RESEARCH ARTICLE

No difference between slow oscillation up- and down-state cueing for memory consolidation during sleep

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Summary

The beneficial effects of sleep for memory consolidation are assumed to rely on the reactivation of memories in conjunction with the coordinated interplay of sleep rhythms like slow oscillations and spindles. Specifically, slow oscillations are assumed to provide the temporal frame for spindles to occur in the slow oscillations up-states, enabling a redistribution of reactivated information within hippocampal–neocortical networks for long-term storage. Memory reactivation can also be triggered externally by presenting learning-associated cues (like odours or sounds) during sleep, but it is presently unclear whether there is an optimal time-window for the presentation of such cues in relation to the phase of the slow oscillations. In the present within-subject comparison, participants ($n = 16$) learnt word-pairs visually presented with auditory cues of the first syllable. These syllables were subsequently used for real-time cueing either in the up- or down-state of endogenous slow oscillations. Contrary to our hypothesis, we found differences in memory performance neither between up- and down-state cueing, nor between word-pairs that were cued versus uncued. In the up-state cueing condition, higher amounts of rapid eye movement sleep were associated with better memory for cued contents, whereas higher amounts of slow-wave sleep were associated with better memory for uncued contents. Evoked response analyses revealed signs of cue processing in both conditions. Interestingly, both up- and down-state cueing evoked a similar spindle response with the induced slow oscillations up-state at ~1000 ms post-cue. We speculate that our cueing procedure triggered generalised reactivation processes that facilitated the consolidation of both cued and uncued memories irrespective of the slow oscillation phase.

KEYWORDS
slow waves, real-time cueing, targeted memory reactivation, auditory cueing, declarative memory

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1 | INTRODUCTION

Sleep supports the consolidation and integration of newly acquired memories into pre-existing knowledge networks (Diekelmann & Born, 2010; Walker & Stickgold, 2010). This process has been proposed to rely on the reactivation of learning-related neuronal representations in a hippocampal–neocortical dialogue (Lewis & Durrant, 2011; Rasch & Born, 2013). Memory reactivation mainly occurs during non-rapid eye movement (NREM) sleep in conjunction with the coordinated interplay of specific oscillatory brain rhythms like neocortical slow oscillations (SOs), thalamo-cortical spindles and hippocampal sharp-wave ripples (SWRs; Klinzing, Niethard, & Born, 2019; Mölle, Marshall, Gais, & Born, 2002). SOs are the hallmark feature of slow-wave sleep (SWS) and are characterised by alternating "up-states" of depolarisation with synchronised neuronal firing and "down-states" of hyperpolarisation associated with widespread neuronal silence (Steriade, McCormick, & Sejnowski, 1993). In the electroencephalogram (EEG), this neural firing pattern translates into a <1 Hz brain oscillation with up- and down-states corresponding to its positive and negative half-waves, respectively (Nir et al., 2011). The synchronising feature of SOs is assumed to provide the temporal frame for spindles and SWRs to occur preferentially in the SO up-states, enabling the reactivated information to be redistributed and strengthened in the hippocampal–neocortical network for long-term memory storage in a process of active system consolidation (Antony, Schönauer, Staresina, & Cairney, 2019; Diekelmann & Born, 2010; Sirota & Buzsáki, 2005; Sirota, Csicsvari, Buhl, & Buzsáki, 2003; Staresina et al., 2015).

Memory reactivation during sleep can also be triggered externally by presenting sensory reminder cues, a procedure that has been termed targeted memory reactivation (TMR; Oudiette, Santostasi, & Paller, 2013). In this procedure, specific stimuli that are associated with a prior learning experience are presented again during subsequent sleep as reminder cues, which facilitate the consolidation and subsequent retrieval of the associated memories (Hu, Cheng, Chiu, & Paller, 2020; Klinzing & Diekelmann, 2019; Schouten, Pereira, Tops, & Louzada, 2017). Different types of stimuli have been used for cueing during sleep, including contextual odours (Rasch, Büchel, Gais, & Born, 2007; Diekelmann et al. 2011) and more item-specific auditory cues like sounds and words (Cairney, Guttesen, El Marj, & Staresina, 2018; Rudoy, Voss, Westerberg, & Paller, 2009; Schreiner & Rasch, 2015; Creery et al. 2015). Most previous studies presented the cues randomly during NREM sleep, without considering ongoing oscillatory activity. Only recently evidence was found that the timing of cue presentation in relation to ongoing sleep oscillations might determine whether or not cueing is effective in improving memory for the associated learning experience (Antony et al., 2018; Göldi, van Poppel, Rasch, & Schreiner, 2019; Shimizu et al., 2018).

Considering that SOs, and particularly their up-states, are assumed to play a key role in orchestrating memory reactivation, the timing of cueing in relation to the phase of SOs may be especially important. Batterink, Creery, and Paller (2016) applied a post hoc approach by classifying the effectiveness of randomly presented

sound cues in relation to ongoing SO activity, observing an optimal phase for cue presentation shortly before the SO down-state. In a more experimental approach, Göldi et al. (2019) directly manipulated the timing of cueing by presenting vocabulary cues in phase with ongoing SO up- or down-states, observing strongest memory benefits for cueing during SO up-states, with weaker effects for down-state cueing. This finding is consistent with theoretical predictions, but contrary to the observations by Batterink et al., leaving it an open question whether there is an optimal time-point for cueing in the SO.

