Local Targeted Memory Reactivation in Human Sleep

Graphical Abstract

Highlights

- Presenting odors to one nostril in sleep achieves local TMR in one brain hemisphere

- Local TMR selectively improves hemisphere-related memories for specific words

- Local TMR differentially modulates slow-wave and spindle power across hemispheres

- Local TMR modulates regional slow-oscillation-spindle phase amplitude coupling (PAC)

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In Brief

Bar et al. perform local targeted memory reactivation (TMR) by associating words with left or right visual field locations plus contextual odor and presenting this odor to one nostril during post-learning naps. Local TMR selectively promotes memory for words processed in the cued hemisphere and modulates local sleep oscillations and coupling.
Local Targeted Memory Reactivation in Human Sleep

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SUMMARY

Memory consolidation can be promoted via targeted memory reactivation (TMR) that re-presents training cues or context during sleep. Whether TMR acts locally or globally on cortical sleep oscillations remains unknown. Here, we exploit the unique functional neuroanatomy of olfaction with its ipsilateral stimulus processing to perform local TMR in one brain hemisphere. Participants learned associations between words and locations in left or right visual fields with contextual odor throughout. We found lateralized event-related potentials during task training that indicate unihemispheric memory processes. During post-learning naps, odors were presented to one nostril in non-rapid eye movement (NREM) sleep. Memory for specific words processed in the cued hemisphere (ipsilateral to stimulated nostril) was improved after local TMR during sleep. Unilateral odor cues locally modulated slow-wave (SW) power such that regional SW power increase was lower in the cued hemisphere relative to the uncued hemisphere and negatively correlated with select memories for cued words. Moreover, local TMR improved phase-amplitude coupling (PAC) between slow oscillations and sleep spindles specifically in the cued hemisphere. The effects on memory performance and cortical sleep oscillations were not observed when unilateral olfactory stimulation during sleep followed learning without contextual odor. Thus, TMR in human sleep transcodes global action by selectively promoting specific memories associated with local sleep oscillations.

INTRODUCTION

Ample evidence suggests that sleep is critical for memory consolidation, in which new and labile memories encoded in wakefulness are transformed into less-labile representations that become integrated into pre-existing knowledge [1–5]. Sleep supports multiple types of subsequent memory performance, including declarative, spatial, procedural, perceptual, and emotional memory (reviewed in [1]).

According to the standard model of systems consolidation, as well as to some of its subsequent variants [2], consolidation of hippocampal-dependent memory occurs in a two-stage process, whereby new memories are initially encoded into the hippocampus during wakefulness and subsequently consolidated in a process that involves cross-talk with neocortex, mostly during non-rapid eye movement (NREM) sleep [3]. Such hippocampal-neocortical dialogue likely involves coordinated coupling between neocortical slow waves (SWs), thalamocortical sleep spindles, and hippocampal sharp-wave ripples (SWRs). SWRs co-occur with reactivation of neuronal ensembles that were selectively engaged in the learning phase [1, 4, 6]. According to this model, cortical SW up-states initiate hippocampal SWRs and neuronal reactivations [7]. Hippocampal reactivation triggers a delayed cortical response that facilitates the transformation to gradually augmented neocortical dependency [8]. At the same time, sleep spindles prime the recipient cortical circuits for plasticity [9], ensuring optimal reception. Thus, hierarchical nesting of SW-spindle-SWR events may promote the transformation of reactivated hippocampal memories to respective neocortical networks [4].

Recent work suggests that memory consolidation during NREM sleep can be externally modulated by re-presentation of contextual cues (e.g., a specific odor or sound associated with select items during learning) [10–12]. This method, known as “targeted memory reactivation” (TMR), promotes memory consolidation and induces hippocampal activity [10], suggesting that it involves reactivation of the fresh engram or part of it. TMR has been implemented successfully in a variety of declarative and non-declarative memory tasks [10, 13–15]. Although odor and sound both serve as effective contextual stimuli, odor entails some advantages for TMR because it rarely wakes up sleeping participants [16–18]. In addition, odors are powerful cues for memories [19, 20], possibly due to direct projections from the olfactory cortex to the hippocampus that largely bypass the thalamus [21, 22]. However, a major unresolved issue in TMR research is whether selective memory benefits entail local or global effects on cortical sleep oscillations. To date, potential local effects were difficult to reveal given that sensory cueing during sleep also modulates cortical sleep oscillations globally [23–25].
addition, typical TMR research compares cortical oscillations across different sleep intervals, inevitably introducing variability in sleep activities that masks potential local modulations. To overcome existing limitations in TMR research, one would ideally cue specific stimuli that trigger processing in select cortical regions, while simultaneous activity in other areas serves as control. Recent studies established the notion of local sleep oscillations and their relation to memory consolidation [26–34]. Thus, in principle, cortical sleep oscillations may differ across regions that undergo local TMR.

