

## Rhinal–hippocampal coupling during declarative memory formation: Dependence on item characteristics

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### Abstract

Lesion and imaging studies have demonstrated that encoding and retrieval of declarative memories, i.e. consciously accessible events and facts, depend on operations within the rhinal cortex and the hippocampus, two substructures of the medial temporal lobe. Analysis of intracranially recorded EEG in presurgical epilepsy patients revealed that successful memory formation is accompanied within one second by a transient enhancement and later decrease of Rhinal–hippocampal phase synchronization in the gamma range, as well as enhanced connectivity in the low-frequency range. In these studies, words with a high frequency of occurrence were used as stimulus material. Here, we re-examined these effects in another group of 10 presurgical epilepsy patients, this time not only for high-frequency, but also for low-frequency words. For successfully memorized compared to later forgotten high-frequency words we again observed an early phase coupling and later decoupling within the gamma range, as well as enhanced coupling within the sub-gamma range. However, for remembered as compared to forgotten low-frequency words clear synchronization increases were only observed for the delta band, but not for the gamma band. Our data suggest, that broadband Rhinal–hippocampal coupling including the gamma range only occurs, when significant semantic associations are processed within rhinal cortex, as is the case for high-frequency words.

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Declarative memory enables us to remember past events and facts [33]. Lesion and neuroimaging data suggest that the medial temporal lobe (MTL) plays a crucial role in declarative memory, especially during the initial steps of memory formation [4,14,15,29,32]. Besides an activity increase of MTL structures, the connectivity between sub-regions appears to be a central factor supporting successful memory encoding. A recent fMRI study, for instance, has revealed an enhanced functional connectivity between the hippocampus and the perirhinal cortex (among other regions) after presentation of line drawings of complex objects, which were later remembered, compared to those, which were forgotten [25].

An important complementary approach to the investigation of declarative memory is the analysis of EEG data that are intracranially recorded from patients with MTL epilepsies dur-

ing presurgical evaluation. In a word memorization paradigm with subsequent free recall, memory formation was found to be associated with the sequential activation of the rhinal cortex and the hippocampus [14]. Analysis of oscillatory processes within the gamma range indicated that successful memory formation is accompanied by an initial stimulus-related increase of phase synchronization between rhinal cortex and hippocampus (time range between 100 and 600 ms) and a later decrease (between 1000 and 1100 ms) [12]. The transient increase and decrease of Rhinal–hippocampal synchronization was interpreted to initiate and later terminate information transfer between both structures [6]. Phase coupling within the gamma range appeared to be escorted by increased low-frequency EEG coherence between rhinal and hippocampal recordings [11]. In these studies only common words, i.e. words with a high frequency of occurrence were used. Thus, it remained an open question, whether Rhinal–hippocampal coupling during memory formation may depend on item characteristics, such as word frequency.

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High-frequency (common) words, as opposed to low-frequency (uncommon) words, have richer semantic contexts [19] and are more meaningful [23], hence give rise to more associative responses with shorter response times [7,23]. This relationship between word frequency and semantic associative processing probably causes the often-observed free recall advantage of high-frequency words [19]. In a prior study high-frequency words led to memory-related ERP effects in both, rhinal cortex and hippocampus, whereas low-frequency words were only associated with a memory-related difference within the hippocampus [17]. This outcome was interpreted to validate the hypothesis that the rhinal cortex participates in semantic operations which indirectly support memory formation, while the hippocampus is directly responsible for the initial steps of memory formation, which are insensitive to item characteristics [5,8,16,22]. Since in case of memorization of low-frequency words fewer associations are processed by the rhinal cortex compared to high-frequency words, one may argue, that much less information has to be transferred from rhinal cortex to the hippocampus. Thus, we wondered whether memory-related Rhinal–hippocampal phase coupling might depend on word frequency and thereby the amount of associative information transferred.

