



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
Main Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2015

Boosting vocabulary learning by verbal cueing during sleep

Schreiner, Thomas ; Rasch, Björn

Abstract: Reactivating memories during sleep by re-exposure to associated memory cues (e.g., odors or sounds) improves memory consolidation. Here, we tested for the first time whether verbal cueing during sleep can improve vocabulary learning. We cued prior learned Dutch words either during non-rapid eye movement sleep (NonREM) or during active or passive waking. Re-exposure to Dutch words during sleep improved later memory for the German translation of the cued words when compared with uncued words. Recall of uncued words was similar to an additional group receiving no verbal cues during sleep. Furthermore, verbal cueing failed to improve memory during active and passive waking. High-density electroencephalographic recordings revealed that successful verbal cueing during NonREM sleep is associated with a pronounced frontal negativity in event-related potentials, a higher frequency of frontal slow waves as well as a cueing-related increase in right frontal and left parietal oscillatory theta power. Our results indicate that verbal cues presented during NonREM sleep reactivate associated memories, and facilitate later recall of foreign vocabulary without impairing ongoing consolidation processes. Likewise, our oscillatory analysis suggests that both sleep-specific slow waves as well as theta oscillations (typically associated with successful memory encoding during wakefulness) might be involved in strengthening memories by cueing during sleep.

DOI: <https://doi.org/10.1093/cercor/bhu139>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-124612>

Journal Article

Published Version

Originally published at:

Schreiner, Thomas; Rasch, Björn (2015). Boosting vocabulary learning by verbal cueing during sleep. *Cerebral Cortex*, 25(11):4169-4179.

DOI: <https://doi.org/10.1093/cercor/bhu139>

Boosting Vocabulary Learning by Verbal Cueing During Sleep

Thomas Schreiner¹ and Björn Rasch^{1,2,3}

¹University of Zurich, Institute of Psychology, Zurich, Switzerland, ²Zurich Center for Interdisciplinary Sleep Research (ZiS), Zurich, Switzerland and ³Department of Psychology, University of Fribourg, Fribourg, Switzerland

Address correspondence to Björn Rasch, Division of Cognitive Biopsychology and Methods, Department of Psychology, University of Fribourg, Rue P.-A.-Faucigny 2, CH-1701 Fribourg, Switzerland. Email: bjoern.rasch@unifr.ch

Reactivating memories during sleep by re-exposure to associated memory cues (e.g., odors or sounds) improves memory consolidation. Here, we tested for the first time whether verbal cueing during sleep can improve vocabulary learning. We cued prior learned Dutch words either during non-rapid eye movement sleep (NonREM) or during active or passive waking. Re-exposure to Dutch words during sleep improved later memory for the German translation of the cued words when compared with uncued words. Recall of uncued words was similar to an additional group receiving no verbal cues during sleep. Furthermore, verbal cueing failed to improve memory during active and passive waking. High-density electroencephalographic recordings revealed that successful verbal cueing during NonREM sleep is associated with a pronounced frontal negativity in event-related potentials, a higher frequency of frontal slow waves as well as a cueing-related increase in right frontal and left parietal oscillatory theta power. Our results indicate that verbal cues presented during NonREM sleep reactivate associated memories, and facilitate later recall of foreign vocabulary without impairing ongoing consolidation processes. Likewise, our oscillatory analysis suggests that both sleep-specific slow waves as well as theta oscillations (typically associated with successful memory encoding during wakefulness) might be involved in strengthening memories by cueing during sleep.

Keywords: high-density EEG, language, sleep, targeted memory reactivations, vocabulary learning

Introduction

Language acquisition is a quintessential human trait and fundamental for every-day communication (Pinker 2000). Learning a new language depends essentially on the learning of new vocabulary, both for learning the native language as an infant as well as during acquisition of foreign languages in school children and adults (Shatz 2001). It has been suggested that sleep may play an important role in language learning (Davis and Gaskell 2009; Margoliash 2010; Margoliash and Schmidt 2010) possibly due to its beneficial role on memory consolidation (Rasch and Born 2013). Sleep appears to facilitate memory for abstract relations of words of an artificial language in infants (Gómez et al. 2006) and benefits the integration of newly learned words into pre-existing knowledge in both school children and adults (Dumay and Gaskell 2007; Henderson et al. 2012). More specifically, Gais et al. (2006) demonstrated that the ability of high school students to remember vocabulary of a foreign language was enhanced when learning was followed by sleep when compared with wakefulness.

According to the active system consolidation hypothesis, the beneficial role of sleep on language acquisition is due to a spontaneous and repeated reactivation of newly acquired information during subsequent non-rapid eye movement (NonREM)

sleep, promoting memory stabilization and integration (Diekelmann and Born 2010; Stickgold and Walker 2013; Genzel et al. 2014). In support of the hypothesis, replay activity during sleep has been consistently reported in memory-related brain structures in rodents and humans, particularly in the hippocampus (Pavlidis and Winson 1989; Wilson and McNaughton 1994; Peyrache et al. 2009; O'Neill et al. 2010). In animal models of language learning, reactivation of song patterns during sleep in birds is assumed to be critical for song learning during development (Dave and Marholias 2000), although mechanisms of memory consolidation during sleep may differ between mammals and birds, particularly with respect to system consolidation (Rattenborg et al. 2011). Furthermore, a series of recent studies has shown that experimentally inducing reactivations during NonREM sleep by using associated memory cues benefits memory consolidation using odors (Rasch et al. 2007; Diekelmann et al. 2011; Ritter et al. 2012; Rihm et al. 2014), sounds (Rudoy et al. 2009; Dongen et al. 2012), or even melodies (Antony et al. 2012; Schönauer et al. 2013), including the successful cueing of hippocampal place cells during sleep in rodents (Bendor and Wilson 2012). In spite of the increasing evidence for the beneficial role of cueing during sleep on various memory processes (e.g., Oudiette and Paller (2013)), it remains an open question whether words can also be used as memory cues during sleep.

Based on studies using event-related potentials (ERPs), it has been suggested that the capacity to establish neural representations of stimuli in sensory memory during sleep is preserved (for a review, see Atienza et al. (2001)). For example, previous studies have shown that several ERP components (such as the auditory N1, the mismatch negativity, the P3a and 2 sleep-specific components, the N350 and the N550) react to a variable degree to different features of the stimuli presented during sleep, such as frequency and significance (e.g., the subjects' own name) (Brualla et al. 1998; Pratt et al. 1999; Perrin et al. 2002). However, it is still unknown whether processing of complex verbal cues during sleep is indeed capable of reactivating associated memories (e.g., the previously learned translation of the foreign word), thereby benefiting the consolidation of foreign vocabulary. Furthermore, it is still unclear whether cueing during sleep is purely beneficial or whether it is associated with "costs" by disturbing ongoing consolidation processes of uncued memories. Finally, the underlying event-related and oscillatory processes of successful reactivations during sleep are basically unknown.

In this study, we directly tested the hypothesis that verbal cueing during postlearning sleep enhances acquisition of foreign vocabulary. We hypothesized that cueing Dutch words specifically improves memory for cued words when compared with uncued words without disturbing consolidation of uncued words. Furthermore, we predict that the improving effect of

cueing is sleep-specific and does not occur after cueing during waking. In addition, we tested the hypothesis that event-related and oscillatory activity associated with cueing during sleep is predictive for cueing-related gains in vocabulary by recording high-density electroencephalography (EEG) during sleep.

Materials and Methods

Subjects

A total of 68 healthy, right-handed subjects (32 female, mean age = 24.61 ± 0.6) with German mother tongue and without Dutch language skills participated in the study. Seventeen subjects participated in each of the 4 experimental groups (e.g., main sleep group, control sleep group, active waking, and passive waking group). Four subjects had to be excluded from both sleep groups due to sleeping problems, resulting in 15 participants in each sleep group (main sleep group: 8 female, mean age = 25.1 ± 1.17 years; control sleep group: 8 female, mean age = 23.87 ± 0.68), 17 subjects in the active waking group (7 female, mean age = 24.7 ± 1.11 years), and 17 subjects in the passive waking group (8 female, mean age = 23.9 ± 0.97 years). Age and gender distribution did not differ between the experimental groups (both $P > 0.75$).

None of the participants were taking any medication at the time of the experiment and none had a history of any neurological or psychiatric disorders. All subjects reported a normal sleep-wake cycle and none had been on a night shift for at least 8 weeks before the experiment. Only subjects with a normal working memory capacity (i.e., minimum OSPAN score of 20, see task description, page 4) were recruited, due to the potential impact of working memory capacity on sleep-dependent declarative memory consolidation (Fenn and Hambrick 2012). On experimental days, subjects were instructed to get up at 7.00 h and were not allowed to take in caffeine and alcohol or to nap during daytime.

The study was approved by the ethics committee of the Department of Psychology, University of Zurich, and all subjects gave written informed consent prior to participating. After completing the whole experiment, participants received 120 Swiss francs (CHF) (sleep groups) or 100 CHF (wake groups), respectively.

