Theta oscillations dictate the evolution of memories across periods of sleep or wakefulness

Dan Denis[1]*, Zhiyi Chen[1], Manroop Kaur[1], Ben Clayden[1], Thomas Schreiner[2], Scott A. Cairney[1,3]

Affiliations:

1 Department of Psychology, University of York, York, United Kingdom

2 Department of Psychology, Ludwig-Maximilians-Universität München, München, Germany

3 York Biomedical Research Institute, University of York, York, United Kingdom

* Corresponding author: Email: dan.denis@york.ac.uk

Abstract

Every day, we are exposed to an enormity of information. But how does the brain select which experiences to strengthen into long-term memories? Using scalp electroencephalography in human participants, we find evidence that memories are tagged at encoding for selective strengthening across sleep or wakefulness. For memories retained over sleep, this process was driven by 3-8 Hz theta rhythms at learning, with the magnitude of this theta response predicting the coupling of slow oscillations to sleep spindles in later sleep (an established neural correlate of sleep-dependent memory processing). In turn, slow oscillation-spindle coupling was associated with superior memory retention at a post-sleep test. These findings offer new insights into the neural mechanisms through which our brains determine which information is retained for the future.

Why do we remember some things and not others? For memories to be successfully retained over long periods of time, they need to be strengthened and stabilized through the process of memory consolidation, including over sleep (1–4). It has long been presumed that overnight consolidation is selective, with certain memories being “tagged” at encoding for preferential processing in later sleep (5–7). However, the nature of this selection process remains unknown.

Theta oscillations (~3-8Hz) are a promising candidate mechanism for the tagging of memories for sleep-dependent consolidation. Theta rhythms at encoding clock the long-term potentiation that is necessary for successful learning (8) and facilitate the binding of new memory elements into a coherent representation (9, 10). Moreover, preliminary work suggests that the efficacy of sleep-related consolidation is determined by theta activity present at prior learning (11).

Sleep spindles (~12-16Hz waxing and waning oscillations) are mechanistically implicated in sleep-dependent memory consolidation through inducing plasticity in learning-related cortical sites (12, 13). Spindles are tuned towards memories that are relevant for the future (14–16) and thus appear to promote selectivity in the sleeping brain. Spindles are temporally coupled with global slow oscillations (SOs, ~1Hz) and hippocampal sharp-wave ripples (~80-150Hz in humans), facilitating a cross-regional dialogue that supports memory reactivation and information transfer within hippocampal-cortical circuits (17–21). Coupled spindles are better predictors of memory than uncoupled spindles, supporting the importance of coupling in facilitating consolidation processes (18, 19). Learning-related theta rhythms correlate with spindle activity in later sleep (11), suggesting that awake learning and sleep-related consolidation processes work synergistically to strengthen certain memories over others.

However, direct evidence that memories are tagged at learning for consolidation during sleep has yet to be established. To obtain this evidence, it is necessary to distinguish between the encoding pattern of a memory that is remembered across a sleep-filled delay from a memory that is remembered across a wake-filled delay, and isolate the brain rhythms that promote this sleep-related memory triage. Using scalp electroencephalography (EEG) and multivariate pattern analysis (MVPA) in humans we set out to test the existence of a tagging mechanism for sleep-dependent memory consolidation, and the relationship between tagging processes at encoding and SO-spindle coupling during subsequent sleep.

Thirty-one healthy adults (M_{age} = 20 years, range = 18 – 23 years, 68% female; **Table S1**) completed a two-visit, within-subjects experiment comprising a sleep and wake condition. Participants began each visit by learning a set of word-object pairs (**Figure 1A**). Immediately after learning, and again following a two-hour delay (**Figure 1B**), participants were presented with the words in isolation, one after another, and for each word were instructed to recall the associated object. To minimize any effects of retrieval practice on our behavioral measures of memory consolidation, participants were tested on different subsets of word-object pairs at the immediate and delayed tests. The two-hour delay was filled with either a daytime nap (**Table S2** for sleep architecture) or time spent awake in the sleep laboratory watching nature documentaries (order counterbalanced).

Confirming the presence of a behavioral benefit of sleep for memory, retention was better when participants slept relative to when they remained awake (t (30) = 2.49, p = .019, d = 0.45; Fig.1C, left). Memory performance did not differ between immediate tests that took place before the sleep or wake delays (t (30) = -0.53, p = .60, d = -0.10; Fig.1C, right). There was no effect of visit number (visit 1 vs 2) on either immediate memory performance (t (30) = -2, p = .055, d = -0.36) or retention (t (30) = 0.16, p = .87). Memory scores are shown in **Table S3**. We next turned our attention to our primary question of whether memories are tagged at encoding for sleep-related consolidation.

Brain activity at encoding dissociates memories that are retained over either sleep or wake

If memories are tagged at learning for sleep-dependent consolidation, then it should be possible to distinguish between memories that are remembered after sleep or wakefulness based on unique patterns of brain activity at encoding. To this end, we trained and tested a multivariate classifier (**Figure 1D**, **Materials and Methods**) on the EEG data at encoding to distinguish between trials that were successfully recalled after sleep from trials that were successfully recalled after wake (trial counts for the EEG analyses are reported in **Table S4**). Compared to a surrogate decoder trained on randomized condition labels (22), significant above chance classification was achieved from 0.03-1.28s after stimulus onset (t_{sum} = 1302, $p_{cluster}$ = .002; Figure S1).

By focusing only on remembered word-object pairs, we could not link these results to mnemonic tagging at encoding because it is plausible that the same pattern of activity could exist for later forgotten pairs. A second classifier was therefore trained and tested to distinguish between word-object pairs at encoding that were later forgotten after either sleep

or wake (i.e., the Forgotten classifier; t_{sum} = 1335, $p_{cluster}$ = .002). On account of the sleep and wake visits