To answer this question, EEG was recorded (sampling rate: 173 Hz; bandpass-filter: 0.03–85 Hz, 6 db/octave; reference: linked mastoids) from 10 patients (four women, mean age  $32.9 \pm 12.6$  years) with pharmacoresistant temporal lobe epilepsy while they performed a single-trial word list-learning paradigm with a free recall test. Multicontact depth electrodes had been implanted stereotactically along the longitudinal axis of each MTL during presurgical evaluation [30], because the zone of seizure onset could not be determined unequivocally by noninvasive investigations. The placement of electrode contacts within the hippocampus and the anterior parahippocampal gyrus, which is covered by rhinal cortex [1], were ascertained by magnetic resonance images in each patient [20]. All patients had a unilateral seizure origin within one MTL based on the analysis of seizure activity in the depth recordings (six patients–right; four patients–left). Only EEG recordings from the MTL contralateral to the zone of seizure origin were analyzed to reduce poorly controllable effects introduced by the epileptic process [9,24]. Experiments were conducted with adequate understanding and written consent of the subjects and in accordance with the Declaration of Helsinki.

Each patient participated in 20 study test blocks each containing nine semantically unrelated German nouns. Of the 180 nouns, 90 had a high frequency of occurrence (mean frequency:  $415.2 \pm 80.1$ /million [3], mean word length  $5.98 \pm 1.16$  letters) and 90 had a low-frequency (mean frequency:  $4.1 \pm 1.0$ /million, mean word length:  $6.13 \pm 1.19$  letters). The order of words was pseudorandomized across subjects under the constraints that each list of nine words contained four or five high-frequency words intermixed with four or five low-frequency words. Word length was balanced between lists, and neither semantic nor phonological similarities occurred within lists. During study, patients were instructed to memorize each word presented sequentially on a computer monitor. To prevent ongo-

ing rehearsal, a distraction task was conducted after each study list (counting backwards in threes starting from a given number between 81 and 99). Thereafter, patients were asked to recall freely the previously displayed words in any order. There was a trend for high-frequency words to be better recalled than low-frequency words (mean recall rates  $39.67 \pm 12.74\%$  versus  $29.89 \pm 10.12\%$ ; paired two-tailed *T*-test:  $t_9 = 2.23$ ;  $p = 0.053$ ). Two training blocks were conducted immediately before the experiment to ensure that each patient had understood the task.

To compare successful and unsuccessful memory encoding, EEG was separated offline into segments for subsequently recalled and unrecalled study items. EEG was then subjected to a continuous complex wavelet transform (Morlet wavelets with 5 cycles length) in the frequency range from 2 to 48 Hz (2 Hz steps). Phases and phase differences between rhinal cortex and hippocampus were extracted for each trial and time point [12,21,27]. Then, Rhinal–hippocampal phase synchronization was quantified by an entropy measure [13]. The higher the synchronization value for a certain time point, the more stable are the phase differences between the two electrodes over all trials of the respective class (remembered/forgotten, low-frequency/high-frequency words). Finally, synchronization values were averaged for consecutive 100 ms time windows from  $-200$  to 1500 ms relative to stimulus onset. Values were divided through the baseline values for the interval  $[-200;0$  ms] and were transformed into percentage change relative to baseline.

For statistical analysis synchronization values were averaged for each subject within the following frequency bands: delta (2 Hz), theta (4–6 Hz), alpha (8–12 Hz), lower beta (14–20 Hz), upper beta (22–30 Hz) and gamma (32–48 Hz). The group average showed that the synchronization difference between remembered and forgotten words in the delta/theta/alpha range is similar for high- and low-frequency words, but differs markedly in the beta and gamma range (see Figs. 1 and 2). Therefore, we performed four-way ANOVAs with word frequency (HF/LF), subsequent recall outcome (MEMORY), time window (TIME) and frequency BAND as repeated measures for both frequency ranges. For the lower bands, we found a main effect of MEMORY ( $p < 0.05$ ;  $F_{1,9} = 7.38$ ) expressing increased synchronization for remembered compared to forgotten words, as well as a BAND\*MEMORY interaction ( $p < 0.01$ ;  $F_{2,18} = 9.02$ ; Huynh–Feldt  $\epsilon = 0.88$ ). This interaction was traced back to a main effect of MEMORY in the delta band ( $p < 0.01$ ;  $F_{1,9} = 13.25$ ), together with a lack of significant effects in the theta or alpha band. No significant HF/LF\*MEMORY ( $p = 0.94$ ;  $F_{1,9} = 0.01$ ) or BAND\*HF/LF\*MEMORY interactions ( $p = 0.54$ ;  $F_{2,18} = 0.57$ ;  $\epsilon = 0.80$ ) were observed indicating that the subsequent memory effect was independent of word frequency in the delta/theta/alpha range.