Design and Procedure

Participants entered the laboratory at 21.00 h. The session started with the application of the electrodes for standard polysomnography,

including electroencephalographic (EEG; 128 channels, Electrical Geodesic, Inc.), electromyographic (EMG), and electrocardiographic (ECG) recordings. Prior to the experiment, participants of the sleep group spent an adaptation night in the sleep laboratory.

In all 4 experimental groups, the learning phase started at ~ 22.00 h with the vocabulary learning task (Dutch–German word pairs, for a detailed description see Vocabulary Learning Task section). After completing the learning task, participants of both sleep groups went to bed at 23.00 h and were allowed to sleep for 3 h, whereas participants in the 2 wake control groups stayed awake (see Fig. 1, for an overview of the procedure). During the 3-h retention interval, a selection of the prior learned Dutch words was presented again during sleep stages N2 and N3 (slow wave sleep, SWS) in the cueing sleep group and during active or passive waking in the wake control groups for a total duration of 90 min (see below for a detailed description of the reactivation phase). In the control sleep group, the same procedure was administered but the selected Dutch words were not replayed during sleep. At ~ 2.00 h, subjects of both sleep groups were awakened from sleep stage 1 or 2 and at ~ 2.15 h, recall of the vocabulary was tested in all experimental groups.

Vocabulary Learning Task

The vocabulary learning task consisted of 120 Dutch words and their German translation, randomly presented in 3 learning rounds (word pairs are listed in the Supplementary Table 1). Dutch words were presented aurally (duration range 400–650 ms) via loudspeakers (70 dB sound pressure level). In the first learning round, each Dutch word was followed by a fixation cross (500 ms) and subsequently by a visual presentation of its German translation (2000 ms). The intertrial interval between consecutive word pairs was 2000–2200 ms. The subjects were instructed to memorize as many word pairs as possible. In a second round, the Dutch words were presented again followed by a question mark (ranging up to 7 s in duration). The participants were instructed to vocalize the correct German word or to say, “next” (German translation: “weiter”). Afterward, the correct German translation was shown again for 2000 ms, irrespective of the correctness of the given answer. In the third learning round, the cued recall procedure was repeated without any feedback of the correct German translation. Recall performance of the third round (without feedback) was taken as pre-entention learning performance. In the third round, participants recalled on average 60.88 ± 1.1 words (range 40–82 words) of the 120 words correctly, indicating an ideal medium task difficulty (recall performance

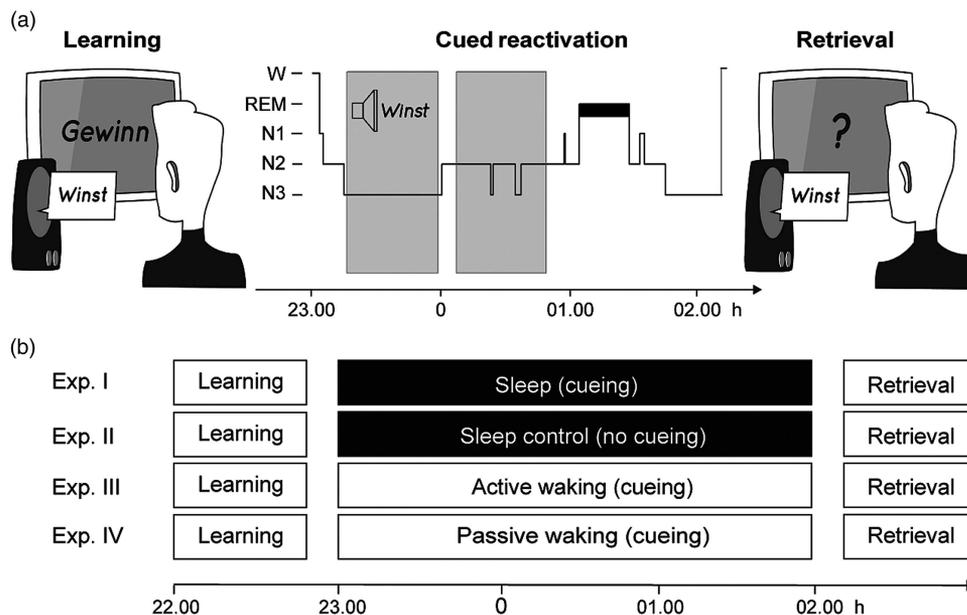


Figure 1. Experimental procedure. (a and b) Participants studied 120 Dutch–German word pairs in the evening. Afterward, participants of the main and the control sleep groups slept for 3 h, whereas 2 other groups stayed awake. During the retention interval, 90 Dutch words (30 prior remembered, 30 prior not remembered and 30 new words) were repeatedly presented again. Cueing of vocabulary occurred during NonREM sleep, during performance of a working memory task, or during rest. The control sleep group did not receive any cues during sleep. After the retention interval, participants were tested on the German translation of the Dutch words using a cued recall procedure.

Table 1

Overview of memory performance

	Cued	Uncued	<i>t</i>	<i>P</i>
Main sleep group				
Cued recall				
Learning	29.87 ± 0.09	33.20 ± 2.54	-1.29	0.22
Retrieval	31.40 ± 0.16	31.33 ± 2.17	0.04	0.97
Change	+1.53 ± 0.79	-1.87 ± 0.70	3.52	0.003**
% Change	105.15 ± 2.64	95.43 ± 2.07	3.43	0.004**
Recognition				
Hits	52.40 ± 0.98	51.20 ± 1.57	1.33	0.80
% Hits	87.33 ± 1.62	85.33 ± 2.62		
<i>d'</i>	2.32 ± 0.15	2.32 ± 0.17	0.00	0.99
Control sleep group				
Cued recall				
Learning	30	31.93 ± 1.84	-1.04	0.31
Retrieval	28.07 ± 0.71	29.27 ± 1.66	-0.77	0.45
Change	-1.93 ± 0.71	-2.66 ± 0.89	0.79	0.44
% Change	93.55 ± 2.37	92.80 ± 3.10	0.24	0.81
Recognition				
Hits	50 ± 1.24	50.60 ± 1.55	-0.64	0.53
% Hits	83.33 ± 2.07	84.33 ± 2.59		
<i>d'</i>	2.01 ± 0.13	2.09 ± 0.16	-0.93	0.36
Active waking group				
Cued recall				
Learning	30.06 ± 0.10	30.59 ± 2.7	-0.19	0.89
Retrieval	25.71 ± 0.83	26.12 ± 2.5	-0.19	0.85
Change	-4.35 ± 0.84	-4.47 ± 0.63	0.12	0.90
% Change	85.53 ± 2.81	84.21 ± 2.16	0.56	0.58
Recognition				
Hits	50.29 ± 1.05	49.35 ± 1.55	0.79	0.43
% Hits	83.83 ± 1.75	82.25 ± 2.59		
<i>d'</i>	1.44 ± 0.15	1.39 ± 0.17	0.65	0.52
Passive waking group				
Cued recall				
Learning	30.35 ± 0.14	27.82 ± 1.75	1.46	0.16
Retrieval	24.24 ± 1.14	22.82 ± 1.78	1.17	0.25
Change	-6.11 ± 1.41	-5.00 ± 0.59	-0.79	0.44
% Change	79.86 ± 4.58	81.25 ± 2.09	-0.35	0.74
Recognition				
Hits	46.53 ± 1.83	43.71 ± 1.85	2.88	0.01*
% Hits	77.54 ± 3.06	72.84 ± 3.08		
<i>d'</i>	1.13 ± 0.17	0.95 ± 0.17	2.41	0.02*

Data are means ± SEM; Numbers indicate absolute or relative values of correctly recalled or recognized words that were presented during the retention interval (cued words, 60 in total) or not (uncued words, 60 in total). For cued recall testing, number of correctly recalled words during the learning phase before and the retrieval phase after the retention interval are indicated. Change (% Change) refers to the absolute (relative) difference in performance between learning and retrieval phases. Hits (% Hits) refers to the absolute (relative) number of correctly recognized words as "old" (since % Hits = Hits × 100/60, statistics are redundant). The sensitivity measure *d'* reflects recognition performance according to signal detection theory based on the proportion of Hits and False Alarms (Macmillan and Creelman 2005). **P* < 0.05; ***P* < 0.01.

50.41%) without any danger of ceiling or floor effects. We observed no difference in preretention memory performance between the 4 experimental groups (main effect of "condition": $F_{3,60} = 0.86$; $P = 0.46$), no difference in presleep memory performance between later cued and uncued words (main effect "cueing": $F_{1,60} = 0.001$; $P = 0.96$) and no interaction between condition and cueing ($F_{3,60} = 0.41$; $P = 0.74$; see Table 1 for descriptive statistics).