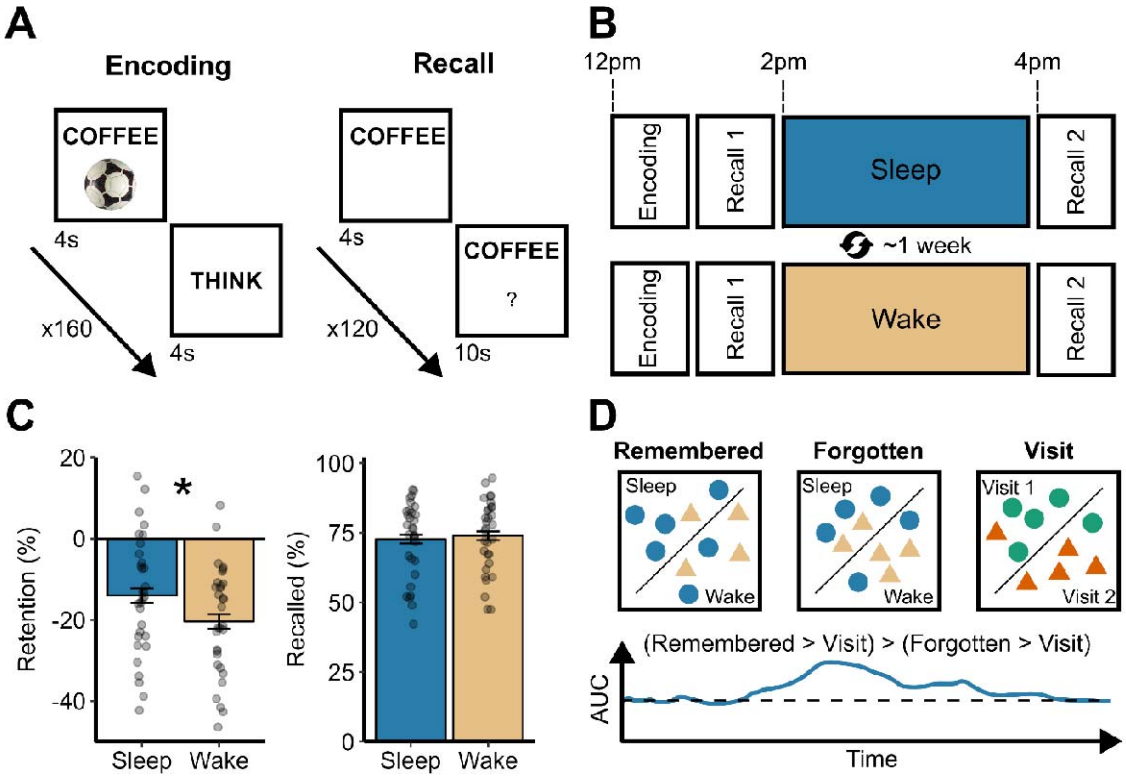

Figure 1. Experimental design and behavior. (A) During encoding, participants were presented with 160 word-object pairs. At recall, memory was probed by way of a cued recall test. (B) Memory was tested immediately (Recall 1) and again following a two-hour delay of either sleep or wake (Recall 2). Sleep and wake visits were manipulated within-subjects and performed one week apart in a counterbalanced order. (C) Retention (the change in performance at Recall 2 compared to Recall 1, with a more negative number indicating more forgetting) was significantly better over the sleep delay compared to the wake delay (* = p < .05). There was no sleep/wake visit difference in the proportion of items correctly recalled at Recall 1 (immediately after learning). Error bars indicate the standard error. (D) Multivariate classification approach. Multivariate classifiers were trained on the EEG time series at encoding to distinguish trials remembered after sleep from trials remembered after wake. As control analyses, additional classifiers were trained to distinguish between trials that were forgotten in the sleep and wake conditions, and also to distinguish between experimental Visits 1 and 2 (independent of the sleep or wake condition). For statistical analysis, a double subtraction was performed, meaning any classifier values significantly above zero reflected a memory-specific signature distinguishing between trials remembered after sleep and trials remembered after wake, independent of any non-specific session differences.

occurring on different days, an additional concern was that the classifier would pick up on non-specific between-visit differences in the EEG signal at encoding (e.g., due to subtle differences in electrode positioning and impedance). To overcome this, we ran a third

classifier (the Visit classifier; t_{sum} = 1087, $p_{cluster}$ = .002) to distinguish Visit 1 from Visit 2 (independent of when the sleep or wake conditions occurred).

To isolate signatures of mnemonic tagging for sleep- and wake-based memory processing, whilst accounting for non-specific between-session differences, a double subtraction was performed (**Figure 1D**; **Materials and Methods**). First, the time series for the Visit classifier was subtracted from the time series for both the Remembered and Forgotten classifiers, generating visit-corrected signals of successful and unsuccessful learning, respectively. The visit-corrected Forgotten classifier was then subtracted from the visit-corrected Remembered classifier, with the remaining metric distinguishing between memories that were tagged at encoding for retention across sleep or wake.

Significant classification was achieved across a time window of 0.87-1.13s after stimulus onset (t_{sum} = 129, $p_{cluster}$ = .014, $d_{cluster}$ = 0.72; **Figure 2A**). Critically, this shows that neural patterns associated with successful encoding differed according to whether the memory was later remembered after sleep or wake. It was not possible to distinguish between the sleep and wake condition when the classifier was trained on trials that were only tested immediately after learning (i.e., before the sleep or wake delay, t_{sum} = 78.36, $p_{cluster}$ = .10; **Figure S2**). Thus, our results suggest a unique, identifiable pattern of brain activity associated with new memories that are subsequently remembered after sleep, consistent with the tagging hypothesis.

Theta oscillations uniquely predict memory retention over sleep

Our classification analysis demonstrates that memories are tagged at learning for processing during later sleep. We next investigated whether theta oscillations at encoding support the tagging of memories for selective sleep-dependent consolidation. We decomposed encoding trials into time-frequency representations (TFRs; see Materials and Methods) and extracted theta (3-8Hz) power for subsequently remembered and forgotten trials (separately for the sleep and wake conditions). Theta power for forgotten trials was then subtracted from theta power for remembered trials to isolate successful learning.

Theta power at learning was significantly higher for word-image pairs remembered after sleep than word-image pairs remembered after wake (0.3-1.05s, t_{sum} = 42.53, $p_{cluster}$= .032, $d_{cluster}$ = 0.54; **Figure 2B**; **Figure S3** for full TFRs and topographic effects, and **Figure S4** showing no difference in the event-related potential). The increase in theta power was significantly greater than zero in the sleep condition (5.68% ± 8.09%; t (30) = 3.90, p < .001,

d = 0.70), but not in the wake condition (-2.73% ± 12.18%; t (30) = -1.25, p = .22, d = -0.22). No difference in learning-related theta power was observed between the sleep and wake conditions for trials successfully recalled at the immediate test (**Figure S2**). Taken together, these findings support the view that theta rhythms at learning coordinate the tagging of memories for selective strengthening across subsequent sleep.

Interestingly, the peak in learning-related theta power for memories retained after sleep emerged ~0.4s before the peak classification accuracy (theta power peak = 0.6s; **Figure 2B**, classifier fidelity peak = 0.98s; **Figure 2A**). This could imply that an early theta surge at learning triggers downstream processes that tag memories for consolidation during later sleep. To test for this, we adopted a cross-correlation approach which determined whether an early increase in theta power predicted later classification accuracy for memories retained after sleep (Materials and Methods). Consistent with this view, significant correlations were observed at lags ranging from -0.95s to -0.05s, peaking at -0.45s (r_{max} = .43, **Figure 2C**), aligning with the time difference in the theta power and classifier peaks.

Theta oscillations predict slow oscillation-spindle coupling activity

If theta rhythms at learning are indeed a tagging mechanism for consolidation during later sleep, theta power at encoding should be related to established oscillatory markers of sleep-dependent memory processing. The coupling between SOs and spindles (SO-spindle coupling) has emerged as a key mechanistic driver of overnight consolidation in recent years (18, 22–24). Therefore, we expected that theta power at encoding would correlate with the amount of SO-spindle coupling during sleep.

SO-spindle coupling events were detected using automated detectors ((23); **Materials and Methods**). Across all participants and electrodes, 16% (SD = 4%) of spindles were coupled to an SO, far exceeding what would be expected by chance (**Supplementary Results**). We observed significant non-uniformity in the preferred phase of the SO to which spindles were coupled at 13/14 electrodes (Zs > 3.49, ps < .03). Sleep spindles preferentially coupled close to the positive peak of the SO (M = 20.96° ± 58.05°), consistent with previous reports suggesting that SO-spindle coupling at this particular phase of the SO is optimal for memory processing in sleep (19, 25).

A robust linear regression revealed a significant association between theta power at learning (for word-image pairs remembered after sleep) and SO-coupled spindle density during later sleep (5 electrodes, t_{sum} = 11.82, $p_{cluster}$ = .028, $r_{cluster}$ = .45; **Figure 2D**). In turn, retention over

sleep was correlated with SO-coupled spindle density (4 electrodes, t_{sum} = 10.62, $p_{cluster}$ = .042, $r_{cluster}$ = .43; **Figure 2E**). No significant cluster emerged between theta power at learning and the density of uncoupled spindles in sleep, nor between uncoupled spindle density and memory performance after sleep. In sum, these results support the hypothesis that theta rhythms present at encoding tag memories for overnight consolidation, which in turn drives the engagement SO-coupled spindles during later sleep, resulting in selective memory strengthening and stabilization.