In contrast, we found a significant HF/LF\*MEMORY interaction ( $p < 0.05$ ;  $F_{1,9} = 9.02$ ) for the beta and gamma range showing that the subsequent memory effect depends on word frequency for the faster oscillations. This effect was not accompanied by a significant BAND\*HF/LF\*MEMORY interaction ( $p = 0.16$ ;  $F_{2,18} = 2.00$ ;  $\epsilon = 1.20$ ). The HF/LF\*MEMORY interaction results from a trend for an increased synchronization for remembered vs. forgotten high-frequency words ( $p = 0.051$ ;  $F_{1,9} = 5.06$ ),

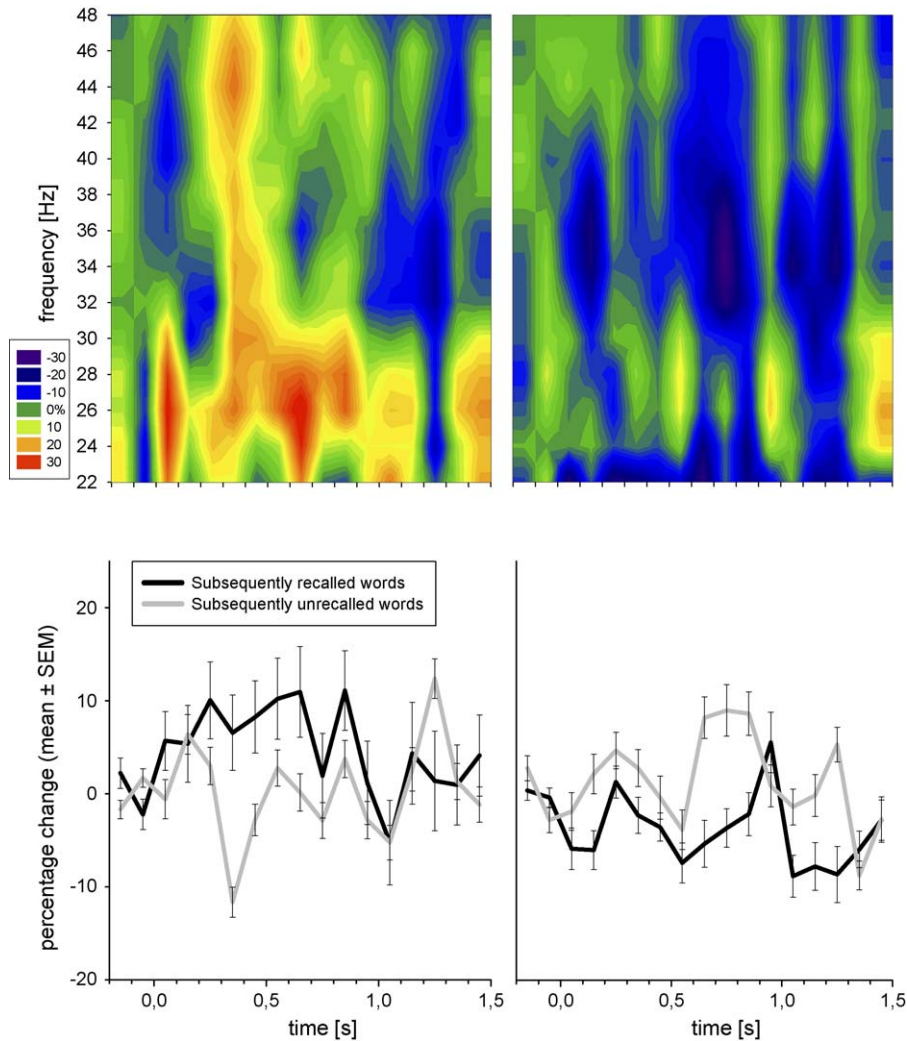


Fig. 1. Rhinal–hippocampal phase synchronization in the upper beta and gamma range (22–48 Hz) for high-frequency words (left) and low-frequency words (right) (grand average). Above: differences of Rhinal–hippocampal synchronization [%] relative to the prestimulus time window [–200;0 ms] for subsequently remembered minus forgotten words. The different EEG frequencies are represented on the *y*-axis and time is depicted on the *x*-axis. Synchronization/desynchronization is coded on a color scale: red areas show an enhancement, blue areas a reduction of synchronization for subsequently recalled versus unrecalled words. Below: changes of Rhinal–hippocampal synchronization [%] relative to prestimulus baseline for subsequently recalled versus unrecalled words. Synchronization values were averaged across the upper beta and gamma range (22–48 Hz).

together with a trend for a decreased synchronization for remembered versus forgotten low-frequency words ( $p=0.087$ ;  $F_{1,9}=3.70$ ). When focusing on the gamma range between 32 and 48 Hz as done in a prior study [12], we observed a significant synchronization increase between 300 and 