In the present study, we applied a real-time cueing approach similar to Göldi et al. (2019), presenting cues either during SO up- or down-states. In a novel learning paradigm of word-pairs associated with spoken syllables, we presented the syllables as cues during subsequent NREM sleep. We expected better memory performance for cued compared to uncued word-pairs, specifically when the cues were presented in the SO up-state.

2 | SUBJECTS AND METHODS

2.1 | Participants

A total of 20 healthy men participated in the study, with data from four being excluded from the final analyses because of sleeping problems (frequent or long-lasting wake phases in three) or technical problems (sound file misfunction in one). Of the remaining 16 subjects (mean +- sem [SD, range]) age 24.4 [0.8, 18–30] years), all subjects were German native speakers and non-smokers, did not take any medication at the time of the experiments, had average hearing abilities, and did not have any history of sleep disturbances and major depression. Subjects reported to have followed a regular sleep–wake cycle for at least 6 weeks prior to the experiments, did not carry out night work or shift work, and did not habitually take naps during the day. On the day of the experiments, subjects were instructed to abstain from caffeinated drinks after 2:00 p.m.; alcohol as well as extreme sports were not permitted for 24 h prior to the experimental sessions.

The study was carried out in accordance with the recommendations of the Declaration of Helsinki. The protocol was approved by the Local Ethics Committee of the Medical Faculty of the University of Tübingen. All subjects gave written informed consent in accordance with the Declaration of Helsinki and were paid for participation.

2.2 | Design and procedure

Each subject participated in two experimental nights in the sleep laboratory in a randomised cross-over design, with the 2 nights being separated by 14 ± 1 day. On the first night, continuous real-time auditory cueing was applied during the "up-state" of simultaneously detected SOs, whereas on the next night cueing occurred during the SOs "down-state". Real-time cueing was applied in a single-blind

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fashion, i.e., participants did not know whether they received "up-state" or "down-state" cueing in a given night. The order of cueing conditions was balanced across subjects.

Prior to the experimental nights, subjects spent an adaptation night in the sleep laboratory to become accustomed with the experimental setup, including the placement of electrodes for polysomnographic recordings as well as in-ear headphones. Additionally, each subject's individual hearing threshold level was tested and each subject's individual SO waveform average was measured (Section 2.4.3).

In the experimental nights, subjects arrived at the sleep laboratory 3.5 h prior to their usual sleeping time (~7:30 p.m., Figure 1a). They were attached to the electrodes for polysomnographic recordings and performed on the control tasks before training on the syllable paired-associates learning task (Section 2.3.1). After learning, in-ear headphones were attached, and subjects went to bed at their usual sleeping time. Real-time auditory cueing started ~5 min after the subject first entered SWS. Cueing was paused whenever signs of arousal, awakening, Stage 1 or REM sleep occurred and resumed

after stable SWS was detected again. Cueing was terminated 210 min after sleep onset.

Subjects were awakened after ~8 h of sleep from sleep Stages 1 or 2. Electrodes were removed, and subjects were allowed to freshen up. Approximately 30 min after awakening, to allow for the dissipation of sleep inertia, subjects performed on the control tasks again and finally recall of the syllable paired-associates learning task was tested.

2.3 | Experimental tasks

2.3.1 | Syllable paired-associates learning task

Subjects were trained on a declarative memory task similar to paired-associates learning tasks used in previous studies (Marshall, 2004; Ngo, Martinetz, Born, & Mölle, 2013; Plihal & Born, 1997). The task included two lists of word-pairs, with the order of lists balanced
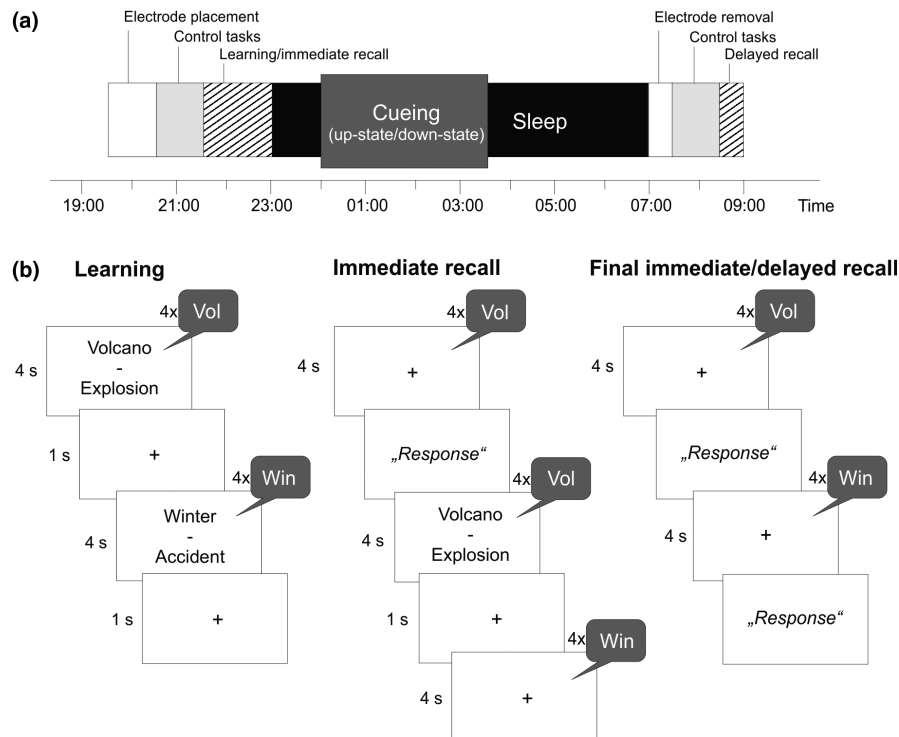