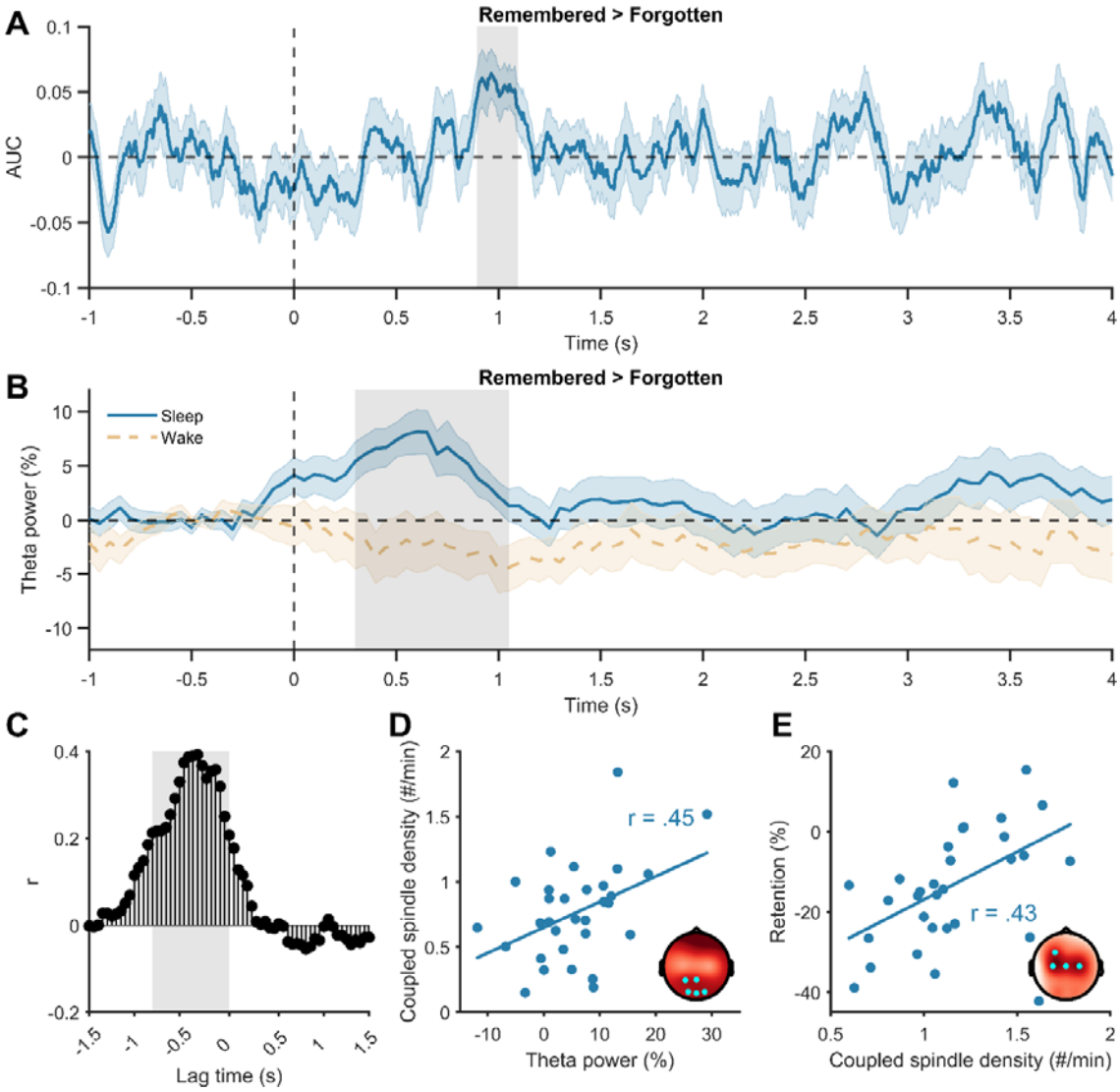

Figure 2. Encoding patterns differentially predict memory retention over a sleep or wake delay. (A) Multivariate classification of encoding data based on items that were subsequently remembered after sleep or wake. Significant classification above zero (extent of the cluster is highlighted in grey, p < .05 corrected) indicates the time points of successful decoding. (B) Theta power at encoding (normalized within-subject and session as a % change relative to the pre-stimulus baseline) was significantly higher for items successfully recalled after sleep compared to wake (significant time points in the cluster are highlighted in grey, p < .05 corrected). (C) Cross-correlation between theta power at encoding (sleep > wake) and the multivariate classifier time series. Significant correlations (highlighted in grey, false discovery rate adjusted) at negative lags indicate that increases in theta power predicted multivariate classification 0.45s later. (D) Theta power at learning predicted a higher

density of SO-coupled spindles during subsequent sleep. The topographical inset highlights significant electrodes in the cluster ($p < .05$ corrected). (E) Higher SO-coupled spindle density during sleep predicted better memory retention after sleep. The topography inset highlights significant electrodes in the cluster ($p < .05$ corrected).

Sleep spindles (and their coupling to SOs) are known to be trait like (26) leaving open the possibility that the correlation between SO-coupled spindles and retention reflects more generalized individual differences. However, no significant correlation was observed between SO-spindle coupling in the sleep condition and retention in the wake condition. Moreover, theta power at learning for word-object pairs remembered after wake was not significantly correlated with SO-spindle coupling, demonstrating that the effects reported in our primary analysis correspond to a unique sleep-specific tagging mechanism and not trait-like oscillatory interactions. In both analyses, no significant clusters formed.

It has long been thought that memory consolidation during sleep is selective, driven by tags set by the brain at learning (5). Yet, the neural basis of this tagging mechanism is poorly understood. Our results provide a novel demonstration that neural activity at encoding can delineate memories that are later remembered after sleep or wakefulness. This shows, for the first time, that the learning brain engages in a selective triage of information that will be strengthened across later sleep, consistent with a dynamic tagging of memory.

We were able to tie the tagging of memories for sleep-dependent consolidation to theta oscillations present during initial learning. That is, theta activity at encoding predicted memory after a delay of sleep, but not wake, and correlated with SO-spindle coupling, a putative marker of memory reactivation and consolidation during sleep. This finding is in line with other work showing that theta activity guides reward learning (27, 28), and mediates stress-related enhancements of emotional memory (29). These bodies of literature suggest a role for theta in prioritizing memories that are most relevant for the future. Our finding extends that idea by implicating it in the tagging of memories for sleep-dependent processing.

Theta oscillations have also been implicated in episodic binding, the amalgamation of different elements of an experience into a single, coherent representation (9). Our finding that theta activity at learning specifically predicts memory after sleep implies that such episodic binding is a major function of sleep-associated consolidation. This idea is consistent with behavioral work demonstrating that the formation of integrated associations at encoding is key to observing a memory benefit of sleep (30, 31). A testable hypothesis for future work

is that forming new associations between schematically incongruent items (32), using an integrative encoding strategy (31), requires a larger theta response at encoding to bind these disparate items together, and to drive selective memory processing in later sleep.

Multiple theoretical models have proposed that neural operations at encoding guide the process of consolidation, tagging memories for selective strengthening across the night (5–7). We have identified, for the first time in humans, the presence of a tagging signal at learning for selective strengthening during later sleep. This tagging signal appears to be driven by a surge in theta oscillations, which in turn guide overnight consolidation mechanisms to preferentially strengthen and stabilize memories deemed relevant for the future.

Materials and Methods

Participants

A total of 44 human participants enrolled in the study, of which 38 met inclusion criteria. Participants had no self-reported history of neurological, psychiatric, or sleep-related disorders, had normal or corrected-to-normal vision, were fluent in English, and scored between 30-80% accuracy on a word pairs task during a preliminary screening session (see **Procedure: Preliminary session**). Seven participants were excluded from the final analysis due to either not sleeping during their nap (n = 3) or being at floor or ceiling on at least one memory test (n = 4). Therefore, 31 participants were entered into the final analysis. Participants took part in exchange for an £80 e-voucher or course credit. The study was approved by the University of York Department Ethics Committee and written informed consent was obtained from all participants.

Stimuli

A set of 480 words and 320 images served as experimental stimuli. Words were all concrete nouns describing natural objects (e.g., plants, animals, foodstuffs). Images were all of manmade objects presented on a plain white background, and were taken from the Bank of Standardized Stimuli (BOSS) database (*33*).