500 ms (paired one-tailed *T*-tests; each  $p<0.05$ ) for remembered vs. forgotten high-frequency words, but no significant synchronization enhancement for low-frequency words. The later desynchronization, which occurs between 1000 and 1300 ms for high-frequency words, emerges already between 600 and 900 ms for low-frequency words (600–700 ms:  $p=0.055$ ; 700–800 ms:  $p=0.064$ ). Compared to the gamma range, the synchronization enhancement in the sub-gamma range is less defined in time and rather seems to be broadly distributed across the trial.

In general, our findings for high-frequency words replicate the data of a prior study [12] indicating that successfully memorized compared to later forgotten items are accompanied by

an early Rhinal–hippocampal phase coupling and later decoupling within the gamma range. In the previous investigation, the gamma synchronization increase showed an even earlier onset already in the [100;200] time window. This discrepancy may in part result from the fact that we used broader wavelets for time-frequency analysis in the prior study compared to the present investigation (7 cycle versus 5 cycle wavelets), which was adapted to concomitant evaluation of the sub-gamma range. Another difference between both studies is that we previously presented 240 word stimuli, whereas in the current study only 90 words were used for each category (high-frequency, low-frequency). Because considerably more trials entered the individual averages, the prior findings [12] have to be regarded as more reliable.

The phase coupling and decoupling in the gamma range for later remembered high-frequency words is associated with broadly distributed synchronization enhancements in