FIGURE 1 Experimental procedure and learning task. (a) Subjects participated in two experimental nights (up-state versus down-state cueing) with otherwise identical procedures, separated by ~14 days. After arrival in the sleep laboratory, electrodes were attached, and subjects performed on the control tasks before starting the learning and immediate recall session. This session ended with the final immediate recall. Subjects went to bed at ~11:00 p.m. and were allowed to sleep for 8 h. In the first night-half, cue syllables were presented continuously in phase with slow oscillation up-states (in one experimental night) or down-states (in the other experimental night) during periods of slow-wave sleep (SWS) and Stage 2 sleep. Cues were presented only for half of the learnt word-pairs while the other half of word-pairs remained uncued. After awakening in the morning, electrodes were removed, and the control tasks were applied again before delayed recall was tested. (b) During learning of the syllable paired-associates learning task, subjects were presented with 40 word-pairs once consecutively. While each word pair was presented, the first syllable of the first word was played four times via in-ear headphones. During the immediate recall test, the syllable associated with a specific word-pair was played again four times. Subsequently, subjects were asked to give their response by naming the corresponding word pair (depicted as "*Response*"). There was no time limit for the response. Correct feedback was then presented, including the syllable being played another four times. Immediate recall was repeated until subjects reached a 60% learning criterion at which point the final immediate recall test ensued. At this final test no feedback was presented. Delayed recall in the morning after sleep was identical to the final immediate recall

across subjects and experimental nights. Each list contained 40 moderately semantically related German words, e.g., volcano – explosion (German: "Vulkan – Explosion"). During learning, all 40 word-pairs were presented once successively in randomised order on a computer screen for 4 s (Figure 1b left panel). Simultaneously with the presentation of each word pair, the first syllable of the first word was played four times over in-ear headphones, pronounced slowly and clearly by a neutral female voice (in the above example "vol", German: "Vul"). Each syllable had a length of ~500 ms, with 500 ms breaks in between repetitions.

After learning, a maximum of four immediate recall runs on the whole list followed, for which only the first syllable of each word pair was played four times and subjects had to verbally name both corresponding words (Figure 1b, middle panel). There was no time limit for the response. Irrespective of whether the response was correct or not, subjects then received feedback, with the correct word pair being presented again together with the syllable being played another four times. The whole word-pair list was repeated in this way until the subject reached a learning criterion of 60% correct responses, with a correct response referring to both words of a pair being remembered correctly. In this case, another final immediate recall test of the list ensued, which was identical to the immediate recall runs but without any feedback being presented (Figure 1b, right panel). Again, there was no time limit for the response. The next morning, memory of the word-pairs was tested with a delayed recall test that was identical to the final immediate recall test, i.e. without any feedback. To prevent serial learning, the order of word-pairs was randomised within each run.

To analyse performance changes across sleep, the retention rate was calculated as the number of correctly recalled word-pairs in the delayed recall test relative to the number of correctly recalled word-pairs in the final immediate recall test, with performance at final immediate recall set to 100%. This measure was calculated separately for cued and uncued word-pairs.

2.3.2 | Control tasks

General mood was assessed with the German version of the Positive and Negative Affect Schedule, including a list of 20 adjectives measuring positive and negative affect on 5-point scales (Krohne, Egloff, Kohlmann, & Tausch, 1996). Subjective sleepiness was assessed with the Stanford Sleepiness Scale on an 8-point scale (Hoddes, Dement, & Zarcone, 1972). Vigilance was measured with a modified version of the Psychomotor Vigilance Task that lasted 5 min and included a red millisecond timer counting upward on a computer screen (Roach, Dawson, & Lamond, 2006). Subjects had to press a key as soon as the timer started (randomly every 2–10 s), and reaction times were recorded. Working memory capacity was assessed with a computerised version of the Digit Span Task (Sterne, 1969), in which subjects were required to memorise and recall sequences of single-digit numbers either in the order as presented (forward span) or in reverse order (backward span). The sequences ranged from three to a maximum of nine digits. Finally, word fluency was tested with the Regensburg Word Fluency Test (Aschenbrenner, Tucha, & Lange, 2000), in which subjects were asked to write down as many words as possible for a specific category within 2 min. Note that data from four subjects are missing for vigilance and working memory due to technical problems.

2.4 | Electrophysiological data

2.4.1 | Polysomnographic recordings

During experimental nights, sleep was recorded with standard polysomnography, including EEG (positions F3, Fz, F4, C3, Cz, C4, P3, Pz, P4) with two reference electrodes at the mastoids, electromyography (two electrodes on the chin) and electro-oculography (two electrodes diagonally above and below the left and right eyes). Two ground electrodes were placed at the forehead. During adaptation nights, a similar setup was applied with a reduced number of EEG electrodes (positions Fpz, C3, Cz, C4). Signals were continuously recorded with a "BrainAmp" DC Amplifier (Brain Products, Germany), sampled at 500 Hz, filtered between 0.03 and 250 Hz, and stored on a personal computer for later offline analysis together with the cueing triggers.

Sleep stages were scored offline by two experienced scorers who were blind to the experimental conditions. Scoring was done according to standard criteria as Wake, Stage 1, Stage 2, SWS and rapid eye movement (REM) sleep (Rechtschaffen & Kales, 1968).