Procedure

Preliminary session

Participants completed a preliminary visit to assess their eligibility for the study. Following informed consent, participants completed questionnaires assessing their subjective sleep quality (Pittsburgh Sleep Quality Index (*34*)), morning-evening preference (Morningness-eveningness questionnaire (*35*)) and daytime sleepiness (Epworth Sleepiness Scale (*36*)). They then performed an initial memory assessment, where they learned associations between 84 semantically unrelated word pairs (*30*, *31*). Each word pair was presented for five seconds, and participants were instructed to form a vivid mental image combining the two word-pair objects together in a scene. Immediately following encoding, memory for all word pairs was tested via a cued recall procedure. The first word was presented on the screen, and participants had ten seconds to respond with the second word in the pair. Participants scoring between 30% and 80% were invited back for the main experiment.

Experimental sessions

Overview: The study consisted of two experimental sessions (sleep and wake condition) spaced 5 - 14 days parts. The order of the two sessions was counterbalanced across participants. Participants arrived at the sleep laboratory between 11am and 1pm and completed a retrospective sleep diary for the previous three nights to assess sleep quality in the nights leading up to the experimental session. There were no differences in self-reported sleep quality in the nights prior to the sleep or wake visit (**Table S1**). Next, participants were wired for electroencephalography (EEG) recordings (see EEG section below). EEG was monitored throughout the entire protocol. Following EEG setup, subjective alertness levels were assessed using the Stanford Sleepiness Scale (SSS) (*37*). Next, participants were familiarized with the images before encoding the word-object pairs (see task details below). Following an immediate memory test, participants either took a nap (120-minute sleep opportunity), or remained awake in the lab for 120 minutes, watching nature documentaries (depending on the experimental session). Afterwards, subjective alertness was assessed again using the SSS, before memory performance was tested for a second time. All experimental tasks were presented using Psychtoolbox 3 (*38*) in MATLAB (MathWorks, Natick, MA, USA). Across all experimental tasks, stimulus presentation order was randomized across participants.

Familiarization: The experiment began with a familiarization phase, where participants were shown all of the to-be-encoded objects along with their descriptions. This was done to facilitate subsequent word-object learning and to provide the proper object names for later

cued recall. Each trial started with a fixation cross for 1 (± 0.2s jitter) second, followed by an object for 2 seconds accompanied by a short description presented above the object. For each trial, participants were asked to think how often they would use or interact with the object in everyday life.

Encoding: During encoding, participants learned 160 word-object pairs. On each trial, a fixation cross was presented for 1 (± 0.2s jitter) second, followed by one word-object pair for four seconds. To ensure all participants followed a similar strategy, they were told to visualize a scene that combined the referent of the word and the object into a single coherent scene (e.g., if the word DOG was paired with an image of a hat, participants might visualize an image of a dog wearing the hat). This type of strategy has been shown to enhance sleep-associated memory consolidation in previous work (*30*, *31*). After four seconds, the word-object pair faded away from the screen and was replaced by an instruction to 'Think' for a further four seconds. During this period, participants were instructed to hold the mental image they had formed in their mind. This was done to facilitate encoding. At the end of the trial, participants were instructed to indicate the vividness of the mental image on a scale from 1 (no image formed) to 4 (very vivid visualization). Each trial was shown once during encoding, and participants were told beforehand that their memory for the associations would be tested.

Retrieval: One half of the word-object pairs were tested at the pre-delay test and the other half at the post-delay test. This permits an assessment of sleep vs wake on memory retention whist preventing retrieval practice effects that might emerge when testing the same items at the pre- and post-delay assessments (*39*). Other than that, the pre-and post-delay memory tests were identical. Each test comprised 80 randomly chosen "old" words from the encoding phase intermixed with 40 "new" words not seen by the participant during encoding. Each trial began with a fixation cross (1s ± 0.2s jitter), after which a word was displayed on the screen for four seconds. During this time, participants were instructed to bring to mind the object originally paired with the word (if they recognized the word). After four seconds, participants indicated whether the word was "old" (i.e., seen during encoding) or "new" (i.e., not seen during encoding). A "don't know" option was also presented. To minimize guessing, participants were instructed to only select "old" or "new" if they were confident in their judgement and to press the "don't know" option when they were not confident. For each identified "old" trial, participants were then given 10 seconds to type a description of the object that was originally paired with the word.

Sleep delay: Participants were given a 120 min opportunity to nap in a laboratory bedroom whilst EEG was monitored. After waking up, participants were given 10 minutes to mitigate the effects of sleep inertia before continuing with the post-sleep memory test.

Wake delay: Participants remained in the sleep lab for 120 minutes watching nature documentaries. They were prohibited from other tasks such as reading or using their cell phones.

Electroencephalography: EEG was acquired with either an Embla NDx or Embla N7000 system using RemLogic 3.4 software. For all participants, the same EEG device was used for both experimental sessions. Gold-plated electrodes were attached to the scalp for EEG (14 electrodes, positioned according to the 10-20 system), above the right eye and below the left eye for EOG, and on the left and right side of the chin for EMG. Ground and reference electrodes were placed above the left and right eyebrow (corresponding to Fp1 and Fp2 of the 10-20 system). Finally, two electrodes were positioned on the left and right mastoid for offline re-referencing (see EEG analysis below). Data were recorded at 256Hz (Embla NDx, later downsampled to 200 Hz) or 200Hz (Embla N7000), and impedances were kept below 5kΩ.

Data analysis

Behavior

To assess memory performance, we calculated the proportion of correctly recalled objects relative to the number of correctly recognized words (i.e., the number of hits). In other words, object memory was only considered when participants correctly recognized the word. An object was considered correctly recalled if either 1) the participant typed the same caption that was shown during the familiarization phase or 2) the description provided unambiguously matched the object. Correctly recalling the object when cued with the word indicates successful associative memory retrieval. Because previous studies using nap paradigms have found sleep effects only for associative memory accuracy (*22*, *40*), all analyses here were formed solely on associative memory judgements. For completeness, item memory scores (hit and false alarm rates) are provided in **Table S3**. To quantify retention over the delay period, we calculated a relative change in image recall (*30*) as [(delayed recall – immediate recall) / immediate recall]. This measure quantifies how

memory changes from the immediate to delayed test while controlling for immediate memory performance.

EEG

Preprocessing - wake: EEG data were preprocessed using functions from the FieldTrip toolbox for MATLAB (*41*) and custom MATLAB scripts. All data collected from the Embla NDx devices were downsampled to 200Hz to match the Embla N7000 recordings. A notch filter was applied at 50Hz to remove electrical line noise and a high-pass filter was applied at .3Hz to remove low frequency artifacts. EEG channels were then re-referenced to the average of the two mastoid channels, before being epoched from -2 to +6 seconds around stimulus pair onset at encoding. Noisy channels and epochs were identified and removed via visual inspection (*ft_visreject* function in FieldTrip) and bad channels were interpolated using a weighted average of the neighboring channels. To correct for ocular artefacts, the epoched data were subjected to an independent component analysis, with ICA components reflecting eye blinks and movements being rejected.

Preprocessing - sleep: Nap data were first manually scored in 30-second epochs in accordance with AASM criteria (*42*). Artifactual epochs were detected using an automated algorithm (43). For each EEG channel, we calculated per-epoch summary metrics of three Hjorth parameters (signal activity, mobility, and complexity). Any epochs in which at least 1 channel displayed > 3 standard deviations from the mean on any of the three parameters were marked as artifact. Artifact detection was performed twice (in case of extreme outliers), and performed separately for each sleep stage (given inherent differences in the EEG signal between different sleep stages) (*43*). Subsequently, all artifact free NREM (stage N2 + N3) epochs were retained for further analysis.