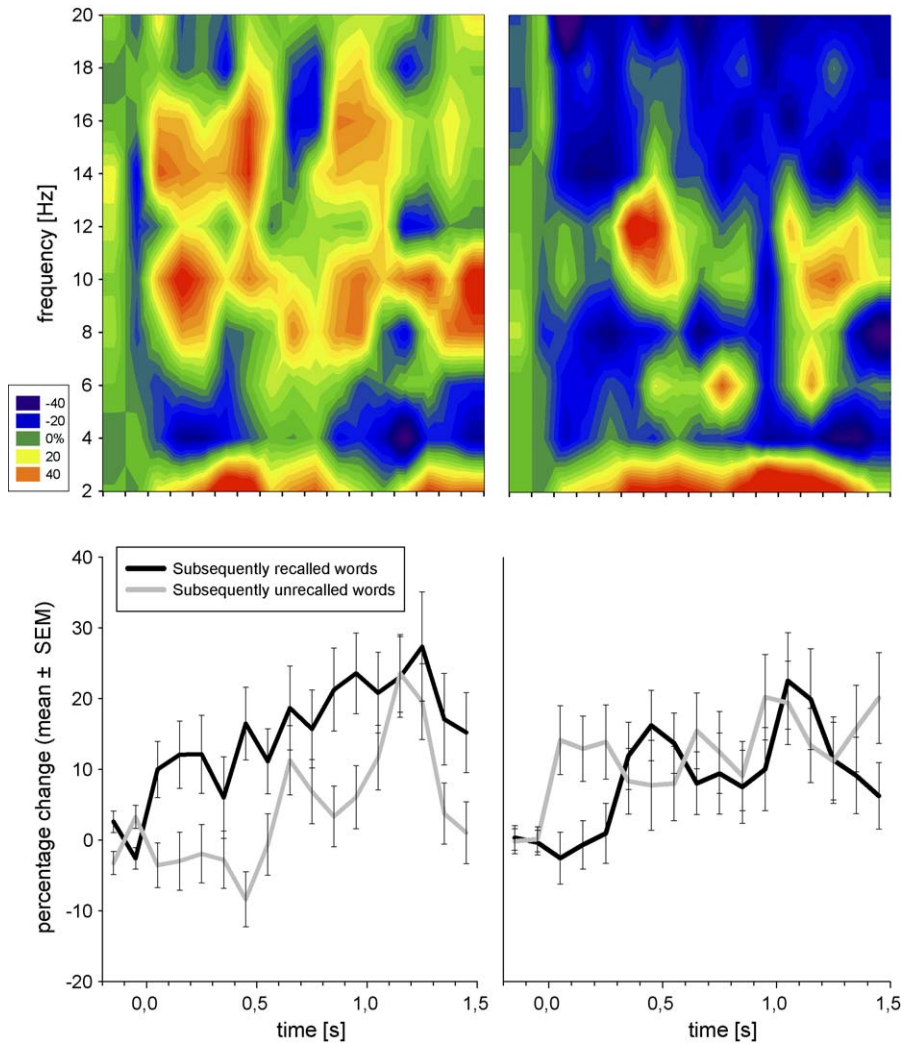


Fig. 2. Rhinal–hippocampal phase synchronization in the 2–20 Hz range for high-frequency words (left) and low-frequency words (right) (grand average). Above: differences of Rhinal–hippocampal synchronization [%] relative to the prestimulus time window [–200;0 ms] for subsequently remembered minus forgotten words. Below: changes of Rhinal–hippocampal synchronization [%] relative to prestimulus baseline for subsequently recalled versus unrecalled words. Synchronization values were averaged across the 2–20 Hz range.

the sub-gamma range, which again are in accordance with previous data [11]. In other studies synchronization effects in the sub-gamma range have, for instance, been attributed to working memory processes [26,28,31], which could accompany long-term memory formation also in the present experiment, at least during the encoding phase. The synchronization increase in the delta range probably corresponds to the slow positive event-related component, which is observed within the hippocampus during declarative memory formation [14]. In the frequency range above delta even an early memory-related desynchronization seemed to occur for low-frequency words. The functional significance of this early Rhinal–hippocampal desynchronization is an open question.

The most important finding is, that the memory-related increase of beta and gamma synchronization is absent for low-frequency words. This outcome is in line with models suggesting that different MTL substructures contribute to behaviorally different operations [5,8,16,22]. One hypothesis regarding the formation of new memories is that the hippocampus carries out an

exclusively mnemonic operation in a serially organized declarative memory system, which is insensitive to item characteristics and modality. According to this hypothesis, the subordinate parahippocampal region feeds the hippocampus with information and supports memory formation by semantic processes, thus indirectly facilitating the transformation of experiences into long-term memory [16].

Our data suggest that a broadband Rhinal–hippocampal coupling including the gamma band only occurs, when significant semantic associations are processed within rhinal cortex, as is the case for high-frequency words. These data do not exclude that for low-frequency words still some isolated memory-related coupling occurs within the gamma-band – but this effect is not evident with regard to macroscopically recorded EEG. In the context of the present study, phase synchronization of gamma oscillations and the accompanying correlated neural firing have been suggested to subserve two basal functions. One is establishing temporal windows for neural communication, which are optimal for the transmission of information [10,18]. The other

one is the formation of Hebbian assemblies due to the precise timing of action potentials [2]. According to this framework, broadband Rhinal–hippocampal synchronization including the gamma range may on the one hand support the memory related transfer of semantic information from rhinal cortex to hippocampus. On the other hand, Rhinal–hippocampal synchronization may enable the formation of associative Hebbian links between rhinal cortex and hippocampus.

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