To perform TMR for specific memories associated with activity in select brain areas, we took advantage of the unique functional neuroanatomy of the olfactory system, where stimulus processing is largely ipsilateral [21, 35]. Although odor information does reach the contralateral hemisphere [36, 37], the first interhemispheric connections in mammals occur at the olfactory cortex. Interhemispheric connections emerge in the anterior olfactory nucleus (AON) and the piriform cortex and cross to the other hemisphere via anterior commissural pathways [21, 35, 37, 38] (but see [39]). Accordingly, olfactory memories can be stored, at least temporally, in one hemisphere, accessible to the other hemisphere only through commissural pathways [40]. Indeed, in newborn rats, memory for learned odor is confined to one hemisphere if a single nostril is stimulated during learning, but this phenomenon ceases when the pups reach the age of 12 days, simultaneously with maturation of the anterior commissure [40, 41]. Given that during NREM sleep effective connectivity across cortical regions is reduced [42], we hypothesized that integration across cortical hemispheres during sleep may be jeopardized in a manner akin to an undeveloped commissure. Hence, we predicted that presenting contextual odor cues to a single nostril in sleep would benefit memories and modulate sleep oscillations associated with the ipsilateral hemisphere.

Based on the aforementioned rationale, we developed a novel method to locally reactivate memories in a single hemisphere, using odor stimulation to a single nostril during sleep (Figure 1). This approach allows us to assess the behavioral and electrophysiological influences of local cueing on memories related to one hemisphere, while memories and activities related to the other hemisphere serve as control (Figure 1A). We used a spatial memory task that requires encoding of paired associations between words and specific locations in either the left or the right visual hemifields, associated with processing in the contralateral cerebral hemisphere (Figures 1C, 1D, and 1F). The results establish that local TMR in sleep via unilateral olfactory stimulation goes beyond global effects and elicits selective memory enhancement associated with regional cortical sleep oscillations.

RESULTS

We performed local TMR via unilateral olfactory stimulation in sleep to test how it may affect memory for cued items and corresponding regional sleep oscillations. To this end, we presented odors that served as contextual cues during pre-sleep object-location associative learning to a single nostril during sleep (Figures 1A–1F). We use the terms cued/uncued hemisphere throughout to refer to the hemisphere ipsilateral/contralateral to the stimulated nostril during sleep, respectively, regardless of the anatomical cueing side (left/right, counterbalanced across participants). The terms cued/uncued words denote word stimuli presented at the visual hemifield contralateral/ipsilateral to the cued hemisphere, respectively, reflecting our prediction that unilateral cueing will improve memory consolidation for items primarily processed in the hemisphere of the stimulated nostril, presented at the contralateral visual hemifield (Figure 1F). This terminology is used to describe the procedure but does not imply that processing of odors or individual words is exclusively confined to one hemisphere. In addition, we conducted a control experiment in which participants learned identical associations with no contextual odor during the memory encoding, followed by unilateral olfactory stimulation during sleep (Figure 1G). This control allowed us to determine which behavioral and electrophysiological effects originate from unilateral olfactory stimulation in sleep versus those caused by unilateral TMR.