Multivariate analysis: Our primary research question was whether EEG encoding patterns that predicted subsequent memory following sleep differed from EEG encoding patterns that predicted subsequent memory after wake. Our primary assessment of this was through classification of single-trial EEG data during encoding using the MVPA-Light toolbox for MATLAB and an LDA classifier (*44*). The classifier was trained and tested to discriminate between encoding trials subsequently remembered after either a sleep or wake delay. Encoding data were baseline corrected (-1 to 0 seconds relative to stimulus onset), linear trends were removed, and data were z-scored across all trials within each condition (sleep or wake) and for each timepoint separately. The two datasets were then smoothed using running average windows of 150ms. The 14 EEG channels served as features and a

different classifier was trained and tested on every time point from -1 to 4 seconds around stimulus onset. Area under the ROC curve was our classification metric, which indexes the mean accuracy with which a randomly chosen pair of sleep and wake trials could be assigned to their correct class (0.5 = chance level performance). To avoid overfitting, data were split into training and test sets using a fivefold cross-validation. Because cross-validation results are stochastic due to the random assignment of trials to folds, the analysis was repeated ten times and the results averaged. To account for differences in the number of trials remembered after sleep compared to after wake, the number of trials per condition were equated by subsampling a random selection of trials from the higher trial count condition to match the condition with the fewer trials. To assess whether classifier accuracy was significantly above chance, surrogate decoding performance was calculated by shuffling the training labels 250 times, and taking the average of these permutations (*22*). This provides, for each participant, a baseline decoding performance that can be compared to the classifier run on the real class labels. Multivariate classification was performed on the preprocessed EEG time series, rather than the theta time-frequency power time series (see next section), so as not to filter out potentially informative information at other frequencies present in the multivariate signal.

An important caveat of our approach is that, by necessity, the sleep and wake conditions were performed on separate experimental days. Non-specific between-session differences, such as small differences in impedance levels and the exact positioning of electrodes could thus be picked up by the classifier. It was therefore crucial to ensure that any signal discriminating between the sleep and wake conditions did not more parsimoniously reflect significant classification of the experimental sessions. To account for this, a set of control analyses were carried out. First, we ran the classifier again, but this time distinguishing trials that were *forgotten* after sleep compared to *forgotten* after wake (i.e., the Forgotten classifier). By comparing this classifier to the first Remember classifier, we could look for periods where classification accuracy was significantly higher for remembered vs not remembered trials. If classification accuracy was higher for one vs the other, this would speak against more generic between visit differences, because we would not expect broad between-sessions differences to impact the memory conditions differently. As a second control analysis, we ran a third classifier to discriminate between Visit 1 and Visit 2 (the Visit classifier). This is dissociable from the sleep and wake condition because the assignment of visit order (sleep first or wake first) was counterbalanced across participants. For our primary analysis, we then performed a double subtraction of the classifier time series. First, we subtracted the Visit classifier time course from the Remember classifier and from the

Forgotten classifier. Thus, any remaining values above zero would reflect genuine sleep vs wake differences for subsequently remembered and forgotten word-image pairings, having removed more generic between-visit factors. Second, we subtracted the visit-corrected Forgotten classifier time series from the visit-corrected Remember classifier. This resulted in a single time series where values above zero would reflect a remember specific signal that differed depending on whether encoding occurred prior to sleep or wake. As a third control, we wanted to rule out any possibility that significant classification could be explained by participants simply engaging in a different cognitive strategy or were in a broadly different mental state when they knew that they would be taking a nap or not. If this were the case, then significant classification between sleep and wake condition would also be possible on encoding trials that were tested at the immediate, pre-delay test. Therefore, we repeated the above procedure but focused on encoding trials that were remembered/forgotten at the immediate, pre-delay test.

Time-frequency analysis: Preprocessed data were convolved with a five-cycle Hanning taper (*ft_freqanalysis* function in FieldTrip, using the *mtmconvol* method) from -2 to 6 seconds relative to stimulus onset in steps of 50ms, and from 3 - 30Hz in steps of 1Hz. To avoid edge artifacts, subsequent analyses focused on the -1 to 4 second time window (consistent with the classification analysis). Artifact rejection was performed on single trial time-frequency representations (TFRs) using a data-driven approach (*45*): power values that exceeded the 85th percentile across all time/frequency points and trials were rejected and removed from subsequent analyses. TFRs were converted into a percent power change relative to a baseline interval of -0.4 seconds to -0.2 seconds before stimulus onset. This window was chosen to mitigate baseline contamination by post stimulus activity while preserving proximity to stimulus onset (*45*). The trial-averaged signal for subsequently forgotten word-object pairs was subtracted from the trial-averaged signal for subsequently remembered word-object pairs, separately for the sleep and wake delay conditions. Finally, theta power estimates were extracted by averaging power values between 3-8Hz. Thus, the final TFR values entered into statistical analysis reflect theta power signatures of successful learning prior to a delay containing sleep or wake.

Slow oscillation-spindle event detection: First, each individual's peak spindle frequency was identified through visual inspection of the NREM power spectrum. The largest, most prominent peak in the 12-16 Hz range was considered that individual's spindle peak frequency. Spindles were then automatically detected using a wavelet based detector (*23*, *46*, *47*). The raw EEG signal was convolved with a complex Morlet wavelet, with the wavelet

peak frequency set at that individual's spindle peak frequency, and the bandwidth of the wavelet (FWHM) set at a 1.3Hz range centered on the peak frequency (*23*, *48*). A spindle was detected whenever the wavelet-filtered signal exceeded a threshold of 6 times the median signal amplitude for a minimum of 0.4s. The threshold of 6 times the median has been empirically determined to maximize between-class (spindle, non-spindle) variance in the wavelet-filtered signal in previous work using a nap paradigm and healthy young adults (*23*). Slow oscillations were then detected using a second automated algorithm (*49*). Data were initially bandpass filtered between 0.5 - 4Hz and all positive-to-negative zero crossings were identified. Candidate slow oscillations were marked if two such consecutive crossings fell 0.8 – 2 seconds apart (i.e., 0.5 – 1.25Hz). Peak-to-peak amplitudes of all candidate oscillations were determined, and those in the top quartile (i.e., with the highest amplitudes) were retained as SOs. For SO-spindle coupling detection, the Hilbert transform was applied to extract the instantaneous phase of the SO signal and the instantaneous amplitude of the spindle signal. For each detected spindle, its peak amplitude was determined. If the spindle peak was found to occur during the time course of any detected 0.5 – 1.25Hz slow oscillations (i.e., between the two positive-to-negative crossings), the event was marked as a SO-spindle coupling event. Our primary measure of interest was the coupled and uncoupled spindle density (number of coupled/uncoupled spindles per minute of NREM sleep) (*23*).

Statistical analysis

Differences between the sleep and wake conditions for a) retention over the delay and b) immediate recall performance were assessed with paired-samples *t*-tests. For multivariate classification analyses, comparison of classifier accuracy against the surrogate baseline distribution was performed using a paired-samples *t*-test at each time point. Analysis of the classifier time series following the double subtraction was achieved via a 1-sample *t*-test against zero at every time point. To examine sleep-wake differences in TFRs, between-condition differences were compared by way of a paired-samples *t*-test conducted at every time point.

To test for time-lagged associations between TFR power and multivariate classification, the cross-correlation between the TFR time series and lagged copies of the classifier time series (from -1.5 to 1.5s lags, in 50ms intervals) were computed. Correlation analyses between theta power at encoding, SO-spindle coupling during sleep, and memory retention were performed as robust linear regressions to minimize the influence of outliers.

To control for multiple comparisons across timepoints/electrodes, we used a cluster-based permutation method (*50*). For each analysis, clusters were formed from adjacent timepoints/electrodes that met an uncorrected threshold of $p < .05$. Permutation distributions were then created by randomly shuffling condition labels 1000 times at each timepoint/electrode and retaining the cluster with the maximum statistic for each permutation. The cluster-level corrected *p*-value is the probability that the cluster would be found by chance within the permutation distribution. A cluster-corrected $p < .05$ was deemed statistically significant. Multiple comparisons across multiple lags in the cross-correlation analysis were controlled using the False Discovery Rate (*51*). All effect sizes were reported using Cohen's d or Pearson's r as appropriate. For cluster-based permutation analyses, effect sizes were derived by averaging values (e.g., theta power, spindle density) across all significant time/electrode points that contributed to the cluster.