Reactivation of Vocabulary

In the reactivation phase during the 3-h retention interval, Dutch words were presented aurally without the German translation. The presentation occurred via loudspeakers (50-dB sound pressure level). Of the 120 words learned before the retention interval, 60 words were cued and 60 were not cued during the subsequent retention interval. The 60 cued words consisted of 30 words that participants remembered during the preretention learning phase (cued hits), and 30 words that participants did not remember before the retention interval (cued misses). The words were individually and randomly chosen for each participant using an automatic MATLAB algorithm. In addition, 30 new words were presented during the retention interval that had not been included in the preretention learning list, serving as

Table 2

Sleep and reactivation parameter

	Main sleep group	Control sleep group	<i>P</i>
Duration (min)			
N1	7.76 ± 1.66	5.20 ± 1.46	0.16
N2	93.16 ± 5.93	100.27 ± 4.71	0.71
SWS	62.26 ± 5.8	57.93 ± 5.37	0.94
REM	22.13 ± 3.18	22.07 ± 2.73	0.37
WASO	4.66 ± 1.71	0.37 ± 0.14	0.03
Duration (%)			
N1	4.02 ± 0.84	2.72 ± 0.70	0.31
N2	48.70 ± 2.64	53.73 ± 2.95	0.25
SWS	33.11 ± 3.26	31.13 ± 2.95	0.72
REM	11.38 ± 1.59	11.65 ± 1.36	0.89
WASO	2.35 ± 0.82	0.002 ± 0.00	0.01
Number of reactivations			
N2	442.86 ± 40.68	–	
SWS	508.80 ± 54.42	–	

Data are means ± SEM. N1, N2: NonREM sleep stages N1 and N2; SWS, slow wave sleep/N3; REM, rapid-eye movement sleep; WASO, wake after sleep onset.

control stimuli. Thus, in total, 90 Dutch words were presented during the retention interval. Presentation occurred every 2.800–3.200 ms in a randomized order for a total of 90 min, resulting in 10–11 exposures to each word (see Table 2). The rationale of repeated cueing during sleep was derived from previous studies using olfactory cues which were repeated several times successfully induces memory reactivation during sleep (Rasch et al. 2007; Diekelmann et al. 2011; Rihm et al. 2014). Furthermore, we aimed at obtaining a sufficient number of trials for detailed EEG analysis. In the main sleep group, exposure to Dutch words occurred during sleep stages 2 and SWS. Sleep was continuously monitored by the experimenter, and the stimulation was interrupted whenever polysomnographic signs of REM sleep, arousal, or awakenings occurred. On average, the presentation of Dutch words during sleep was interrupted 5.2 ± 0.5 times. In the control sleep group, Dutch words were also classified as "cued" and "uncued" words using the same procedure as in the main experiment, but the verbal cues were not administered during sleep. In the active waking group, cueing of Dutch words occurred during performance on a computerized *n*-back task. The 3-h wake retention interval was divided into 30-min periods. In the first, third, and fifth 30-min period, participants performed on the *n*-back task (including a total of 27 67-s blocks of 0-back, 1-back, and 2-back blocks, in a randomized order, for more details see task description). Subjects were instructed to focus on the task and were given feedback on accuracy after each 30-min period. While subjects accomplished the *n*-back task, Dutch words were played in the same manner as in the sleep group, resulting in a total exposure time of 90 min. Between the 3 blocks of word reactivation, subjects completed questionnaires and played an online computer game (Bubble shooter). In the passive waking group, Dutch words were played during passive waking of the participants, allowing full attention on the replayed Dutch words. Participants were re-exposed to the Dutch words in the first, third, and fifth 30-min period of the 3-h retention interval. They were instructed that they would hear some of the Dutch words again and should attentively listen to the words. In the remaining 30-min periods, the participants performed on the *n*-back task and filled out questionnaires, without any auditory stimulation.

Recall of Vocabulary after the Retention Interval

During the recall phase, the Dutch words were presented aurally in a randomized order. In addition to the 120 words included in the preretention learning list, the 30 control words from the reactivation phase and 30 entirely new words were tested. After listening to the word, participants had to indicate whether the word was old (part of the learning material) or new. If the current word was recognized as old, they were asked to give the German translation.

As index of memory recall of German translations across the retention interval, we calculated the relative difference between the number of correctly recalled words before and after the retention interval, with the preretention memory performance set to 100%. For recognition

memory of Dutch words, we calculated the sensitivity index d' [i.e., $z(\text{Hits}) - z(\text{False Alarms})$] according to signal detection theory. Proportions of 0 and 1 were replaced by $1/2N$ and $1-1/2N$, respectively, with N representing the number of trials in each proportion (i.e., $N=60$, see Macmillan and Creelman (2005)). The memory indices for cued recall and recognition were calculated separately for cued and uncued words.

OSPAN Task

The OSPAN task was administered to assess the subjects' working memory capacity (Unsworth et al. 2005). Each trial included an equation succeeded by a letter. The subjects had to indicate if the answer to a given equation was correct and had to remember the letter afterwards. Every 3–6 trials, 12 letters appeared on the screen and subjects had to select those that had been shown before.

n-Back Test

Subjects of both waking groups accomplished intermixed 0-, 1-, and 2-back versions of the *n*-back working memory task (Gevins and Smith 2000). In this task, different letters appear successively in the center of the screen. In the 0-back version, subjects had to press a key whenever the letter “x” appeared on the screen. In the 1-back version, subjects had to respond to a letter repetition (h-f-f-k), while the 2-back version requires subjects to respond to a letter repetition with one intervening letter (h-f-s-f).

Sleep EEG

Sleep was recorded by standard polysomnography including EEG, EMG, and ECG recordings. EEG was recorded using a high-density 128-channel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR, USA). High-density EEG was used to obtain a reliable estimation of possible topographical distributions to the reactivation-related effects. Impedances were kept below 50 k Ω . Voltage was sampled at 500 Hz and initially referenced to the vertex electrode (Cz). Additionally to the online identification of sleep stages, polysomnographic recordings were scored offline by 3 independent raters according to standard criteria (Iber et al. 2007). In order to exclude the possibility of sleep onsets in the waking groups, EEG of the waking reactivation phase was also scored offline.

Event-Related Potentials

Offline EEG analysis was realized using Brain Vision Analyzer software (version: 2.0; Brain Products, Gilching, Germany). Data were referenced to averaged mastoids, low-pass filtered with a cutoff frequency of 30 Hz (roll-off 24 dB per octave), and high-pass filtered with a cutoff frequency of 0.1 Hz (roll-off 12 dB per octave). The EEG data were epoched into 1700 ms segments beginning 200 ms before stimulus onset. The 200-ms interval preceding stimulus onset served as baseline and was used for baseline correction. Epochs were categorized based on performance between pre- and postsleep tests yielding the following categories of ERPs: first, we analyzed ERPs for later remembered when compared with later forgotten cued words. In addition, we separated later remembered words in “Gains” (i.e., cued Dutch words not remembered before sleep but correctly recalled after sleep) and “HitHit” words (i.e., cued Dutch words remembered before and after sleep). Later forgotten words were separated in “Losses” (i.e., cued words correctly retrieved before sleep but not remembered after sleep) and “MissMiss” words (i.e., cued Dutch words not remembered before and after sleep). The control stimuli presented during the retention interval entered the category “Control.”

Signal averaging was carried out separately per subject and per condition and grand averages of all conditions were calculated. For statistical analysis, average EEG amplitudes measured over the interval from 800 to 1.100 ms after stimulus onset were compared. To protect against error inflation due to multiple testing of multiple electrodes, we used a false discovery rate of $P < 0.05$. For illustration of the results, we present the ERP of the electrode with the highest significance (for sleep stage-specific ERP analyses, see Supplementary Results and Fig. 2).

Slow Oscillations Analysis

Artifact-free EEG data, ranging from -300 to 1500 ms with respect to the gain and loss trials, were low-pass filtered at 30 Hz and band-pass filtered between 0.5 and 4.0 Hz (stopband 0.1 and 10 Hz) using a Chebyshev Type II filter (MATLAB, The Math Works, Inc., Natick, MA, USA). Slow oscillations were then identified visually at electrode site Fz as well as electrode sites F3 and F4 as waves of a total duration >500 ms and a minimal amplitude of 75 μV , starting in a time window between 0 and 800 ms poststimulus.

Analysis of Power Changes

We analyzed average power differences between Gains and Losses using a fast Frequency Transformation implemented in Brain Vision Analyzer with a Hanning Window of 10% during the 2.5 s after each word. Power values were analyzed for slow spindle activity (11–13 Hz) and fast spindle activity (13–15 Hz), as these frequency bands have been implicated in processes of memory consolidation (Antony et al. 2012; Fuentemilla et al. 2013; Rasch and Born 2013; Cairney et al. 2014). Frequency bands corresponding to slow wave activity (0.5–4 Hz) were not measured because of the limited number of possible cycles in the short trial length and border effects.

Theta oscillations (5–7 Hz) were analyzed using a Continuous Wavelet Transformation as implemented in Brain Vision Analyzer (complex Morlet waveform, frequency range from 5 to 7 Hz in 10 logarithmic steps, Morlet parameter $c=7$). In order to avoid edge effects, the trials entering the wavelet transform were segmented from -0.7 to 1.9 s with respect to stimulus presentation. An interval of 0.4 s at the beginning and the end of the trials was discarded afterward. A total of both induced and evoked activity was calculated by performing the wavelet analysis on single trials, after normalization with respect to the prestimulus time window from -300 to -100 ms (for the results of the total theta power calculation see Supplementary Fig. 1). Subsequently, the resulting single-trial frequency spectra were averaged. This procedure provides the overall power of a given frequency range. In order to obtain the induced power, which is thought to play a role in binding distributed cortical representations (Düzel et al. 2005), we subtracted the theta effects of the average ERP (evoked power) from each single trial before calculating the time–frequency analysis and averaging the single trials. Statistical analysis was performed for a time window of 700–900 ms after stimulus onset. Additionally, the same procedure was performed for slow spindles (11–13 Hz) and fast spindles (13–15 Hz), due to their assumed involvement in processes of sleep-dependent memory consolidation (for sleep stage-specific oscillatory analyses, see Supplementary Results and Fig. 3). As with the calculation of average oscillatory activity, frequency bands corresponding to slow wave activity (0.5–4 Hz) were not measured because of the limited number of possible cycles in the short trial length and border effects.