Lateralized EEG Activity upon Learning Indicates Unihemispheric Memory Processes

First, we tested whether our task elicited lateralized brain activity during memory encoding. A pilot eye-tracking experiment, with a separate group of subjects, verified that participants successfully followed task instructions to visually fixate at the screen center, even when words moved to peripheral target locations (Figure S2). Next, we examined whether the electroencephalogram (EEG) during task learning (memory encoding in Figures 1A–1C) shows lateralized activity and its dynamics with the progression of learning. We collected EEG data in 30 participants (those participating in the control experiment) while they memorized 32 word-location associations that repeated in six learning rounds. We compared event-related potentials (ERPs) in central electrodes (C3 and C4) contralateral/ipsilateral to each word’s target location, separately for trials in the first three learning rounds (“early learning rounds”) and for trials in the last three learning rounds (“late learning rounds”). We found that contralateral and ipsilateral ERPs’ amplitudes were significantly different during both early and late learning rounds, indicating lateralized EEG activity during task learning (Figures 2A and 2B; early: t = 8.938, p < 10^-5; late: t = 8.064, p = 2 × 10^-5; via Monte-Carlo permutation test [MCPT]; STAR Methods). We then tested the three different trial periods to examine their contribution to this effect: word at center (0–800 ms); word moving to target location (target enlargement + word moving; 800–1,350 ms); and word at target location (1,350–1,750 ms). During early learning rounds, contralateral and ipsilateral ERPs were statistically different only during word moving (Figure 2A; t = 3.706; p = 0.006) and word at target periods (t = 11.261; p < 10^-5; MCPT), likely reflecting lateralized visual processing as the word moves and reaches its target location. However, during late learning rounds, the contralateral and ipsilateral ERPs were significantly different for all three trial periods (Figure 2B; t = 2.985, p = 0.018; t = 6.879, p = 2 × 10^-5; and t = 9.509, p < 10^-5 for word at center, word moving, and word at target, respectively). Importantly, laterality appeared earlier in late-learning trials and was significantly larger just before/during word movement (Figures 2C and 2D; t = 2.477, p = 0.007 and t = 2.321, p = 0.016 for the 600- to 800-ms window during word at center period and word moving, respectively; MCPT). Thus, in late-learning rounds, once some learning had occurred, the EEG showed earlier lateralized activity, already at periods when the word was still at the screen center. This
Figure 1. Experimental Design

(A) Schematic timeline illustrating the experimental procedure. (1) Participants first learned word-location associations in the presence of a rose odor delivered via mask to both nostrils. (2) Immediately after learning, memory retrieval was tested. Next, (3) participants took a short (~1-2 h) nap in the lab. During NREM sleep, odor was delivered unilaterally via a special mask to a single nostril. Finally, (4) a post-sleep test of memory retrieval was performed. (B) From left to right: participant during the learning task, with mask providing odor stimulation to both nostrils; participant during the memory retrieval test, without any mask or odor delivery; sleeping participant with a “separate nostril mask” providing odor stimulation to single nostril; participant during the memory retrieval test, without any mask or odor delivery.

(C) Learning trial timeline. Words appeared sequentially at the center of the screen and moved toward one of eight circular targets.

(D) Memory retrieval trial timeline. Words appeared sequentially at the center of the screen, and participants chose the target location associated with each word using the cursor. Green dashed line illustrates an example cursor trajectory.

(E) The nasal sleep mask enabling unilateral odor stimulation.

(F) Illustration of the terminology: odor cues delivered during sleep to the cued nostril (in this example, the right nostril; anatomical sides were counterbalanced across participants). The ipsilateral hemisphere is the cued hemisphere (olfactory pathways are mainly ipsilateral). Words associated during learning with target locations in the hemifield contralateral to the cued hemisphere (in this example, targets in the left hemifield) are cued words (visual pathways are mainly contralateral).

(G) Schematic timeline for the control experiment. Memory encoding occurred without any contextual olfactory stimulation, followed by unilateral odor cuing in sleep as in the main TMR experiment.

See also Figure S1.
earlier lateralization constituted an electrophysiological correlate of a learned association between that word and its corresponding spatial location in left or right visual field. Frontal and parietal-occipital EEG showed qualitatively similar results (Figure S3). Overall, we find robust lateralization in the EEG during the task that increases with learning progression, supporting our assumption that the task involves unihemispheric memory processes.