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Competing interests

Authors declare that they have no competing interests.

References

1. S. Berres, E. Erdfelder, The sleep benefit in episodic memory: An integrative review and a meta-analysis. *Psychol Bull* **147**, 1309–1353 (2021).
2. S. Brodt, M. Inostroza, N. Niethard, J. Born, Sleep—A brain-state serving systems memory consolidation. *Neuron* **111**, 1050–1075 (2023).
3. L. Himmer, M. Schönauer, D. P. J. Heib, M. Schabus, S. Gais, Rehearsal initiates systems memory consolidation, sleep makes it last. *Science Advances* **5**, eaav1695 (2019).
4. X. L. Liu, C. Ranganath, Resurrected memories: Sleep-dependent memory consolidation saves memories from competition induced by retrieval practice. *Psychon Bull Rev*, doi: 10.3758/s13423-021-01953-6 (2021).
5. J. Born, I. Wilhelm, System consolidation of memory during sleep. *Psychol Res* **76**, 192–203 (2012).
6. R. Stickgold, M. P. Walker, Sleep-dependent memory triage: evolving generalization through selective processing. *Nat Neurosci* **16**, 139–145 (2013).
7. E. T. Cowan, A. C. Schapiro, J. E. Dunsmoor, V. P. Murty, Memory consolidation as an adaptive process. *Psychon Bull Rev* **28**, 1796–1810 (2021).
8. P. T. Huerta, J. E. Lisman, Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. *Neuron* **15**, 1053–1063 (1995).
9. B. J. Griffiths, M. C. Martín-Buro, B. P. Staresina, S. Hanslmayr, Disentangling neocortical alpha/beta and hippocampal theta/gamma oscillations in human episodic memory formation. *NeuroImage* **242**, 118454 (2021).
10. A. Clouter, K. L. Shapiro, S. Hanslmayr, Theta Phase Synchronization Is the Glue that Binds Human Associative Memory. *Curr Biol* **27**, 3143-3148.e6 (2017).
11. D. P. J. Heib, K. Hoedlmoser, P. Anderer, G. Gruber, J. Zeitlhofer, M. Schabus, Oscillatory theta activity during memory formation and its impact on overnight consolidation: a missing link? *J Cogn Neurosci* **27**, 1648–1658 (2015).
12. M. Rosanova, D. Ulrich, Pattern-Specific Associative Long-Term Potentiation Induced by a Sleep Spindle-Related Spike Train. *J. Neurosci.* **25**, 9398–9405 (2005).
13. J. Seibt, C. J. Richard, J. Sigl-Glöckner, N. Takahashi, D. I. Kaplan, G. Doron, D. de Limoges, C. Bocklisch, M. E. Larkum, Cortical dendritic activity correlates with spindle-rich oscillations during sleep in rodents. *Nat Commun* **8**, 684 (2017).
14. I. Wilhelm, S. Diekelmann, I. Molzow, A. Ayoub, M. Mölle, J. Born, Sleep selectively enhances memory expected to be of future relevance. *J Neurosci* **31**, 1563–1569 (2011).
15. K. Igloi, G. Gaggioni, V. Sterpenich, S. Schwartz, A nap to recap or how reward regulates hippocampal-prefrontal memory networks during daytime sleep in humans. *eLife* **4**, e07903 (2015).
16. S. Studte, E. Bridger, A. Mecklinger, Sleep spindles during a nap correlate with post sleep memory performance for highly rewarded word-pairs. *Brain Lang* **167**, 28–35 (2017).
17. N. Niethard, H.-V. V. Ngo, I. Ehrlich, J. Born, Cortical circuit activity underlying sleep slow oscillations and spindles. *Proceedings of the National Academy of Sciences* **115**, E9220–E9229 (2018).
18. D. Denis, S. A. Cairney, Neural reactivation during human sleep. *Emerging Topics in Life Sciences* **7**, 487–498 (2023).
19. D. Denis, S. A. Cairney, Electrophysiological Mechanisms of Memory Consolidation in Human Non-rapid Eye Movement Sleep. *Curr Sleep Medicine Rep* **10**, 181–190 (2024).
20. R. F. Helfrich, J. D. Lendner, B. A. Mander, H. Guillen, M. Paff, L. Mnatsakanyan, S. Vadera, M. P. Walker, J. J. Lin, R. T. Knight, Bidirectional prefrontal-hippocampal dynamics organize information transfer during sleep in humans. *Nature Communications* **10**, 3572 (2019).
21. H.-V. Ngo, J. Fell, B. Staresina, Sleep spindles mediate hippocampal-neocortical coupling during long-duration ripples. *eLife* **9**, e57011 (2020).
22. T. Schreiner, M. Petzka, T. Staudigl, B. P. Staresina, Endogenous memory reactivation during sleep in humans is clocked by slow oscillation-spindle complexes. *Nature Communications* **12**, 3112 (2021).
23. D. Denis, D. Mylonas, C. Poskanzer, V. Bursal, J. D. Payne, R. Stickgold, Sleep Spindles Preferentially Consolidate Weakly Encoded Memories. *J. Neurosci.* **41**, 4088–4099 (2021).
24. A. Solano, L. A. Riquelme, D. Perez-Chada, V. Della-Maggiore, Motor Learning Promotes the Coupling between Fast Spindles and Slow Oscillations Locally over the Contralateral Motor Network. *Cerebral Cortex* **32**, 2493–2507 (2022).

25. T. Ng, E. Noh, R. M. Spencer, Does slow oscillation-spindle coupling contribute to sleep-dependent memory consolidation? A Bayesian meta-analysis. *eLife* **13** (2024).
26. R. Cox, D. S. Mylonas, D. S. Manoach, R. Stickgold, Large-scale structure and individual fingerprints of locally coupled sleep oscillations. *Sleep* **41**, zsy175 (2018).
27. M. J. Gruber, A. J. Watrous, A. D. Ekstrom, C. Ranganath, L. J. Otten, Expected reward modulates encoding-related theta activity before an event. *Neuroimage* **64**, 68–74 (2013).
28. J. F. Cavanagh, C. M. Figueroa, M. X. Cohen, M. J. Frank, Frontal Theta Reflects Uncertainty and Unexpectedness during Exploration and Exploitation. *Cerebral Cortex* **22**, 2575–2586 (2012).
29. H. Heinbockel, C. W. E. M. Quaedflieg, T. R. Schneider, A. K. Engel, L. Schwabe, Stress enhances emotional memory-related theta oscillations in the medial temporal lobe. *Neurobiology of Stress* **15**, 100383 (2021).
30. D. Denis, A. C. Schapiro, C. Poskanzer, V. Bursal, L. Charon, A. Morgan, R. Stickgold, The roles of item exposure and visualization success in the consolidation of memories across wake and sleep. *Learn. Mem.* **27**, 451–456 (2020).
31. D. Denis, R. Bottary, T. J. Cunningham, M.-C. Tcheukado, J. D. Payne, The influence of encoding strategy on associative memory consolidation across wake and sleep. *Learn. Mem.* **30**, 185–191 (2023).
32. J. E. Ashton, B. P. Staresina, S. A. Cairney, Sleep bolsters schematically incongruent memories. *PLOS ONE* **17**, e0269439 (2022).
33. M. B. Brodeur, E. Dionne-Dostie, T. Montreuil, M. Lepage, The Bank of Standardized Stimuli (BOSS), a New Set of 480 Normative Photos of Objects to Be Used as Visual Stimuli in Cognitive Research. *PLOS ONE* **5**, e10773 (2010).
34. D. J. Buysse, C. Reynolds, T. Monk, S. Berman, D. Kupfer, The Pittsburgh Sleep Quality Index (PSQI): A new instrument for psychiatric practice and research. *Psychiatry Research* **28**, 193–213 (1989).
35. J. A. Horne, O. Ostberg, A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol* **4**, 97–110 (1976).
36. M. W. Johns, A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* **14**, 540–545 (1991).
37. E. Hoddes, W. Dement, V. Zarcone, The development and use of the Stanford sleepiness scale. *Psychophysiology* **9**, 150 (1972).
38. M. Kleiner, D. Brainard, D. Pelli, A. Ingling, R. Murray, C. Broussard, What's new in psychtoolbox-3. *Perception* **36**, 1–16 (2007).
39. K.-H. T. Bäuml, C. Holterman, M. Abel, Sleep can reduce the testing effect: It enhances recall of restudied items but can leave recall of retrieved items unaffected. *Journal of Experimental Psychology: Learning, Memory, and Cognition* **40**, 1568–1581 (2014).
40. S. Studte, E. Bridger, A. Mecklinger, Nap sleep preserves associative but not item memory performance. *Neurobiology of Learning and Memory* **120**, 84–93 (2015).
41. R. Oostenveld, P. Fries, E. Maris, J.-M. Schoffelen, FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci* **2011**, 156869 (2011).
42. C. Iber, S. Ancoli-Israel, A. Chesson, S. F. Quan, *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specification* (Americian Academy of Sleep Medicine, Westchester, IL, 2007).
43. S. M. Purcell, D. S. Manoach, C. Demanuele, B. E. Cade, S. Mariani, R. Cox, G. Panagiotaropoulou, R. Saxena, J. Q. Pan, J. W. Smoller, S. Redline, R. Stickgold, Characterizing sleep spindles in 11,630 individuals from the National Sleep Research Resource. *Nat Commun* **8**, 15930 (2017).
44. M. S. Treder, MVPA-Light: A Classification and Regression Toolbox for Multi-Dimensional Data. *Frontiers in Neuroscience* **14** (2020).
45. A. á V. Guttesen, M. G. Gaskell, E. V. Madden, G. Appleby, Z. R. Cross, S. A. Cairney, Sleep loss disrupts the neural signature of successful learning. *Cerebral Cortex*, bhac159 (2022).
46. D. Mylonas, C. Tocci, W. G. Coon, B. Baran, E. J. Kohnke, L. Zhu, M. G. Vangel, R. Stickgold, D. S. Manoach, Naps reliably estimate nocturnal sleep spindle density in health and schizophrenia. *Journal of Sleep Research* **29**, e12968 (2019).
47. E. J. Wamsley, M. A. Tucker, A. K. Shinn, K. E. Ono, S. K. McKinley, A. V. Ely, D. C. Goff, R. Stickgold, D. S. Manoach, Reduced sleep spindles and spindle coherence in schizophrenia: mechanisms of impaired memory consolidation? *Biol Psychiatry* **71**, 154–161 (2012).