Statistical Analysis

Data were analyzed using repeated-measures analyses of variance (ANOVA). Where appropriate, significant interactions were further evaluated with Fisher's least significant difference post hoc tests. The level of significance was set to $P=0.05$.

Results

Effects of Verbal Cueing on Memory for Dutch Vocabulary

As expected, re-exposure to Dutch words improved later memory for the German translation of the cued words, when cueing occurred during sleep. Participants correctly recalled $105.14 \pm 2.64\%$ of the cued words, whereas only $95.43 \pm 2.07\%$ of the uncued words were remembered after sleep, with memory performance before sleep set to 100% (Fig. 2, see Table 1 for absolute values). The improvement of almost 10% points of vocabulary learning by cueing during sleep when compared with uncued words was highly significant ($t_{14} = 3.43$;

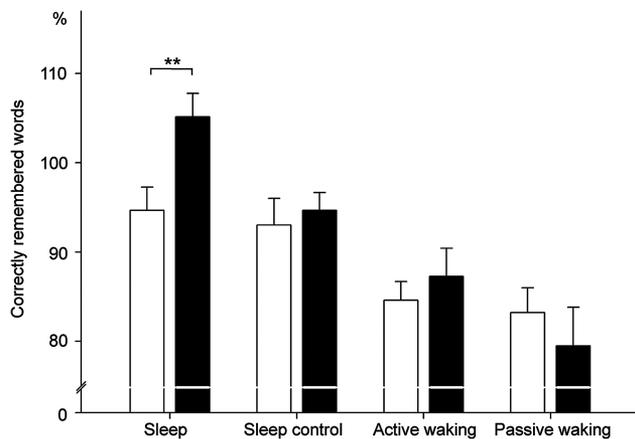


Figure 2. Behavioral results. In the main sleep group, memory for cued word pairs (black bar) was significantly improved when compared with uncued pairs (white bar). Recall of uncued word pairs in the main sleep group was comparable with recall performance of word pairs in the control sleep group, which did not receive any cues during sleep. No enhancing effects of cueing on later memory retrieval occurred in both waking control groups. Retrieval performance is indicated as percentage of recalled German translations with performance before sleep set to 100%. Values are mean ± SEM. ** $P \leq 0.01$.

$P = 0.004$). In fact, cueing during sleep even induced a 5% increase in memory for cued Dutch words above presleep performance levels, and this increase reached a statistical trend ($+5.14 \pm 2.64\%$; $P = 0.072$, one-sample t -test, two-sided). In contrast, German translations of uncued Dutch words were significantly more forgotten when compared with recall performance before sleep ($-4.75 \pm 2.07\%$; $P = 0.045$). Thus, reactivation of vocabulary during sleep did not only prevent forgetting of German translations, but showed a trend of improving memory beyond baseline levels. On the individual level, 12 of 15 participants benefited from cueing (range +1 to +11 words, for the absolute difference between cued and uncued words), whereas 3 participants did not (range 0 to -1 words).

To test whether the observed benefits of cueing during sleep disturbed the consolidation of uncued words or not, we conducted an independent control experiment without presenting any verbal cues during sleep after learning (sleep control group). After learning, words were also classified as cued and uncued words using the same algorithm as in the main experiment (see Materials and Methods), but no verbal cues were replayed during sleep. As expected, recall of words classified as cued and uncued did not differ (93.55 ± 2.37 vs. $92.80 \pm 3.10\%$; $t_{14} = 0.24$; $P = 0.81$). More importantly, memory performance in the sleep control group after sleeping without any verbal cues was highly comparable with the recall performance for uncued words observed in the main experiment with verbal cues during sleep (93.55 ± 2.37 vs. $95.43 \pm 2.07\%$; $t_{14} = 0.71$; $P = 0.48$), and was significantly lower when compared with memory for cued words (92.80 ± 3.10 vs. $105.14 \pm 2.64\%$; $t_{14} = 3.26$; $P = 0.003$, Fig. 2, see Table 1, for absolute values).

In the 2 waking groups, cueing did not reveal any beneficial effect on memory for Dutch vocabulary, neither in the active waking group (85.53 ± 2.8 vs. $84.2 \pm 2.16\%$, for cued and uncued words, respectively; $t_{16} = 0.56$; $P = 0.58$) nor in the passive waking group (79.86 ± 4.58 vs. $81.25 \pm 2.09\%$, for cued and uncued words, respectively, $t_{16} = -0.35$, $P = 0.74$; see Table 1 for absolute values). Thus, even with the availability of

attentive processing resources in the passive waking group, re-exposure to Dutch words during waking failed to improve memory for the German translations.

In addition to sleep-specific improvement by cueing, recall of German translation was generally better in the 2 sleep groups when compared with the 2 waking control groups, reflecting the well-known beneficial effect of retention intervals filled with sleep when compared with waking on memory consolidation (main effect condition; $F_{3,60} = 13.06$; $P < 0.001$; see Fig. 2). Post hoc tests revealed that recall performance in both sleep groups independent of cueing was better when compared with the active waking and the passive waking group ($t_{62} = 5.61$; $P < 0.001$).

While cueing during sleep improved memory for German translation of Dutch words as tested by cued recall, we observed no sleep-specific benefit of cueing on recognition of Dutch words. The interaction remained nonsignificant ($F_{3,60} = 1.35$; $P = 0.15$). However, sleep improved recognition of Dutch words independently of cueing (main effect condition; $F_{2,46} = 15.87$, $P < 0.001$): both sleep groups showed a significantly higher recognition performance (main sleep group: $d' = 2.32 \pm 0.13$; sleep control group: $d' = 2.04 \pm 0.14$) when compared with the active waking group ($d' = 1.42 \pm 0.16$) and the passive waking group ($d' = 1.05 \pm 0.16$; all $P < 0.001$), while neither the 2 waking groups ($P = 0.10$) nor the 2 sleep groups ($P = 0.68$) differed significantly among each other. In fact, recognition of cued and uncued Dutch words was basically identical in the main sleep group (see Table 1), safely excluding that recognition testing prior to cued recall might have confounded the reported beneficial effect of cueing during sleep as tested by cued recall. While cueing also did not affect recognition in the active waking group, cued words were better recognized in the passive waking group in an exploratory analysis, possibly reflecting the fact that the participants in the latter group attended the cued Dutch words during the retention interval (see Table 1).

Sleep and Cueing

The beneficial effect of cueing on memory during NonREM sleep cannot be explained by general alterations in sleep as the effect was specific for cued when compared with uncued words, while the general improving effect of sleep on memory was present for both word categories. Sleep architecture was not altered by cueing, as sleep parameters recorded in the main sleep group did not differ from those of the control sleep group (see Table 2). In addition, we did not observe any increases in alpha power 1000 ms before (indicative of brief awakenings (Rudoy et al. 2009)) and after the auditory stimulation at electrode site Oz, excluding that cueing of words induced short lasting arousal responses (alpha power before ($2.12 \pm 0.41 \mu\text{V}$) and after the auditory cue ($2.01 \pm 0.5 \mu\text{V}$), respectively, $t_{14} = 0.31$, $P = 0.75$). Still participants of the main sleep group spent more time awake than subjects of the control sleep group (4.66 vs. 0.55 min; $t_{14} = 2.86$, $P = 0.013$), indicating that auditory cueing slightly interrupted sleep. Note that auditory presentation of words was stop whenever signs of arousal or awakenings were detected. Importantly, performance levels of uncued words in the main sleep group and in the sleep group without cueing were almost identical, indicating that increases in wake time did not impair ongoing and spontaneous processes of memory consolidation.

We did not observe any significant associations between the memory advantage induced by cueing (i.e., by subtracting memory for cued minus uncued words (Antony et al. 2012)) and the relative time spent in a certain sleep stage (N1: $r=0.18$, $P=0.50$; N2: $r=-0.360$, $P=0.18$; SWS: $r=0.18$, $P=0.51$; REM: $r=0.24$, $P=0.93$). Cueing was monitored online and was restricted to sleep stages N2 and SWS. The total number of cueings did not differ between N2 and SWS (Table 2), and we did not observe any significant association between the memory advantage induced by cueing and number of cueings in N2 or SWS (N2: $r=-0.39$, $P=0.14$; SWS: $r=0.1$, $P=0.72$; for a more detailed description and analysis see Supplementary Table 2 and Results). Additionally, EEG offline scoring of the waking groups revealed no signs of sleep onsets, indicating that the subjects of both waking groups were awake throughout the reactivation phase.