**Local TMR during Sleep Selectively Improves Hemisphere-Related Memories**

Memory for word-location associations was evaluated immediately (~5 min) after training (pre-sleep) and ~2.5 h later, following a nap (post-sleep). To test for selective TMR effects on memory for cued words, we conducted a two-way, repeated-measures ANOVA with conditions of time (pre-sleep/post-sleep) and cueing (cued/uncued). We found no main effect of time ($F(1,18) = 2.847; p = 0.109$) and no main effect of cueing ($F(1,18) = 0.049; p = 0.827$) but a significant interaction between time and cueing ($F(1,18) = 8.579; p = 0.009$). Post hoc comparisons revealed that this interaction reflects memory maintenance for cued words (two-tailed paired $t$ test; $t(18) = −0.956; p = 0.352$), whereas memory for uncued words significantly deteriorated after sleep (two-tailed paired $t$ test; $t(18) = 2.842; p = 0.011$; effect size: Cohen’s $d = 0.652$). Nonparametric statistics further confirmed that memory was stable for cued words (Wilcoxon sign-rank = 48.5; $Z = 1.024; p = 0.306$) but deteriorated for uncued words (Wilcoxon sign-rank = 114; $Z = −2.415; p = 0.016$; effect size: rank biserial correlation [RBC] $r = 0.484$). To further assess memory performance, we normalized post-sleep memory performance of each participant to her pre-sleep performance (set to 100%). The results (Figure 3A) revealed that, after sleep, memory for cued words (mean ± SD = 106% ± 17.5%; median = 100%) was higher ($t(18) = 3.05, p = 0.007$, effect size: Cohen’s $d = 0.699$; Wilcoxon sign-rank = 145, $Z = 2.593, p = 0.009$, effect size: RBC $r = 0.674$) than for uncued words (mean ± SD = 89.7% ± 17.0%; median = 91.7%), and this was evident in most (13/19) participants. No significant correlations were

Figure 2. EEG Lateralization during Task Learning

(A) Central EEG ERP (n = 30 participants) time locked to word presentation during the first three rounds of the learning task for ipsilateral electrodes (pink) and contralateral electrodes (green) to the target at each trial. Shading around ERP traces represents standard error. Yellow highlight marks areas with significant differences between ipsilateral and contralateral ERPs ($p < 0.05$; STAR Methods). Time zero marks the beginning of each trial: 0–800 ms, a word appears in the center of the screen; 800–1,350 ms, target enlargement and the word moves toward the target location; and 1,350–1,750 ms, the word appears at the target location. Significance levels between ipsilateral and contralateral ERPs of each time interval are marked in black arrows in bottom. (B) Same as (A) but for the last three rounds of the learning task. Note that only late-learning rounds are associated with early differences between ipsilateral and contralateral ERPs (<800 ms, before words start moving, without any difference in visual input). (C) Comparison of ERP laterality (root mean square [RMS] of the difference between the ipsilateral and contralateral ERPs) for early- versus late-learning rounds. Black bars denote jackknife estimates of the standard error (STAR Methods). (D) Mean difference (red bars) between ipsilateral and contralateral ERPs in individual subject data for word at center (600–800 ms) and target enlargement and word moving (800–1,350 ms) in early (light green circles) and late (dark green circles) rounds of the learning task. Note that the difference between ipsilateral and contralateral ERPs is higher for the late-learning rounds, reflecting lateralized memory processes.

See also Figures S2 and S3 and Data S1.
found between the time spent in each sleep stage or the number of odor cues received in sleep to memory performance.

By contrast, unilateral odor stimulation in the control experiment did not show significant behavioral effects. Due to one participant with extreme scores (marked in black in Figure 3 C) that shifted the distribution from normality, we report parametric tests without his data, as well as nonparametric tests that include this participant. A two-way, repeated-measures ANOVA did not reveal significant interaction between time and olfactory stimulation (F = 1.74; p = 0.205). Similarly, normalized post-sleep memory performance was not significantly different between “cued” (mean ± SD = 85.24% ± 19.345%; median = 83.33%) and “uncued” words (mean ± SD = 97.49% ± 21.081%; median = 100%; Figure 3 C; t test t(17) = −1.566, p = 0.136; we use the terms cued and uncued here for simplicity, although odors presented in sleep were not used as cues during prior learning). Nonparametric analysis also failed to reveal significant differences between memory for cued and uncued words (Figure 3 C; Wilcoxon sign-rank = 40; Z = −1.728; p = 0.084). To formally establish that the behavioral effect we observe is a result of the unilateral TMR manipulation, we conducted a mixed ANOVA with parameters of experiment (TMR/control) and visual field (cued/uncued), finding a significant interaction (F(1,36) = 9.257; p = 0.004). This interaction was also significant using a non-parametric test comparing the differences in scores between visual fields for each participant across experiments (Z = 3.008; ranked sum = 474; p = 0.003; via Wilcoxon ranked sum test).