48. R. Cox, A. C. Schapiro, D. S. Manoach, R. Stickgold, Individual differences in frequency and topography of slow and fast sleep spindles. *Front Hum Neurosci* **11**, 433 (2017).
49. B. P. Staresina, T. O. Bergmann, M. Bonnefond, R. van der Meij, O. Jensen, L. Deuker, C. E. Elger, N. Axmacher, J. Fell, Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature Neuroscience* **18**, 1679–1686 (2015).
50. E. Maris, R. Oostenveld, Nonparametric statistical testing of EEG- and MEG-data. *J Neurosci Methods* **164**, 177–190 (2007).
51. Y. Benjamini, Y. Hochberg, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* **57**, 289–300 (1995).
52. M. Gilson, A. Tauste Campo, X. Chen, A. Thiele, G. Deco, Nonparametric test for connectivity detection in multivariate autoregressive networks and application to multiunit activity data. *Network Neuroscience* **1**, 357–380 (2017).

Supplementary Materials

Supplementary Results

To determine whether the co-occurrence of SOs and spindles reflects a “true” coupling of the two oscillations, we needed to ensure that the number of coupling events exceeded what would be expected by chance, given the amount of time spent in non-rapid eye movement sleep, and the number of detected slow oscillations and spindles. To this end, the observed SO and spindle signal was compared to a randomized signal where the spindle signal was circularly shifted, and coupling to SOs was re-calculated (*23*, *52*). The start of the shifted signal is set to a random position, and early entries are sequentially moved to after the end of the original signal. By shifting the spindle signal in this fashion, the temporal relationship between SOs and spindles is disrupted, whilst retaining the signal properties of the original signal (i.e., the distribution of SOs and spindles). This was performed over 1000 iterations to generate a null distribution of SO-spindle coupling. The null distribution was then compared with the observed number of coupling events. Across each participant/electrode, we calculated the percentage of participants and electrodes where the degree of observed coupling was significantly higher ($p < .05$) than what would be expected by chance. Across all electrodes, 29/31 (94%) of participants exhibited a rate of coupling that significantly exceeded what would be expected by chance (binomial test, $p < .001$).

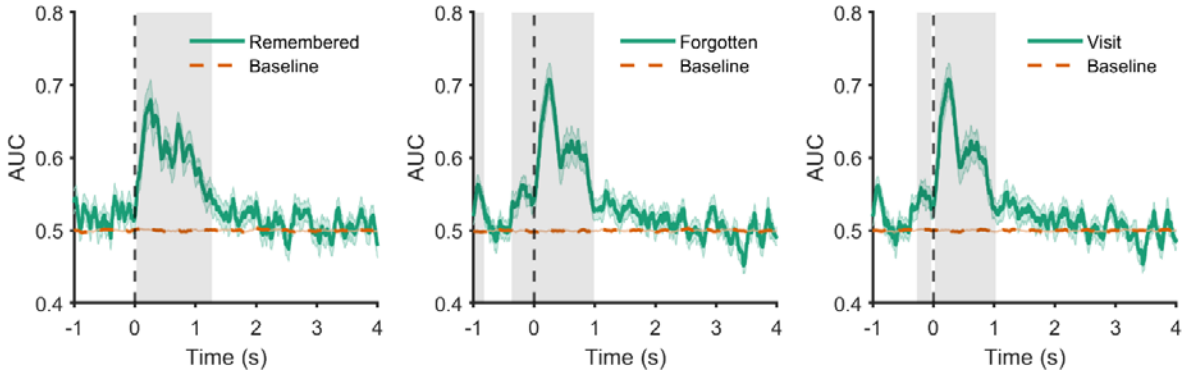

Figure S1. Classifier performance vs baseline. Ability of classifier to decode encoding trials into subsequently remembered across the sleep or wake delay (left); subsequently forgotten across the sleep or wake delay (middle); or decoding of experimental visit independent of delay condition or memory status (right). Classifier accuracy was compared to a surrogate decoding baseline, which was estimated by shuffling the training labels 250 times. Significant clusters (i.e. where classification exceeded chance levels) are highlighted in grey. Shaded areas around the lines indicates the standard error of the mean.