Neural Correlates of Cueing During Sleep

In order to characterize the process of cueing on a neural basis, we analyzed ERPs and oscillatory responses to vocabulary cues during sleep. First, we analyzed ERPs for later remembered when compared with later forgotten cued words. In addition, we separated later remembered words in Gains (i.e., cued Dutch words not remembered before sleep but correctly recalled after sleep) and HitHit words (i.e., cued Dutch words remembered before and after sleep). Later forgotten words were separated in Losses (i.e., cued words correctly retrieved before sleep but not remembered after sleep) and MissMiss words (i.e., cued Dutch words not remembered before and after sleep). Please note that the categories Gains and Losses reflect a clear behavioral change after cueing, therefore best representing the neural pattern associated with processes underlying successful versus unsuccessful cueing for later memory retrieval. In contrast, neural correlates of HitHit and MissMiss words are more difficult to interpret, as cueing during sleep might be ineffective for sufficiently strong memory traces (cases of HitHit) or nonexistent associations (cases of “LossLoss”) after encoding before sleep (for the behavioral analysis of Gains and Losses please see Supplementary Results and Table 2).

Remarkably, the EEG analysis of the average ERP amplitudes in the main sleep group clearly revealed a more pronounced negativity for subsequently remembered versus subsequently forgotten cued words at electrode site Fz ($t_{14} = -2.85$, $P=0.013$). We further explored this difference by separately analyzing Gains and “HitHits” as well as Losses and MissMiss. Similar to the previous analysis, the difference between the ERP responses associated with HitHits when compared with “MissMisses” was significant ($t_{14} = 2.45$, $P=0.028$). More importantly, we observed the largest negative amplitude associated with cueing of “Gain” words. Neural correlates of Gains represent a memory gain induced by cueing during sleep (i.e., successful verbal cueing during sleep), and the amplitude was significantly increased when compared with all other word categories at electrode site Fz in a time interval from 800 to 1100 ms after word onset ($F_{6,84} = 4.52$, $P=0.001$), all pairwise post hoc tests $P<0.04$, see Fig. 3a,b). As Losses are the most suitable control category for Gains (i.e., behavioral change in memory induced by cueing, relatively similar number of occurrences, etc.), we focused on the comparison between Gains and Losses in all subsequent analyses.

The analysis of all electrode revealed that the amplitude difference between Gains and Losses had a stable fronto-central distribution (see Fig. 3c) comparable with distributions of subsequent memory effects observed during waking (Werkle-Bergner et al. 2006). Furthermore, in a single-trial analysis, we counted the number of clearly identifiable slow waves (negative amplitude $>75 \mu\text{V}$ with a duration of >500 ms starting in a time window 0–800 ms poststimulus, see Materials and Methods) that followed cueing of Gain words when compared with Losses during sleep. This analysis revealed, that Gains were significantly more often followed by slow oscillations ($31.09 \pm 3.6\%$ of all cueing trials of Gains) when compared with Losses ($18.48 \pm 3.4\%$ cueing trials of Losses; $t_{14} = 5.35$, $P<0.001$). This result was found at electrode site Fz, as well as F3 and F4 indicating a stable frontal distribution of this effect. This result is compatible with the assumption that the presence of a slow oscillation after the presentation of a Dutch word during sleep plays an important role for successfully stabilizing the associated memory trace, reactivated by the memory cue presented during sleep. As both slow oscillations and sleep spindles are critically involved in processes of memory consolidation during sleep (Rasch and Born 2013), we also analyzed possible differences in average oscillatory power between Gains and Losses for slow spindles (11–13 Hz) and fast spindle activity (13–15 Hz). However, we did not observe any difference between Gains and Losses in this analysis (all $P>0.10$).

We further explored difference between Gains and Losses in time–frequency space. We controlled for a possible contribution of the evoked brain response by subtracting the average ERP (evoked power) from each single trial before calculating the time–frequency analysis (induced power) (Klimesch et al. 1998). In contrast to our expectations, the time–frequency analysis revealed no significant increase in oscillatory power in the spindle band related to Gains versus Losses, neither in the fast spindle band (13–15 Hz) nor in the slow spindle band (11–13 Hz). However, sleep stage-specific analyses revealed a significant increase in slow spindle power during SWS (but not during stage N2) in a time window 600–800 ms after the cue ($P<0.05$, for details see Supplementary Results and Fig. 3). Please note that the analysis of power changes in the slow oscillations/delta band was not possible due to the relatively small intertrial interval between verbal cues.

Finally, we also analyzed power changes for the theta band. Theta activity is prevalently linked to successful memory encoding during waking (Nyhus and Curran 2010) and poststimulus increases in induced theta power have been specifically linked to processes of recollection (Düzel et al. 2005). Interestingly, induced theta power associated with verbal cueing during sleep differed significantly between conditions ($F_{4,56} = 7.38$, $P=0.002$). Gains were associated with an increase in induced theta power in a time window of 700–900 ms after stimulus onset. The increase in induced theta power was particularly strong in right frontal as well as left parietal electrodes (e.g., electrode FC6: $t_{14} = 3.68$; $P=0.009$), strongly suggesting that a transient increase in theta power is critical for successful cueing during sleep (see Fig. 3d–f; see Supplementary Fig. 1 for total power changes). Interestingly, increases in theta activity for Gains when compared with Losses were more pronounced during stage 2 sleep, but were also reliably observed during SWS (see Supplementary Results and Fig. 3).