2.4.2 | Real-time cueing setup

For detection of SOs, an additional electrode was attached to position AFz on the scalp (on the connecting line between nasion and inion centred between Fpz and Fz) and referenced to linked mastoids. The corresponding prefrontal EEG signal was conducted to a second EEG recording system with a "Digitimer D360" amplifier (Digitimer Ltd., Hertfordshire, UK) for signal amplification and filtering between 0.25 and 4 Hz. A high-performance data acquisition interface "Power 1401mk-II" (Cambridge Electronic Design Ltd.) sampled the filtered signal at 200 Hz and performed the SO detection and cue presentation based on a custom-made script running under "Spike2 Software Version 7" (Cambridge Electronic Design Ltd.; Besedovsky et al., 2017; Ngo et al., 2013).

2.4.3 | Auditory cueing

Auditory cues were presented binaurally via customary in-ear headphones (MDR-EX35, Sony). The timing of cue presentation was adjusted to each subject's individual SO morphology to ensure cue onset in phase with SO positive or negative peaks. A SO was detected once the filtered prefrontal EEG signal passed a threshold

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of −80 μV and after a change in gradient from negative to positive had occurred, i.e. a local minimum was present. Upon detection of such a SO negative peak, the cue was presented immediately (for down-state cueing) or after each subject's individual delay time between the negative peak and the following positive peak (for up-state cueing). To determine each subject's individual delay times, the SO detection algorithm was applied during the first SWS epoch of each subject's adaptation night and the average delay between on-line identified SO negative peaks and the subsequent positive peaks was extracted. After cue presentation, there was a non-stimulation interval of at least 2.5 s before the next cue could be presented. Please note, both up- and down-state cueing are based on an online identification of SO down-state troughs due to its marked morphology (high [negative] amplitudes and a distinct waveform), which is more accessible with a real-time algorithm (Bergmann et al., 2012; Ngo et al., 2013, 2015). Although, the individual delay time introduced in the up-state cueing condition thus leads to a certain phase variability with respect to the SO up-state peak, a previous account has demonstrated that the susceptibility to auditory stimuli is ensured across a window of 600 ms (Navarrete et al., 2020).

For both cueing conditions, the cues consisted of the syllables as presented during the learning phase. Of the 40 syllables in each condition, 20 syllables were chosen to be cued, with the other 20 syllables serving as uncued controls. The 20 cue syllables were individually determined for each subject by randomly selecting half of the syllables of word-pairs correctly remembered at the final immediate recall test, and half of the word-pairs not remembered at this final test. During cueing, the 20 cue syllables were played in cycles of random order. The sound volume for cueing started at 15 dB sound pressure level above each subject's individual hearing threshold level as determined during the adaptation night. If this volume resulted in a clearly visible event-related potential (ERP) without any signs of arousal, it remained constant throughout the cueing protocol. In case of no visible ERP, the volume was calibrated upward in steps of 3 dB until an ERP without arousals was obtained. If the volume increase resulted in arousals or awakening, the volume was decreased to the last level without arousal responses. On average each cue was presented 22.02 ± 1.83 times.

2.4.4 | EEG data analysis

All EEG analyses were performed on data pre-processed by band-pass filtering between 0.3 and 30 Hz and limited to artefact-free NREM epochs. To confirm the overall responsiveness to auditory cueing, the EEG signals were first averaged based on a time window from −1.5 to 1.75 s relative to the cue onset. Furthermore, to confirm a selective cueing during SO up- and down-states, the instantaneous phase was extracted from the EEG signals band-pass filtered between 0.3 and 1.25 Hz using a Hilbert transform and the phase distribution during the cue onsets was determined.

Next, to examine frequency-resolved responses, specifically in the 12–16 Hz spindle range, time-frequency representations (TFRs) were calculated based on Morlet wavelets time-locked to the cue onset from −1.5 to 1.75 s in 50-ms steps and a frequency range from 5 to 20 Hz in 0.5-Hz steps (Ngo, Fell, & Staresina, 2020). The number of wavelet cycles was set adaptively to half of the corresponding frequency (or rounded up to the next integer value) but at least five cycles, resulting in time windows of approximately 500 ms. Finally, all TFRs were transformed to the relative change with respect to a baseline from −2 to −1.5 s.

2.5 | Statistical analysis

Data were analysed with two-tailed paired sample t tests and repeated measures analysis of variance (ANOVA), with the factors "up-state/down-state" and "cued/uncued" for the memory data and the factors "up-state/down-state" and "pre/post" for the control tasks. Correlation analyses explored the association between memory change across sleep (retention rate) and the amount of Stage 2, SWS and REM sleep. These were performed using Pearson's product-moment correlation coefficient and are reported uncorrected. Phase-distribution of cue onsets for each subject was examined for non-uniformity with a Rayleigh test for circular data. Finally, EEG averages and TFRs were statistically assessed for differences in amplitude or relative power between the up- and down-state condition with a two-tailed paired sample t test (significance threshold $p < 0.05$), including a Monte-Carlo based cluster-permutation procedure to correct for multiple comparisons (cluster threshold $p < 0.05$). The significance level was set to $p = 0.05$.

3 | RESULTS

3.1 | Memory

During learning, subjects needed a mean (SEM) of 2.94 (0.25) and 3.00 (0.24) immediate recall trials to reach the learning criterion in the up- and down-state cueing condition, respectively ($p = 0.75$). Performance at the end of learning, i.e., in the final immediate recall test, was also comparable between conditions (mean [SEM] number of correctly recalled word-pairs: up-state 32.13 [0.89], down-state 33.31 [1.11]; $p = 0.16$). There was also no difference between cued and uncued word-pairs in final immediate recall (mean [SEM] up-state: cued 16.19 [0.44], uncued 15.94 [0.47]; down-state: cued 16.63 [0.57], uncued 16.67 [0.55]; all $p > 0.16$).