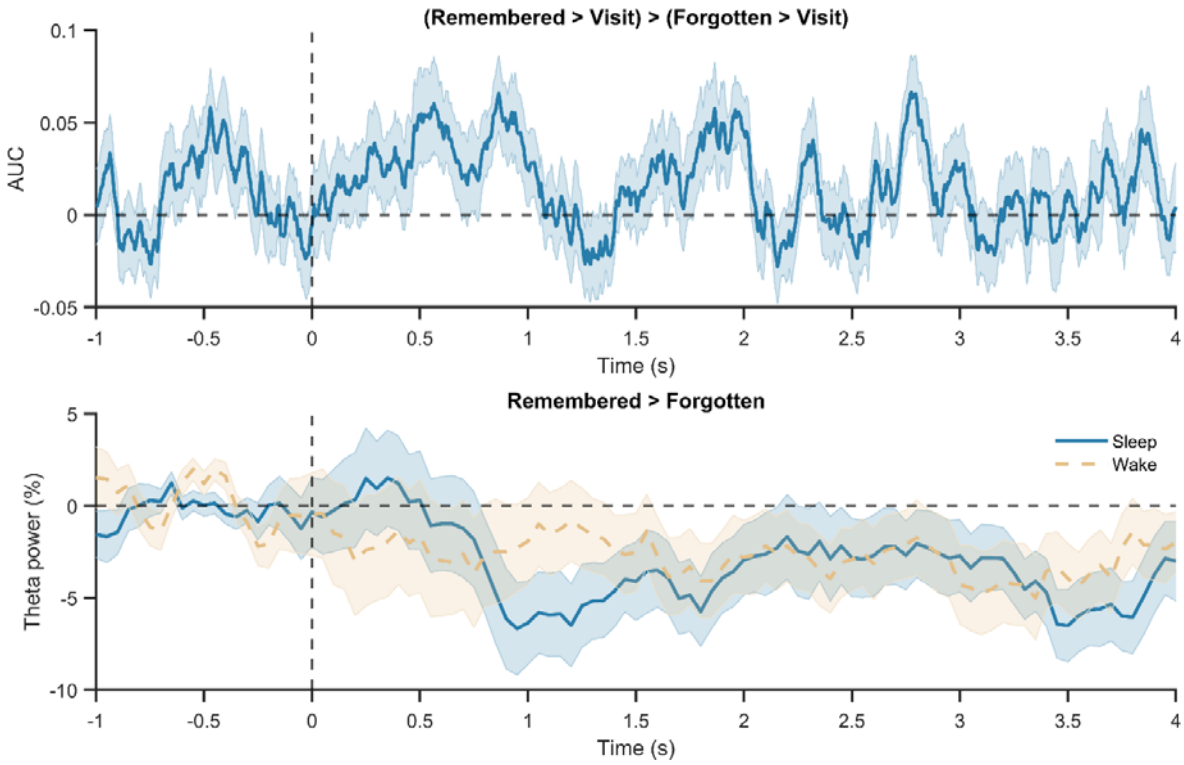

Figure S2. Top. Multivariate classification could not classify delay type (sleep or wake) based on encoding trials that were remembered at the immediate test. Bottom – No theta power differences at encoding for trials recalled at the immediate test prior to the sleep or wake delay.

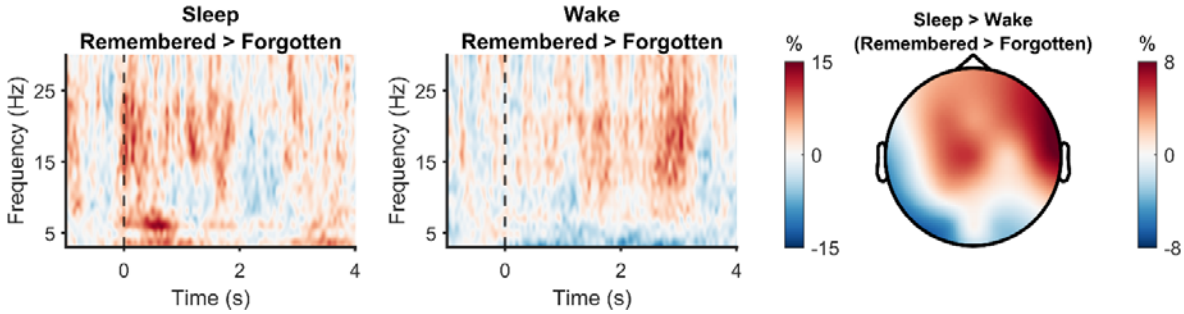

Figure S3. Time-frequency representations at learning prior to sleep (left) and wake (middle). Warmer colors indicate a greater % increase (relative to pre-stimulus baseline) in spectral power for remembered compared to forgotten trials. The right hand plot depicts the spatial extent of encoding activity for word-object pairs that were remembered (> forgotten) after sleep (> wake).

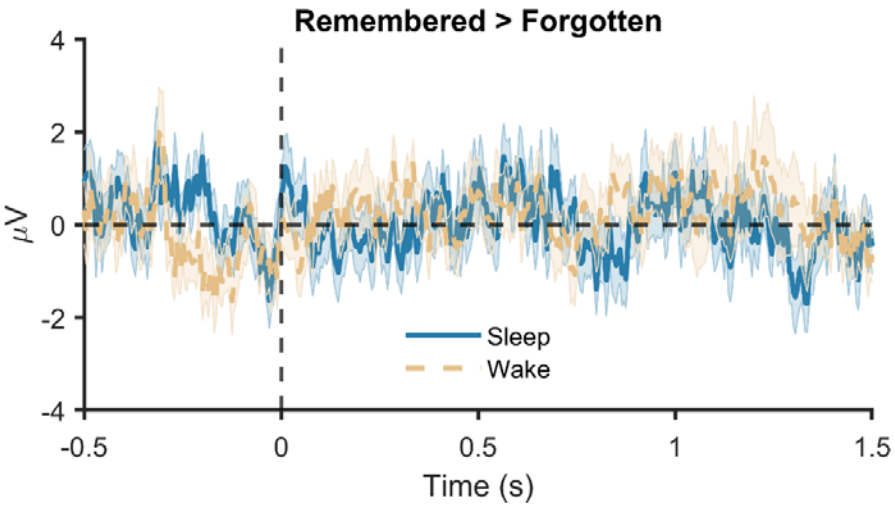

Figure S4. Event-related potentials during encoding. No difference in ERP activity between the sleep and wake conditions were observed. Shaded area around the line indicates the standard error.

Table S1.

Participant demographics and questionnaire measures

	M	SD
Age (years)	20	1
Sex	N	%
Female	21	68
Male	10	32
	M	SD
PSQI	4.45	1.95
MEQ	48.7	7.38
ESS	5.23	2.88

	Sleep visit		Wake visit		*t*	*p*
	M	SD	M	SD		
Sleep diary						
TST (hours)	8	2	8	2	0.33	.75
SOL (minutes)	20	22	21	29	1.28	.21
WASO (minutes)	7	8	8	10	0.90	.38
SE (%)	95	4	94	5	0.82	.42
Stanford sleepiness scale						
Encoding	2.19	0.83	2.03	0.84	0.41	.69
Recall 2	2.81	1.01	2.71	1.19	0.33	.75

Note. M = Mean, SD = standard deviation. PSQI = Pittsburgh sleep quality index global score, MEQ = Morningness-eveningness questionnaire, ESS = Epworth sleepiness scale. TST = total sleep time, SOL = sleep onset latency, WASO = Wake after sleep onset, SE = Sleep efficiency (% of time in bed that was spent asleep). Sleep diary measures calculated as the average of the three nights prior to the experimental day.

Table S2.

Sleep architecture

	M	SD
Total sleep time (min)	84	31
N1 (%)	13.52	8.25
N2 (%)	54.78	12.23
N3 (%)	19.98	14.59
REM (%)	11.71	12.07
Sleep spindles (N)	367	178
Slow oscillations (N)	303	134
SO-spindle coupling events (N)	57	28

Note. M = mean, SD = standard deviation. SO = slow oscillation. Spindle, SO, and SO-spindle coupling counts derived from electrode Cz.

Table S3.

Raw memory scores

	Recall 1		Recall 2	
	M	SD	M	SD
Hit rate				
Sleep	90.92	5.96	85.81	7.09
Wake	89.93	5.97	80.39	7.09
False alarm rate				
Sleep	5.21	5.43	6.08	4.83
Wake	5.15	5.52	6.12	4.83
Associative memory				
Sleep	72.74	8.65	62.84	8.87
Wake	73.91	8.65	59.15	8.88

Note, M = mean, SD = standard deviation. Associative memory calculated as % of correctly identified old words for which the associated image was recalled.

Table S4

Trial counts for EEG analyses

	Recall 1		Recall 2	
	Remembered	Forgotten	Remembered	Forgotten
	M (SD)	M(SD)	M (SD)	M (SD)
Sleep	47 (16)	38 (20)	38 (18)	36 (18)
Wake	48 (15)	38 (22)	32 (15)	41 (15)

Note. M = mean, SD = standard deviation. Average trial counts presented for information only. Note that for all analyses, trial-counts were matched per-participant by randomly selecting a subset of trials from the higher count condition to match the lowest trial condition.