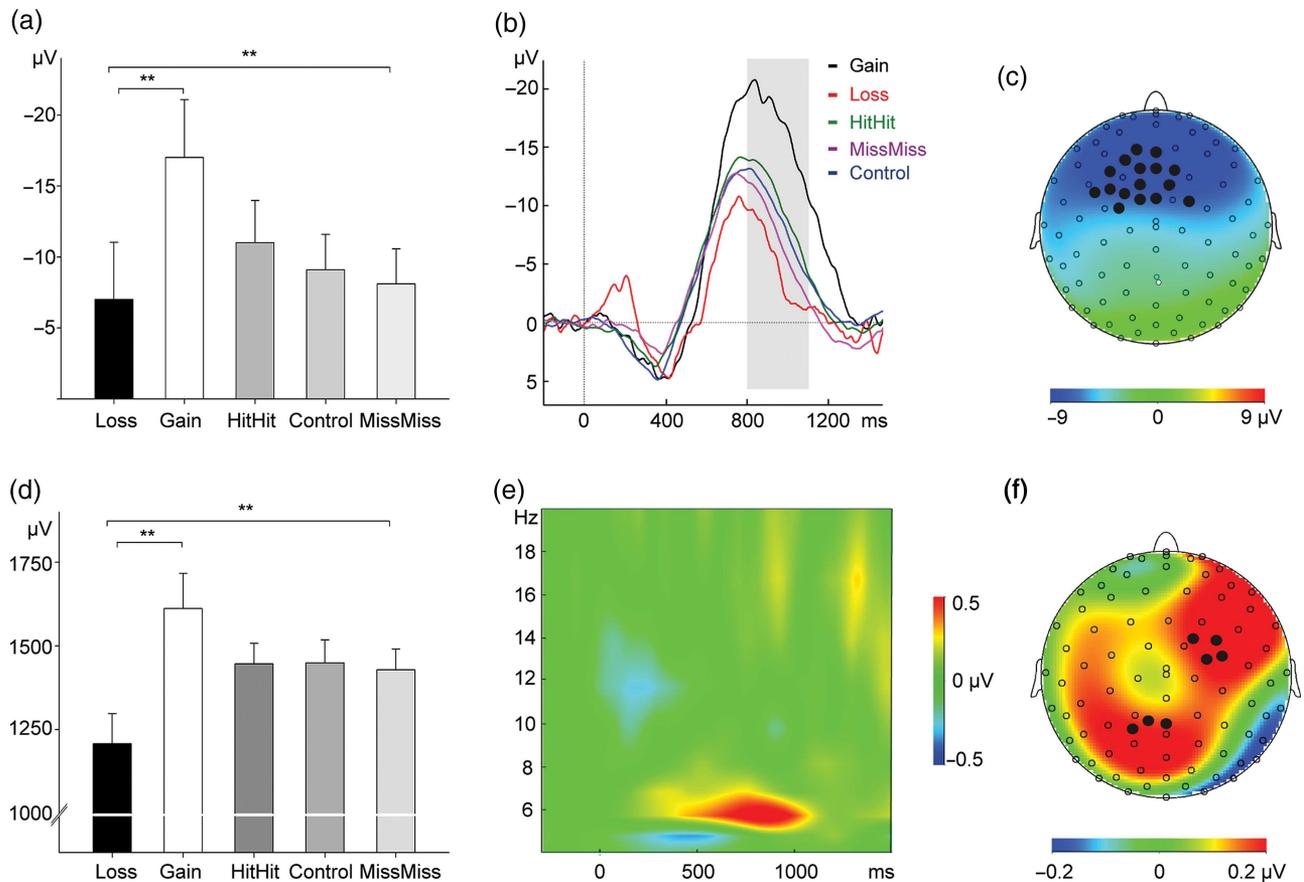


Figure 3. Electrophysiological results. ERPs and oscillatory theta power recorded during cueing in the sleep group were computed for words, for which cueing during sleep led to a change in memory performance. “Gains” reflect cued words not remembered in the presleep test but correctly recalled in the postsleep test. “Losses” refer to cued words remembered in the presleep test but not in the postsleep test. Words remembered before and after the retention interval were labeled “HitHit” and words not remembered both before and after the retention interval were labeled “MissMiss.” The new 30 Dutch words formed the “Control” condition. (a and b) Successful cueing was associated with a more pronounced negativity at frontal electrode sites (representative electrode Fz). The rectangle illustrates the time window used for waveform quantification. (c) Scalp map representing the topographical distribution for the difference between “Gains” and “Losses” in the time window between 800 and 1100 ms, indicating a pronounced frontal distribution (all electrodes entered the analysis; black dots indicate significant electrodes at $P < 0.05$, false discovery rate) corrected for multiple comparisons). The following electrodes were significant: E4, E5, E6, E11, E12, E13, E16, E19, E20, E23, E24, E28, E29, E35, E112 (see Supplementary Fig. 2 for the exact electrode positions). (d and e) Induced theta power for the difference between “Gains” and “Losses” (electrode FC6), indicating a distinct increase in induced theta power associated with successful cueing. (f) Scalp map depicting the distribution of theta power increase for “Gains” relative to “Losses” in the time window between 700 and 900 ms. The following electrodes were significant: E53, E60, E61, E62, E111, E117 (FC6), E118). $***P \leq 0.01$.

Discussion

Our findings show for the first time that cueing prior learned foreign vocabulary during sleep improves later recall. Furthermore, memory performance for uncued words in the main sleep group resembled memory performance of participants who did not receive any verbal cues during sleep, suggesting that cueing led to a real gain in memory performance. In addition, successful cueing during sleep, which resulted in later memory gains during retrieval testing, was associated with an increased late negativity and increased theta activity during NonREM sleep.

The beneficial effect of cueing during sleep is consistent with the active system consolidation hypothesis, which assumes that spontaneous memory reactivations during sleep are critical for the enhancing effect of sleep on memory consolidation. In fact, recent studies have successfully used memory-associated odors, sounds, or melodies (Rasch et al. 2007; Rudoy et al. 2009; Antony et al. 2012) to cue and strengthen memories during sleep. Here, we go an important step beyond these previous results by showing that also complex stimuli like foreign vocabulary can be successfully used to reactivate memories during

sleep, leading to an enhanced memory for vocabulary the next day. Importantly, our results are highly relevant for vocabulary learning in an educational setting, because our procedure of reactivating foreign vocabulary could be easily applied to these every-day learning contexts. However, as retrieval was tested in the night after only a few hours of sleep in the current study, future studies should test the memory-improving effects of cueing during sleep the next day or after several days. In addition, it still needs to be determined whether or not the beneficial effects of cueing during sleep are possibly accompanied by any detrimental effects on sleep-dependent memory consolidation of other material learned during the day. Finally, future studies need to examine whether cueing of vocabulary during sleep indeed facilitates foreign language learning.

In our experiment, we explicitly chose Dutch as a foreign language to achieve sufficiently few learning trials required for our analysis. Due to the close relation of Dutch to German or English, German-speaking participants could more easily learn the vocabulary and might even be able to correctly guess the meaning of some words. However, guesses cannot explain our reported improved effect of cueing during sleep, as words

were randomly assigned to the cued and uncued conditions. Furthermore, we can exclude that cueing simply increased perceptual fluency (Jacoby and Dallas 1981), because mere exposure to the words during waking similarly increases perceptual fluency and had no effect on memory for the vocabulary in our study. Still, the degree of prior knowledge of related languages, learning difficulty, and memory strength during encoding might be important factors determining the effectiveness of cueing during sleep, requiring further examination. Most importantly, the close relationship of the languages Dutch and German might have considerably affected the successful effect of cueing during sleep in our study. Thus, replicating our results with more distant languages is necessary to generalize our findings.

In contrast to the beneficial effect of cueing during sleep on recall of German translations, recognition of Dutch words was not affected by cueing during sleep. This result suggests that cueing during sleep specifically strengthens the association between the Dutch words and the German translations in memory, thereby facilitating later recall. However, recognition was only tested once (and not before and after the retention interval), which might have reduced the sensitivity of this test for possible beneficial effects of cueing during sleep on memory consolidation. Importantly, the null effect on recognition safely excludes that the reported beneficial effect of cueing during sleep on later recall might be confounded by prior recognition testing or higher familiarity with the cued words. Interestingly, sleep in general (independent of cueing) improved both recognition of Dutch words and recall of German translations, suggesting a broader role of sleep in memory consolidation when compared with experimental cueing during sleep.

Moreover, our results provide first evidence that the beneficial effects of cueing during sleep exceed the normal consolidation effects of sleep on memory, since recall of uncued words in the main sleep group was almost identical to memory performance of sleeping control participants who did not receive any cues during sleep. Thus, verbal cueing during sleep appears to benefit later recall of cued memory associations without disturbing ongoing consolidation processes during sleep. Hence, from a behavioral level, it appears as if the beneficial effect of cueing during sleep on memory occurs without any obvious costs. However, future studies in animal models or using intracranial recordings might additionally examine, in order to get a more comprehensive view, whether verbal cueing during sleep does not interfere with ongoing reactivation and consolidation processes also on the neural level. In contrast to our finding for verbal cues, others (Antony et al. 2012; Schönauer et al. 2013) reported some evidence for costs of cueing of procedural memories during sleep, as performance on the uncued sequence after receiving cues during sleep was lower when compared with performance in a separate group which did not receive any cues during sleep. Also here, future studies need to determine the mechanisms underlying a potential biasing of consolidation processes of cueing procedural memories during sleep when compared with the benefits of verbal cueing during sleep.

In the wake groups, the lack of beneficial memory effects by cueing was independent of the availability of attentional resources: both unattended cueing (active wake group) as well as attended cueing (passive wake group) during wakefulness failed to improve later retrieval of cued words. Thus, even though several rodent studies have reported the existence of spontaneous replay activity during periods of quiet (passive) waking (Gerrard et al. 1986; Kudrimoti et al. 1999), it may not

serve the same function as replay during NonREM sleep, as inducing reactivation during this behavioral state does not improve memory at least in humans. The lack of a memory effect by cueing during wakefulness is well in line with recent findings emphasizing the critical role of active and effortful retrieval to strengthen memories during wakefulness, whereas pure repeated study of words (without active retrieval testing) is not sufficient to improve memory (Karpicke and Roediger 2008). Please note that cued words were played rather fast in our study (one word every 3 s), possibly not leaving enough time for active retrieval attempts.

Still our results concerning the sleep specificity and the lack of beneficial effects of cueing in the waking groups should be interpreted with caution, because reactivation in both wake groups occurred during the night (11.00–02.00 AM) to exclude circadian factors on learning and retrieval. Thus, tiredness by partial sleep deprivation might have influenced the effects of cueing on memory performance. However, young participants (and particularly students) are typically quite used to stay up until 2.00 AM on weekends, so we consider the possible impact of tiredness on memory performance in the wake groups to be rather small. Furthermore, even if testing participants in the afternoon would result in a beneficial effect of cueing on memory, one could speculate that the underlying processes of this advantage are different from those acting during sleep: partial sleep deprivation mostly affects prefrontal functions like attention, working memory and possibly also task-related motivation. These processes are apparently not relevant for the benefits of cueing during sleep. One might hypothesize that cueing during sleep appears to benefit memory consolidation in an automatic, effortless and involuntary way, whereas benefits of cueing during wakefulness might possibly depend on the availability of attentional resources, high motivation, and active re-encoding of cued words. In contrast to this hypothesis, a recent study demonstrated beneficial effects of cueing in the afternoon during performance of a working memory task (Oudiette et al. 2013), possibly suggesting that cueing during wakefulness might improve memory even in the absence of attentional resources. Thus, an alternative explanation could be that the beneficial effects of cueing during wakefulness depend on an optimal circadian time, and that cues delivered during wakefulness at nighttime cannot be successfully processed as the brain is already overloaded by information encoded during prolonged prior wakefulness. As the memory mechanisms underlying cueing during wakefulness are still unclear, further investigation regarding the sleep specificity of cueing benefits are clearly needed.

In contrast to previous reactivation studies, we administered reactivation cues during both N2 sleep and SWS instead of restricting reactivation to SWS. The rationale for including N2 sleep was that 1) reactivation studies in rats do not differentiate between N2 sleep and SWS and 2) no previous reactivation study in humans has explicitly tested the effects of reactivation during N2 sleep on memory. Thus, we included N2 to obtain more time for repeated reactivation of Dutch words. In our view, early N2 sleep and SWS differ rather quantitatively (with respect to the occurrence of slow oscillations) than qualitatively, and our results suggest that cueing during N2 sleep might have at least no detrimental effects or even support memory consolidation during sleep.