Contrary to our hypothesis, memory retention across sleep did not differ between up- and down-state cueing, or between cued and uncued word-pairs. Following up-state cueing, subjects remembered a mean (SEM) of 95.96% (2.82%) of the cued word-pairs and 95.53% (2.30%) of the uncued word-pairs (with the number of word-pairs recalled at the final immediate recall test before sleep representing 100%). After down-state cueing, they remembered a mean (SEM) of 93.98% (2.39%) of the cued and 96.50% (2.87%) of the uncued word-pairs (Figure 2; main effect "up-state/down-state":
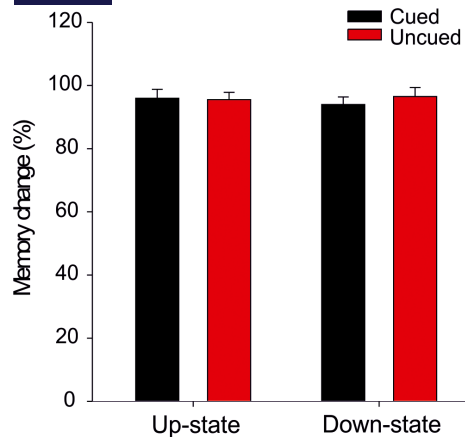
FIGURE 2 Effects of up- and down-state cueing on memory. Memory retention across sleep did not differ following cueing in phase with the slow oscillation up- and down-state. There was also no difference between cued and uncued word pairs. Memory retention refers to performance at delayed recall relative to final immediate recall, with final immediate recall set to 100%

$F_{(1,15)} = 0.06$, $p = 0.81$; main effect "cued/uncued": $F_{(1,15)} = 0.15$, $p = 0.70$; interaction: $F_{(1,15)} = 0.51$, $p = 0.49$. This pattern of results remained the same when only a subgroup of subjects was analysed, who showed a manifest evoked response to the presentation of the cues during sleep in both cueing conditions ($n = 12$, see below Section 3.2 and Figure S1). Subjects in this subgroup remembered a mean (SEM) of 96.93% (2.67%) and 95.12% (3.04%) of the cued and uncued word-pairs following up-state cueing and 95.47% (1.72%) and 98.07% (3.27%) of the cued and uncued word-pairs following down-state cueing (all $p > 0.40$).

An exploratory item-based analysis revealed that successful recall was mostly due to memory maintenance, i.e., word-pairs that were correctly recalled at the final immediate recall before sleep as well as at delayed recall after sleep (on average 14.9 word-pairs). There were only very few gains in memory, i.e., word-pairs that were not remembered before sleep but after sleep (on average 0.6 word-pairs), and very few losses, i.e., word-pairs that were remembered before sleep but not after sleep (on average 1.5 word-pairs). There were no differences between conditions in memory maintenance, gains, and losses ($p > 0.25$ for all main effects and interactions; all data are presented in Table S1).

3.2 | Cue-related responses

Examining the distribution of cue onsets with respect to the instantaneous SO phase confirmed a selective presentation of cues during the SO up-states in the up-state condition (resultant vector = 0.96, mean phase = −0.42, $p < 0.001$) and concurrent with SO down-states in the down-state condition (resultant vector = 0.99, mean phase = −2.93, $p < 0.001$) across participants (Figure 3a inset). Similarly, a non-uniform phase distribution was confirmed in each individual participant (all $p < 0.001$). Averaging the EEG signal time-locked to the cue
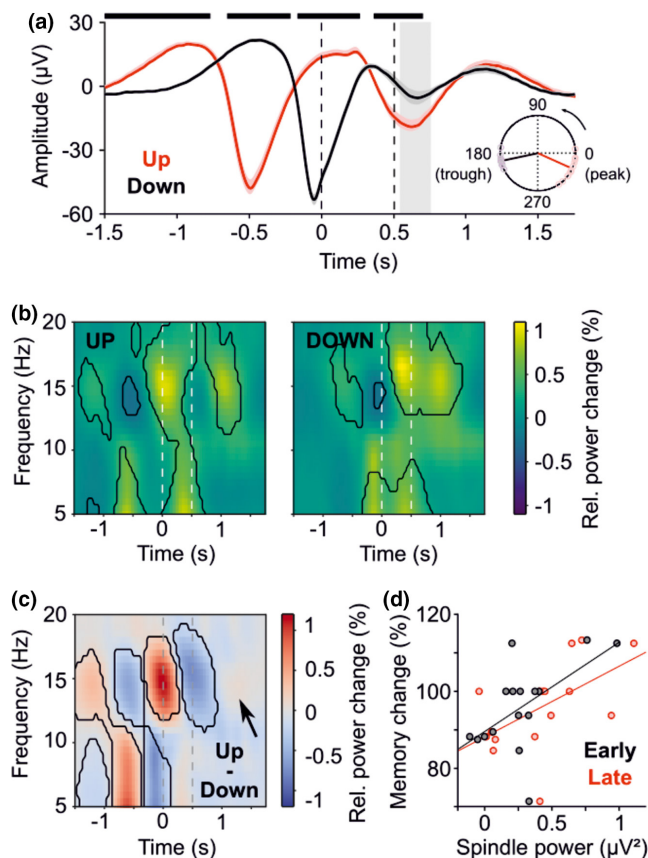