To test whether olfactory stimulation during sleep influences additional cognitive aspects, we measured the perceived intensity of the odor after sleep via subjective ratings. In the main TMR experiment, perceived odor intensity positively correlated with the number of 30-s odor stimulation epochs in N2 sleep (parametric: r_p = 0.672, p = 0.001; non-parametric: r_s = 0.649, p = 0.002; Figure 3 B). This correlation was not observed in the control experiment (parametric: r_p = 0.083, p = 0.734; non-parametric: r_s = 0.033, p = 0.892; Figure 3 D). We also verified that memory performance was not significantly different between cueing in left versus right nostrils or between associations in left versus right visual hemifields (Data S1).

Together, the behavioral results indicate that, when an odor serves as a contextual stimulus during learning and is re-presented unilaterally in sleep (main TMR experiment), it covertly
affects the representation of the odor and improves memories associated with hemisphere-specific processing.

**Local TMR during Sleep Differentially Modulates EEG SW and Spindle Power across Hemispheres**

To test how unilateral odor cueing modulated cortical sleep oscillations, we first evaluated the EEG spectral power changes during 30-s odor stimulation (“odor-on”) epochs compared to baseline (“odor-off”) epochs (Figures 4A and 4E; STAR Methods). In central EEG electrodes, we found that odor stimulation significantly increased SW (0.5–4 Hz) power over both hemispheres and both experiments (TMR experiment, Figure 4B: cued hemisphere: Wilcoxon sign-rank = 144, Z = 1.972, p = 0.049, effect size: RBC r = 0.516; uncued hemisphere: Wilcoxon sign-rank = 159, Z = 2.576, p = 0.01, effect size: RBC r = 0.674; control experiment, Figure 4F: cued hemisphere: Wilcoxon sign-rank = 167, Z = 2.897, p = 0.004, effect size: RBC r = 0.758; uncued hemisphere: Wilcoxon sign-rank = 175, Z = 3.219, p = 0.001, effect size: RBC r = 0.842). Both slow oscillations (SOs) (0.5–1 Hz) and spindle (12–16 Hz) frequency ranges color bar legend on right in SD of baseline). Red contours mark significant differences between the hemispheres using cluster-based permutation testing (**p < 0.001).

**Control experiment**

To test how unilateral odor cueing modulated cortical sleep oscillations, we first evaluated the EEG spectral power changes during 30-s odor stimulation (average of n = 761 epochs and 19 participants). Thick black bar on top represents 30-s odor stimulation. Hot colors mark power increases in the SW (0.5–4 Hz) and spindle (12–16 Hz) frequency ranges (color bar legend on right in SD of baseline). Red contours mark significant differences between the hemispheres using cluster-based permutation testing (**p < 0.001).
and delta oscillations (1–4 Hz) showed increased power during odor cueing (Data S1).

In contrast to SW power that was elevated during olfactory stimulation in both experiments, spindle (12–16 Hz) power significantly increased in both hemispheres only in the TMR experiment (Figures 4B and 4F: cued hemisphere: Wilcoxon sign-rank = 31; Z = −2.575; p = 0.01; effect size: RBC r = 0.674; uncued hemisphere: Wilcoxon sign-rank = 157; Z = 2.495; p = 0.013; effect size: RBC r = 0.653). In the control experiment, unilateral olfactory stimulation did not significantly modulate spindle power (Data S1). Thus, SW power is affected by olfactory stimulation, whereas spindle power is elevated only when memory reactivation is involved.