In accordance to the active system consolidation, which assumes a critical role of slow oscillatory activity in synchronizing hippocampal memory reactivations with thalamo-cortical spindle

activity (Bergmann et al. 2012; Dongen et al. 2012; Ritter et al. 2012; Oudiette et al. 2013; Rasch and Born 2013; Rihm et al. 2014), successful cueing in our study was accompanied by an increased number of poststimulus slow oscillations. However, and in contrast to our expectations, this difference was not accompanied by an increase in sleep spindle activity, when analyzing sleep stage N2 and SWS together. Interestingly, the SWS-specific analysis revealed enhanced oscillatory power in the slow spindle band (11–13 Hz) succeeding the replay of Gains with regards to Losses. Both slow and fast sleep spindles have been related to memory improvement (e.g., Schabus et al. 2008), while some recent study claimed that especially slow spindles during SWS seem to play a crucial role for memory consolidation (Cox et al. 2012), which led the authors to suggest that the possible potentiating effects of spindles for memory consolidation are tied to their co-occurrence with slow oscillations. This interpretation would fit to our data, since successful cueing was, as mentioned above, accompanied by an increased number of poststimulus slow oscillations as well as an enhanced oscillatory power in the spindle band.

Slow oscillations have been shown to play a causal role in processes of declarative memory consolidation during sleep (Marshall et al. 2006; Ngo et al. 2013), and might therefore also provide an important temporal time frame for stabilizing and consolidating externally induced memory reactivations by verbal cueing. To further examine the exact temporal relationships between verbal cueing during sleep and slow oscillations, future studies will need to systematically vary the onset of verbal cues presented during sleep in accordance to the up and down states of the ongoing slow oscillations.

Additionally, the results of the EEG time–frequency analysis indicate that successful cueing during sleep (i.e., cueings leading to enhanced memory performance) is accompanied by poststimulus increase in induced theta power at right frontal and left parietal regions. Induced theta during waking has been linked to the encoding and retrieval of new declarative information (Klimesch 1999; Nyhus and Curran 2010). In addition, theta oscillations have been suggested to play a functional role in controlling, maintaining and storing memory content during wakefulness (Nyhus and Curran 2010; Lisman and Jensen 2013 for reviews). During sleep, ongoing theta rhythms have been mainly associated with hippocampal activity during REM sleep, whereas the role of theta activity during NonREM sleep is less clear (Cantero et al. 2003). However, some recent studies have indeed implicated theta activity during NonREM sleep in processes of memory consolidation. Faster theta frequency or increased theta power during NonREM sleep predicted better subsequent memory performance in patients with Alzheimer’s disease or amnesic mild cognitive impairment (Hot et al. 2011; Westerberg et al. 2012). Schabus et al. (2005) observed a similar results pattern in healthy subjects, leading to the author’s speculation that increased theta activity during NonREM sleep might be associated with the reactivation of newly encoded information and as a consequence with improved memory performance. Our results partly support this notion emphasizing the importance of increases in theta power after reactivation for successful memory consolidation during sleep. However, whether these processes observed during sleep are indeed similar to theta increases underlying successful memory encoding during wakefulness and whether or how they relate to hippocampal theta rhythms require further examination.

In general, the results reported here also indicate that complex auditory cues like foreign vocabulary are indeed capable of reactivating associated memories during sleep, suggesting that some processing of the presented words is preserved during sleep (at least to some extent). Similarly, previous studies presenting verbal material during sleep have suggested a preserved capacity to discriminate semantic incongruity as well as the participants own name from other names during sleep (Brualla et al. 1998; Perrin et al. 1999; Pratt et al. 1999; Ibáñez et al. 2006). The successful reactivation of memories during NonREM sleep was accompanied by an increased negativity over frontal brain regions, resulting in improved retrieval after sleep. The observed time interval, as well as the frontal topography associated with this “subsequent reactivation effect,” is similar to ERPs typically observed during encoding for later remember items (i.e., the subsequent memory effect). In particular, an increased negativity has been reported during encoding of subsequently remembered stimuli using auditory presentations (Cycowicz and Friedman 1999; Guo et al. 2005), whereas subsequent memory for visually presented items is typically accompanied by more positive going ERPs in prefrontal and medio-temporal regions (Friedman and Johnson 2000; Werkle-Bergner et al. 2006). In spite of these morphological similarities, it remains an open question whether neural generators and mechanisms underlying the subsequent reactivation effect observed during sleep are indeed similar to processes underlying encoding and retrieval during wakefulness.

To better understand the underlying function of the reported enhanced late negativity associated with successful cueing during sleep, we can only refer to studies using auditory stimuli to investigate the extent of information processing during sleep. Some of those studies focused on the formation of stimulus representations in sensory memory by performing different kinds of oddball paradigms (for a review see Atienza et al. 2001). In a study by Niiyama et al. (1995), participants were trained to react to rare sound stimuli during wake. Re-exposure to rare sounds during sleep stage N2 was associated with an enhanced late negativity over frontal electrodes (labeled as N350 and N550) when compared with frequent tones. The authors interpreted this component as part of elicited K-complexes, which might reflect a certain level of information processing. In a similar oddball study (Karakas et al. 2007), the same results concerning the late negativity with regards to rare stimuli were obtained during sleep stage N2 and even SWS. Additionally, the authors reported that enhanced theta power was associated with the processing of rare stimuli, suggesting that theta power during sleep might be related to sensory/attentional processing of auditory stimuli. However, it is still a matter of debate whether these findings are really specific for sensory memory (Ibáñez et al. 2009). Our results extend this interpretation by suggesting that large negativities after auditory stimuli presented during sleep might also support processes of long-term memory formation.

In sum, our results demonstrate that cued reactivation of foreign words during sleep enhances vocabulary learning and that these processes are accompanied by distinct neuronal activities which involve sleep-specific slow oscillatory mechanism but possibly also share some properties with theta-related oscillations typically observed during successful encoding during wakefulness. Our findings suggest that verbal cueing of foreign vocabulary during postlearning sleep might be an

efficient and effortless tool to improve foreign vocabulary learning in educational settings as well as every-day life.

Supplementary Material

Supplementary material can be found at: <http://www.cercor.oxfordjournals.org/>.

Funding

This work was supported by a grant from the Swiss National Foundation (SNF) (PP00P1_133685) and the Clinical Research Priority Program “Sleep and Health” of the University of Zurich.

Notes

We thank Niki Hug, Janina Leeman, and Rebecca Paladini for assistance in data collection and analysis, Tobias Egli and Maurice Göldi for help in programming and Ines Wilhelm for helpful comments on earlier versions of the manuscript. *Conflict of Interest*: None declared.