FIGURE 3 Electrophysiological effects of up- and down-state cueing. (a) Averaging the EEG signal time-locked to cue onset (here shown at Cz as a representative channel) revealed successful targeting of up-states (red line) and down-states (black line). Statistical comparison between conditions is indicated by the top black lines, whereas the cue on- and -offset are marked by the vertical dashed lines. Differences between conditions occur mainly before and during cueing. Additionally, up-state cueing evoked a stronger negative deflection at ~500 ms after cue onset (grey box). The inset depicts the distribution of the slow oscillation (SO) phase during cue onset across subjects as a phase plot, which confirms the cue presentation during either the up-state (red) or down-state (black). Note, the arrow marks the counter clockwise transition from up- to down-state. (b) Time-frequency representations (TFRs) determined for the up-state (left) and down-state cueing conditions (right) reveal several power clusters between 5 and 8 Hz as well was between ~12 and 16 Hz, reflecting the occurrence of SO down-states and SO up-state locked spindle activity, respectively. White vertical dashed lines mark cue on- and offset. Colour bar depicts the relative change in power with respect to a baseline interval from −2 to −1.5 s before the cue. (c) Time-frequency representation of the difference between up- and down-state cueing (red indicating higher power in the up-state condition and vice versa for blue) reveal that significant clusters (indicated by black contours) emerge mainly before and during cueing due to the targeting of SO up- and down-states. Only a brief difference in spindle power is found directly after the cue, whereas the evoked spindle response with the subsequent SO up-state between 1 and 1.5 s is comparable between conditions (black arrows). (d) For the up-state cueing condition, memory retention for cued pairs is predicted by the early (at ~500 ms with $r = 0.58$, $p = 0.02$, black circles) and late spindle power (at ~1000 ms with $r = 0.55$, $p = 0.027$, red circles)

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onset revealed an evoked response with a negative deflection maximal at ~500–600 ms after cue onset in both cueing conditions (Figure 3a, see top horizontal black line for statistical comparison). Contrasting these averages to post hoc identified sham trials, confirmed that these deflections deviate from unstimulated sham events (Figure S2a,b), confirming that the cues were processed at least on the sensory level. Inspection of the negative deflection in the initial averages ~500 ms post-cue revealed a stronger trough after up-state cueing (Figure 3a, light grey box). However, a follow-up analysis, in which the sham traces were subtracted from the corresponding cueing condition in order to derive the pure auditory evoked response revealed comparable responses between cueing conditions (Figure S2C), confirming that, on the one hand, in both conditions cues were processed, but on the other hand, this perception is not modulated by the targeted SO phase. Of note, four subjects did not show a pronounced evoked response in at least one of the experimental conditions (Figure S1). However, excluding these subjects from the analyses did not affect the behavioural outcome (Section 3.1).

Inspecting the spectral composition of potential effects of auditory cueing with time-frequency representations (aligned to cue onset) from 5 to 20 Hz revealed power differences between up- and down-state cueing in repeatedly occurring clusters bound to two frequency ranges. One the one hand, a lower cluster with frequencies between the lower limit of 5 Hz up to ~8 Hz, which is driven by high amplitude SO down-states. On the other hand, a cluster between ~12 and 16 Hz reflecting nested spindle activity concurring with SO up-states. As expected, these differences emerged mainly before or during cue-onset, stemming from the inherent electrophysiological differences caused by targeting up- and down-states (Figure 3b). However, in addition, directly after the cue at ~500 ms, higher spindle power was found in the down-state cueing condition, likely caused by a delayed SO peak compared to the up-state cueing condition. Strikingly, both up- and down-state cueing evoked a similar spindle response with the induced SO up-state at ~1000 ms post-cue (Figure 3c, black arrow). Intriguingly, exploratory analyses revealed a significant correlation between 12 and 18 Hz spindle power for an early (0.35–0.85 s) and late (1–1.5 s) time window in the up-state cueing condition with memory retention for the cued word-pairs (early: $r = 0.58$, $p = 0.02$ and late: $r = 0.55$, $p = 0.027$, Figure 3b). No association was found by correlating the same spindle responses with the performance on uncued word-pairs or the down-state cueing condition (all $p > 0.13$).

The number of cue presentations during sleep was higher in the down-state condition (mean [SEM, range] 477.94 [54.07, 118–850]) than in the up-state condition (mean [SEM, range] 433.50 [56.69, 95–833], $p = 0.005$). However, the number of cue presentations was overall high (on average 22 times/cue) and was not related to memory changes across sleep (all $p > 0.12$).

3.3 | Sleep stages

Subjects slept a mean (SEM) of 458.06 (7.23) and 454.38 (8.62) min in the up- and down-state condition, respectively ($p = 0.58$), and showed comparable amounts of Stage 1, Stage 2, SWS, REM sleep and wake time (all $p > 0.11$; Table 1). Exploratory correlation analyses revealed significant associations in the up-state cueing condition between memory change for the cued word-pairs and the amount of REM sleep ($r = 0.55$, $p = 0.027$), as well as between memory change for the uncued word-pairs and the amount of SWS ($r = 0.53$, $p = 0.036$; Figure 4). Interestingly, a trend towards a similar pattern of results was evident for memory change of the cued word-pairs in the up-state condition and the amount of REM sleep in the down-state condition ($r = 0.49$, $p = 0.054$), as well as for memory change of the uncued word-pairs in the up-state condition and the amount of SWS in the down-state condition ($r = 0.49$, $p = 0.054$). There were no significant correlations for memory change in the down-state cueing condition (all $p > 0.11$).