Next, we tested for power differences between the cued and uncued hemispheres using two approaches: first, a direct comparison revealed a smaller increase in SW power in the cued hemisphere compared to the uncued hemisphere in the TMR experiment (Wilcoxon sign-rank = 31; Z = −2.575; p = 0.01; effect size: RBC r = −0.674). This effect also showed a trend for significance in the control experiment (Figure 4G; Wilcoxon sign-rank = 47; Z = −1.932; p = 0.053; effect size: RBC r = −0.505). Dividing the SW band into SO and delta bands revealed that higher power in the uncued hemisphere was driven by elevation in delta (1–4 Hz) power in both experiments (TMR experiment: Wilcoxon sign-rank = 34; Z = −2.455; p = 0.014; effect size: RBC r = −0.642; control experiment: Wilcoxon sign-rank = 29; Z = −2.656; p = 0.008; effect size: RBC r = −0.695). Baseline odor-off periods were not significantly different between hemispheres (p > 0.2; STAR Methods), indicating that the lower SW power in the cued hemisphere is not a result of baseline modulations. No significant difference between hemispheres was observed in either experiment for spindle power elevation (Data S1). Second, we compared odor-induced power changes between hemispheres using cluster-based permutation across time-frequency bins. In the TMR experiment, we found a significant cluster with higher SW (0.5–4 Hz) power in the uncued hemisphere 13–20 s into the odor-on period (red contour in Figure 4A; cluster statistics = −347.546; p = 0.003). No significant clusters were found in the control experiment (Figure 4E).

To formally assess interaction between experiment type (TMR/control) and hemisphere side (cued/uncued), we subtracted the SW power elevation of the cued side from the uncued side for each participant and compared these difference scores between experiments. We found significant interaction for spindle power elevation (Z = −2.248; ranked-sum = 293; p = 0.025; Wilcoxon rank-sum test; no interaction in SW was found with Wilcoxon rank-sum test) and significant cluster when testing for interaction in SW in the frontal electrodes (cluster statistics = −237.858; p = 0.015; via two-sample cluster permutation test; no significant cluster was found in the central electrodes or when testing the spindles band). These results support the hypothesis that differences between hemispheres in EEG power are due to unilateral TMR. Analysis of EEG power changes in frontal electrodes yielded similar albeit weaker results (Figure S4; Data S1).

In the TMR experiment, we further found a relation between SW power changes and memory, whereby SW power in the cued hemisphere was negatively correlated with memory improvement for cued words (rsw = −0.49; p = 0.033; Figure 4D). Thus, the lower was SW power in the cued hemisphere. The better was the associated memory. By contrast, odor-induced SW power in the uncued hemisphere did not correlate with memory improvement for uncued words (rsw = 0.107; p = 0.664). These two correlations are significantly different from each other (via one-tail Fisher r-to-z transformation z = −1.82; p = 0.034). Correlation between SW power and memory was stronger in the SO frequency band (memory for cued words and SO power in cued hemisphere: rsw = −0.513; p = 0.025; memory for uncued words and SO power in the uncued hemisphere: rsw = 0.165, p = 0.5; difference between correlations via one-tail Fisher r-to-z transformation: z = −2.074, p = 0.019) and did not involve the delta band. In the control experiment, we did not observe any correlation between power changes in sleep oscillations and memory performance (Figure 4H; Data S1). In addition, spindle power as well as the number/density of spindles and SO events was not correlated with memory improvement in both experiments.

Thus, in the TMR experiment, odor cues differentially modulated SW power in the cued versus uncued hemispheres, and smaller SW power increase in the cued hemisphere correlates with memory for select cued words processed in this hemisphere.

Local TMR during Sleep Differentially Modulates Phase Amplitude Coupling between SOs and Spindles across Hemispheres

We tested whether unilateral odor cueing affected the phase amplitude coupling (PAC) between sleep spindles and SO (0.5–1 Hz) up-states in frontal electrodes, implicated in memory consolidation [4, 43]. Analyzing the typical phase of SOs at which sleep spindles preferentially occurred during odor-on intervals (STAR Methods), we observed tight locking of spindles around SO up-states (Figure 5A; mean preferred-phase values around zero as reported previously [43], ranging between −67° and 15° in all experiments and electrodes; Figure 5A). Rayleigh test for non-uniformity confirmed tight SO-spindle coupling (TMR experiment, cued hemisphere: Z = 7.842, p = 1.772 × 10−5; uncued hemisphere: Z = 8.66, p = 5.667 × 10−5; control experiment: cued hemisphere: Z = 5.18, p = 0.004; uncued hemisphere: Z = 4.656, p = 0.008).