References

- Antony JW, Gobel EW, O'Hare JK, Reber PJ, Paller KA. 2012. Cued memory reactivation during sleep influences skill learning. *Nat Neurosci*. 15:1114–1116.
- Atienza M, Cantero JL, Escera C. 2001. Auditory information processing during human sleep as revealed by event-related brain potentials. *Clin Neurophysiol*. 112:2031–2045.
- Bendor D, Wilson MA. 2012. Biasing the content of hippocampal replay during sleep. *Nat Neurosci*. 15:1439–1444.
- Bergmann TO, Mölle M, Diedrichs J, Born J, Siebner HR. 2012. Sleep spindle-related reactivation of category-specific cortical regions after learning face-scene associations. *Neuroimage*. 59:2733–2742.
- Brualla J, Romero MF, Serrano M, Valdizán JR. 1998. Auditory event-related potentials to semantic priming during sleep. *Electroencephalogr Clin Neurophysiol*. 108:283–290.
- Cairney SA, Durrant SJ, Hulleman J, Lewis PA. 2014. Targeted memory reactivation during slow wave sleep facilitates emotional memory consolidation. *Sleep*. 37:701–707.
- Cantero JL, Atienza M, Stickgold R, Kahana MJ, Madsen JR, Kocsis B. 2003. Sleep-dependent theta oscillations in the human hippocampus and neocortex. *J Neurosci*. 23:10897–10903.
- Cox R, Hofman WF, Talamini LM. 2012. Involvement of spindles in memory consolidation is slow wave sleep-specific. *Learn Mem*. 19:264–267.
- Cycowicz YM, Friedman D. 1999. The effect of intention to learn novel, environmental sounds on the novelty P3 and old/new recognition memory. *Biol Psychol*. 50:35–60.
- Dave A, Marholias D. 2000. Song replay during sleep and computational rules for sensorimotor vocal learning. *Science*. 290:812–816.
- Davis MH, Gaskell MG. 2009. A complementary systems account of word learning: neural and behavioural evidence. *Philos Trans R Soc Lond B Biol Sci*. 364:3773–3800.
- Diekelmann S, Born J. 2010. The memory function of sleep. *Nat Rev Neurosci*. 11:114–126.
- Diekelmann S, Büchel C, Born J, Rasch B. 2011. Labile or stable: opposing consequences for memory when reactivated during waking and sleep. *Nat Neurosci*. 14:381–386.
- Dumay N, Gaskell MG. 2007. Sleep-associated changes in the mental representation of spoken words. *Psychol Sci*. 18:35–39.
- Düzel E, Neufang M, Heinze H-J. 2005. The oscillatory dynamics of recognition memory and its relationship to event-related responses. *Cereb Cortex*. 15:1992–2002.
- Fenn KM, Hambrick DZ. 2012. Individual differences in working memory capacity predict sleep-dependent memory consolidation. *J Exp Psychol Gen*. 141:404–410.
- Friedman D, Johnson R. 2000. Event-related potential (ERP) studies of memory encoding and retrieval: a selective review. *Microsc Res Tech*. 51:6–28.
- Fuentemilla L, Miró J, Ripollés P, Vilà-Balló A, Juncadella M, Castañer S, Salord N, Monasterio C, Falip M, Rodríguez-Fornells A. 2013. Hippocampus-dependent strengthening of targeted memories via reactivation during sleep in humans. *Curr Biol*. 23:1769–1775.
- Gais S, Lucas B, Born J. 2006. Sleep after learning aids memory recall. *Learn Mem*. 13:259–262.
- Genzel L, Kroes MCW, Dresler M, Battaglia FP. 2014. Light sleep versus slow wave sleep in memory consolidation: a question of global versus local processes? *Trends Neurosci*. 37:10–19.
- Gerrard JL, Kudrimoti H, McNaughton BL, Barnes CA. 1986. Reactivation of hippocampal ensemble activity patterns in the aging rat. *Behav Neurosci*. 115:1180–1192.
- Gevins A, Smith ME. 2000. Neurophysiological measures of working memory and individual differences in cognitive ability and cognitive style. *Cereb Cortex*. 10:829–839.
- Gómez RL, Bootzin RR, Nadel L. 2006. Naps promote abstraction in language-learning infants. *Psychol Sci*. 17:670–674.
- Guo C, Voss JL, Paller KA. 2005. Electrophysiological correlates of forming memories for faces, names, and face-name associations. *Brain Res*. 22:153–164.
- Henderson LM, Weighall AR, Brown H, Gareth Gaskell M. 2012. Consolidation of vocabulary is associated with sleep in children. *Dev Sci*. 15:674–687.
- Hot P, Rauchs G, Bertran F, Denise P, Desgranges B, Clochon P, Eustache F. 2011. Changes in sleep theta rhythm are related to episodic memory impairment in early Alzheimer's disease. *Biol Psychol*. 87:334–339.
- Ibáñez A, López V, Cornejo C. 2006. ERPs and contextual semantic discrimination: degrees of congruence in wakefulness and sleep. *Brain Lang*. 98:264–275.
- Ibáñez AM, Martín RS, Hurtado E, López V. 2009. ERPs studies of cognitive processing during sleep. *Int J Psychol*. 44:290–304.
- Iber C, Ancoli-Israel S, Chesson A, Quan SF. 2007. The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specification. Westchester (IL): American Academy of Sleep Medicine.
- Jacoby LL, Dallas M. 1981. On the relationship between autobiographical memory and perceptual learning. *J Exp Psychol Gen*. 110:306–340.
- Karakaş S, Cakmak ED, Bekçi B, Aydın H. 2007. Oscillatory responses representing differential auditory processing in sleep. *Int J Psychophysiol*. 65:40–50.
- Karpicke JD, Roediger HL. 2008. The critical importance of retrieval for learning. *Science*. 319:966–968.
- Klimesch W. 1999. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res Rev*. 29:169–195.
- Klimesch W, Russegger H, Doppelmayr M, Pachinger T. 1998. A method for the calculation of induced band power: implications for the significance of brain oscillations. *Electroencephalogr Clin Neurophysiol*. 108:123–130.
- Kudrimoti HS, Barnes CA, McNaughton BL. 1999. Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. *J Neurosci*. 19:4090–4101.
- Lisman JE, Jensen O. 2013. The theta-gamma neural code. *Neuron*. 77:1002–1016.
- Macmillan NA, Creelman CD. 2005. *Detection theory: a user's guide*. Cambridge, UK: Cambridge University Press.
- Margoliash D. 2010. Sleep, learning, and birdsong. *ILAR J*. 51:378–386.
- Margoliash D, Schmidt MF. 2010. Sleep, off-line processing, and vocal learning. *Brain Lang*. 115:45–58.
- Marshall L, Helgadóttir H, Mölle M, Born J. 2006. Boosting slow oscillations during sleep potentiates memory. *Nature*. 444:610–613.
- Ngo HV, Martinetz T, Born J, Mölle M. 2013. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*. 78:545–553.
- Niiyama Y, Fushimi M, Sekine A, Hishikawa Y. 1995. K-complex evoked in NREM sleep is accompanied by a slow negative potential

- related to cognitive process. *Electroencephalogr Clin Neurophysiol.* 95:27–33.
- Nyhus E, Curran T. 2010. Functional role of gamma and theta oscillations in episodic memory. *Neurosci Biobehav Rev.* 34:1023–1035.
- O'Neill J, Pleydell-Bouverie B, Dupret D, Csicsvari J. 2010. Play it again: reactivation of waking experience and memory. *Trends Neurosci.* 33:220–229.
- Oudiette D, Antony JW, Creery JD, Paller KA. 2013. The role of memory reactivation during wakefulness and sleep in determining which memories endure. *J Neurosci.* 33:6672–6678.
- Oudiette D, Paller KA. 2013. Upgrading the sleeping brain with targeted memory reactivation. *Trends Cogn Sci.* 17:142–149.
- Pavlides C, Winson J. 1989. Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *J Neurosci.* 9:2907–2918.
- Perrin F, Bastuji H, Garcia-Larrea L. 2002. Detection of verbal discordances during sleep. *Neuroreport.* 13:1345–1349.
- Perrin F, Garcia-Larrea L, Mauguiere F, Bastuji H. 1999. A differential brain response to the subject's own name persists during sleep. *Clin Neurophysiol.* 110:2153–2164.
- Peyrache A, Khamassi M, Benchenane K, Wiener SI, Battaglia FP. 2009. Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nat Neurosci.* 12:919–926.
- Pinker S. 2000. Survival of the clearest. *Nature.* 404:441–442.
- Pratt H, Berlad I, Lavie P. 1999. "Oddball" event-related potentials and information processing during REM and non-REM sleep. *Clin Neurophysiol.* 110:53–61.
- Rasch B, Born J. 2013. About sleep's role in memory. *Physiol Rev.* 93:681–766.
- Rasch B, Büchel C, Gais S, Born J. 2007. Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science.* 315:1426–1429.
- Rattenborg NC, Martinez-Gonzalez D, Roth TC, Pravosudov VV. 2011. Hippocampal memory consolidation during sleep: a comparison of mammals and birds. *Biol Rev Camb Philos Soc.* 86:658–691.
- Rihm JS, Diekelmann S, Born J, Rasch B. 2014. Reactivating memories during sleep by odors: odor specificity and associated changes in sleep oscillations. *J Cogn Neurosci.* 23:1–14.
- Ritter SM, Strick M, Bos MW, Van Baaren RB, Dijksterhuis A. 2012. Good morning creativity: task reactivation during sleep enhances beneficial effect of sleep on creative performance. *J Sleep Res.* 21:643–647.
- Rudoy JD, Voss JL, Westerberg CE, Paller KA. 2009. Strengthening individual memories by reactivating them during sleep. *Science.* 326:1079.
- Schabus M, Hoedlmoser K, Pecherstorfer T, Anderer P, Gruber G, Parapatics S, Sauter C, Kloesch G, Klimesch W, Saletu B et al. 2008. Interindividual sleep spindle differences and their relation to learning-related enhancements. *Brain Res.* 1191:127–135.
- Schabus M, Hoedlmoser K, Pecherstorfer T, Kloesch G. 2005. Influence of midday naps on declarative memory performance and motivation. *Somnologie.* 9:148–153.
- Schönauer M, Geisler T, Gais S. 2013. Strengthening procedural memories by reactivation in sleep. *J Cogn Neurosci.* 26:143–53.
- Shatz M. 2001. Psychology of vocabulary acquisition. *Int Encycl Soc Behav Sci.* 16292–16294
- Stickgold R, Walker MP. 2013. Sleep-dependent memory triage: evolving generalization through selective processing. *Nat Neurosci.* 16:139–145.
- Unsworth N, Heitz RP, Schrock JC, Engle RW. 2005. An automated version of the operation span task. *Behav Res Methods.* 37:498–505.
- Van Dongen EV, Takashima A, Barth M, Zapp J, Schad LR, Paller KA. 2012. Memory stabilization with targeted reactivation during human slow-wave sleep. *Proc Natl Acad Sci USA.* 109:10575–10580. 2012.
- Werkle-Bergner M, Müller V, Li S-C, Lindenberger U. 2006. Cortical EEG correlates of successful memory encoding: implications for lifespan comparisons. *Neurosci Biobehav Rev.* 30: 839–854.
- Westerberg CE, Mander BA, Florczak SM, Weintraub S, Mesulam M-M, Zee PC, Paller KA. 2012. Concurrent impairments in sleep and memory in amnesic mild cognitive impairment. *J Int Neuropsychol Soc.* 18:1–11.
- Wilson MA, McNaughton BL. 1994. Reactivation of hippocampal ensemble memories during sleep. *Science.* 265:676–679.