3.4 | Control tasks

Subjects showed comparable positive and negative affect in the up- and down-state cueing conditions, as well as during the learning and recall sessions (all $p > 0.38$). Subjective sleepiness was also comparable between conditions in both sessions (all $p > 0.32$). There were also no differences in mean reaction times in the vigilance task (all $p > 0.27$) and word fluency (all $p > 0.73$), as well as in working memory capacity, including forward span and backward span (all $p > 0.06$; see Table S2 for all data of the control tasks).

4 | DISCUSSION

In the present study, we used a real-time cueing paradigm to present learning-related syllable cues at different phases of the ongoing SO. Our results show no difference in memory performance depending on whether cues were presented in SO up- or down-states. We also found no difference in memory for cued and uncued word-pairs.

Inspection of the distribution of cue onsets with respect to the SO phase, as well as the evoked responses upon cueing, confirmed the expected electrophysiological effects upon real-time cueing during sleep. These data suggest that the cues were processed in cortical networks at the envisaged phase of the SO, i.e., in the up-state for up-state cueing and in the down-state for down-state cueing. Despite the processing of the cues, we did not observe differences in memory retention across sleep for the cued and uncued word-pairs. This finding is unexpected, considering that a number of previous studies found better memory for cued compared to uncued stimuli in similar experimental setups (Cairney et al., 2018; Rudoy et al., 2009; Schreiner, Lehmann, & Rasch, 2015; Schreiner & Rasch, 2015). It could be speculated that our cueing procedure was not effective in triggering reactivation and improving memory. However, rather than being ineffective, it is also possible that in our specific paradigm, cueing influenced memory for both cued and uncued word-pairs to a similar extent. Overall memory performance was very high for cued word-pairs, as well as for uncued word pairs.

TABLE 1 Sleep data

Variable, min, mean (SEM)	Up-state	Down-state	*p*
TST	458.06 (7.23)	454.38 (8.62)	0.58
S1	29.59 (3.88)	25.69 (2.85)	0.24
S2	228.31 (7.48)	216.56 (9.56)	0.13
SWS	96.44 (8.64)	107.16 (8.60)	0.12
REM sleep	82.59 (6.48)	83.59 (4.94)	0.87
Wake	17.84 (4.61)	17.84 (4.43)	1.00

used for: REM sleep, rapid eye movement sleep; S1, Stage 1 sleep; S2, Stage 2 sleep; SWS, slow-wave sleep; TST, total sleep time; Wake, time awake after sleep onset.

Paired-sample *t* tests *p* values. Note that the difference between TST and the sum of sleep stages reflects epochs scored as movement time (i.e., artefacts).
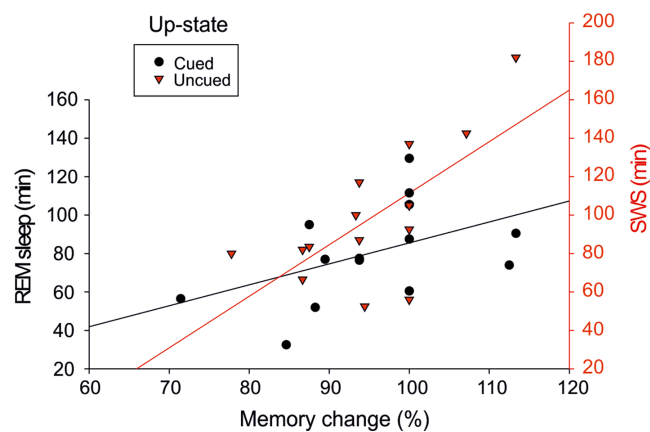

FIGURE 4 Associations between sleep and memory for up-state cueing. In the up-state condition, subjects with high amounts of rapid eye movement (REM) sleep recalled more of the cued word-pairs (left ordinate, black dots and black line, *r* = 0.55, *p* = 0.027) and subjects with high amounts of slow-wave sleep (SWS) remembered more of the uncued word-pairs (right ordinate, red dots and red line, *r* = 0.53, *p* = 0.036). There were no correlations in the down-state condition

Subjects remembered on average 95% of the cued word-pairs and 96% of the uncued word-pairs, showing almost no forgetting in both conditions. These numbers are comparable with performance for cued memories in other studies, ranging from 97% to 99%, whereas performance for uncued memories is typically lower, i.e. ~87%–90%, indicating stronger forgetting of uncued memories (Batterink et al., 2016; Göldi et al., 2019; Schreiner & Rasch, 2015).

It could be speculated that the syllables that served as cues in the present study represent more generalised contextual cues, improving memory for the entire set of learning material rather than individual associated memories. While previous auditory cueing studies have mostly used inherently meaningful stimuli like real-world sounds (e.g., "meow"), the syllable cues in the present study represent rather abstract cues that do not convey any meaning per se (e.g., "vol"). Because of this abstract nature, the syllables may have failed to reactivate the individual associated word-pairs on an item

level but instead may have activated related contents from the same learning experience, including those associations that had not been cued. This explanation is supported by previous evidence for general unspecific cueing effects, showing that the presentation of single cues can in some cases improve uncued memories from the same set of learning material (Cairney et al., 2018; Oudiette et al., 2013). Critically, this interpretation can only be tested by comparing cued and uncued memories to a sham condition without any cueing in the same subjects, which was unfortunately not included in the present study.

