistent with our data (hippocampus-independent WM for subsequently forgotten items; hippocampus-dependent WM for subsequently remembered items). Thus, the primacy effect observed in the behavioral data (Fig. 2) might be interpreted as an indirect measure of WM processes in the current study. For further discussion on the primacy effect as well as other issues, please refer to the supplemental material (available at www.jneurosci.org).

Together, our data suggest that hippocampus-dependent pro-

cesses previously observed during multiitem WM occur in a LTM task with sequential presentation of items as well and are associated with successful encoding into LTM. However, failure to recruit hippocampus-dependent WM processes at early list positions leads to failure of LTM formation. These findings complement previous results indicating that WM maintenance of individual items facilitates LTM encoding by suggesting that only hippocampus-dependent WM is beneficial for LTM encoding.

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