Despite comparable performance for cued and uncued memories, there was no difference between up- and down-state cueing for the cued memories. There is only one previous study that has applied a similar cueing approach targeting SO up- and down-states, which indeed showed stronger memory benefits for up-state cueing (Göldi et al., 2019). However, on a descriptive level, Göldi et al. (2019) observed a similar memory improvement for down-state cueing, with the improvement of up-state cueing and down-state cueing not being significantly different. It could be speculated that in the present study, both cueing conditions effectively facilitated memory reactivation and consolidation. One explanation for this interpretation relates to the question whether the presentation of cues can be precise enough to target one specific phase of the SO exclusively. In the present study, the timing of cue onset was almost perfectly aligned with the SO up- and down-state, respectively. However, the mean duration of the cues was 500 ms, meaning that the cues extended beyond the targeted phase into the transition to the next phase. Thus, in the down-state condition, the cue started in the down-state but continued into the down-to-up transition of the SO, a time window that has been suggested to be most susceptible for the presentation of external reactivation cues (Navarrete et al., 2020). The speculation that both experimental conditions facilitated memory reactivation in the present study, is corroborated by the spectral decomposition of the EEG response to the auditory cueing. Differences in the response, specifically in the spindle band, were mainly found during cue presentation, reflecting targeting of up- and down-states. Importantly, similar to previous work (Cairney et al., 2018; Schreiner & Rasch, 2015) we observed a post-cue spindle response with a long delay of 1–1.5 s that concurred with subsequent SO up-states and has been specifically linked to the initiation of memory reactivation. This delayed spindle response was similarly pronounced in both conditions. The critical role of sleep spindles for memory consolidation is further supported by the observed association between spindle activity and memory change for the up-state cueing condition and is also consistent with a body of evidence for the causal involvement of spindles in memory formation (Latchoumane, Ngo, Born, & Shin, 2017). However, we additionally found an association for spindle activity present at the cue offset, whereas consistent findings have primarily linked later spindle activity to memory processing (e.g., Cairney et al., 2018; Schreiner & Rasch, 2015). Considering that sleep spindles are governed by a refractoriness (Antony et al., 2018; Ngo et al., 2015), it is unlikely that early and late spindle increases emerge after the same cue. While one might think initially that early

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spindle activity counteracts the later spindle expression and thus interferes with memory reactivation, the positive correlation suggests that even this early spindle increase is to some extent functional. Interestingly, a recent TMR study in rodents showed that sensory cueing triggered a cortico–hippocampal–cortical loop (Rothschild, Eban, & Frank, 2017). In other words, reminder cues first activated relevant cortical representations, which preceded hippocampal reactivation. These hippocampal patterns again preceded cortical activation patterns. Moreover, recent reports in humans have provided evidence, that sleep spindles may mediate a cortico–hippocampal communication around hippocampal ripples (Helfrich et al., 2019; Ngo et al., 2020). Considering these findings, one speculative interpretation of our spindle results may be that early spindle increases reflect an activation of cue-informed cortical representation relayed to the hippocampus. Conversely, the late spindle increases mediate a bottom-up process between the hippocampus and neocortex facilitating long-term memory. To what extend this highly speculative conclusion proves to be true and questions like whether these processes represent disjunct events or how they unfold temporally, remain to be answered.

The exploratory analysis of correlations with sleep stages revealed better memory for cued word-pairs in subjects with high amounts of REM sleep and better memory for uncued word-pairs in subjects with high amounts of SWS in the up-state cueing condition. These findings are partly in line with previous findings from Göldi et al. (2019), who likewise observed associations between better memory performance and high amounts of REM sleep, although only in the down-state cueing condition. Considering that cues were only presented during NREM sleep but not during REM sleep, the observed association with REM sleep could be interpreted in line with a two-stage model of active system consolidation, assuming that REM sleep strengthens neocortical memory traces that have been reactivated and reshaped during prior NREM sleep (Almeida-Filho, Queiroz, & Ribeiro, 2018; Diekelmann & Born, 2010; Klinzing et al., 2019; Rasch & Born, 2013). The observed association between memory for uncued word-pairs and SWS supports our view that the presentation of cues in the present study also facilitated the reactivation of uncued word-pairs. The syllable cues may lead to the spreading of activation in the associative network, activating the entire learning material including uncued word-pairs, with these presumably weaker associations possibly benefitting from higher amounts of SWS to increase the number and/or intensity of reactivations. However, additional exploratory analyses revealed similar associations between memory for cued word-pairs in the up-state condition and REM sleep in the down-state condition as well as between memory for uncued word-pairs in the up-state condition and SWS in the down-state condition. This pattern of results may indicate an underlying trait factor for subjects with high amounts of REM sleep and SWS, respectively. However, considering that these associations were only observed for memory benefits in the up-state cueing condition but not for down-state cueing, there seems to be an interaction between trait factors and memory cueing. It could be speculated that subjects with high amounts of REM sleep as a

trait factor show generally higher capacities for neuronal plasticity during sleep, which may be associated with a higher sensitivity for cue-induced reactivations in-phase with the SOs up-state. Subjects with high amounts of SWS as a trait factor, on the other hand, may be more sensitive to unspecific cueing effects with cues triggering more spreading of activation in the associative network to facilitate the reactivation also of uncued items. However, these explanations are very speculative, and the sleep analyses were only exploratory and uncorrected, and should therefore be interpreted with great caution. Moreover, the sample size of the present study was relatively small, calling for replications with larger samples.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Jing-Yi Wang conceived the study. Jing-Yi Wang, Jan Born, Hong-Viet V. Ngo and Susanne Diekelmann planned and designed the study. Jing-Yi Wang, Katharina L. Heck and collected the data. Jing-Yi Wang, Katharina L. Heck, Hong-Viet V. Ngo and Susanne Diekelmann analysed the data, Hong-Viet V. Ngo and SD wrote the manuscript. All authors revised and approved the manuscript.

DATA AVAILABILITY STATEMENT

Datasets are available from the corresponding author on request.

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