Next, to evaluate how unilateral odor cueing may affect PAC, we compared SO-spindle PAC between hemispheres in each experiment separately (Figures 5B and 5C). We employed a within-subject, within-session approach made possible by our experimental design, where PAC in the cued hemisphere was compared to simultaneous PAC in the uncued hemisphere. To facilitate a sensitive paired comparison and go beyond inter-subject variability, we normalized the preferred phase in each hemisphere by subtracting from each hemisphere’s “preferred phase” the “average preferred phase” across both hemispheres for each subject separately (STAR Methods). We tested for differences in normalized preferred phases between hemispheres, using both parametric and non-parametric circular data 2-sample tests. We found that, in the TMR experiment, there was a significant difference in the preferred phases (Watson-Williams test: F = 10.297, p = 0.003; common-median test: p = 12.7368, p = 3.585 × 10−5). Accordingly, for most participants (15/19), the preferred phase in the cued hemisphere occurred later than in the uncued hemisphere (Figure 5B). No significant difference in preferred phase between hemispheres was observed for odor-off periods.
In the control experiment, there was no significant difference in PAC between hemispheres. PAC effects did not exhibit correlation with power modulations, suggesting that these are independent phenomena. Together, we find that unilateral TMR led to a different PAC between SOs and spindles such that spindles tended to occur later over frontal cortex in the cued hemisphere.

Analysis of PAC in central electrodes revealed a similar profile that trended to significance (Figure S5; Data S1).

DISCUSSION

We used local TMR to enhance memories associated with processing in a single brain hemisphere. After confirming that our learning paradigm gives rise to lateralized EEG markers of unihemispheric memory processes, odor presentation to a single nostril during sleep was intended to re-establish the context of learning before sleep and led to differential effects of memory consolidation for select items. Local TMR improved memory performance for a subset of stimuli primarily processed in the cued hemisphere. In parallel, unilateral odor cues modulated regional sleep oscillations across the two hemispheres. Spindle activity and SW power increased in both hemispheres during odor stimulation, but SW power increase was lower in the cued hemisphere and negatively correlated with memory improvement for cued words. Temporal coupling between sleep spindles and SO up-states, implicated in sleep-dependent memory consolidation [1, 4], changed locally such that spindles in the cued hemisphere peaked later and closer to the SO peak. A control experiment tested whether results are due to the local TMR or merely reflect unilateral olfactory stimulation in sleep. When odors were not used as contextual cues during learning, no difference was observed in memory performance between items largely processed in the cued and uncued hemispheres. Odor stimulation in sleep elevated SW power even without TMR, but such power increase was not correlated with memory performance nor was it significantly different between hemispheres. In addition, spindle power was not affected by unilateral olfactory stimulation in sleep, indicating that spindle power effects are related to learning. Finally, SO-spindle PAC in the control experiment did not show consistent changes between cued and uncued hemispheres when conducting a paired (within-subject) analysis. Formal tests for interaction between experiment type (TMR/control) and hemisphere (cued/uncued) support the hypothesis that the observed effects stem from the unilateral TMR manipulation. Together, the results demonstrate that local TMR in human sleep goes beyond the effects of unilateral olfactory stimulation during sleep alone and transcends global effects by selectively promoting consolidation of specific memories associated with regional sleep oscillations.

Lateralized ERPs emerging upon learning in wakefulness support the notion of unihemispheric memory processes. In the last learning rounds, when some learning had already occurred, lateralized ERPs became different across hemispheres already early in the trial (while word stimuli were still presented at the center of the screen), suggesting that memory processes for word-location associations occur predominantly in a unihemispheric manner. Our results join accumulating evidence for lateralized brain activity during maintenance of unilaterally encoded stimuli in visual short-term memory tasks [44–51]. Unihemispheric biases are also observed during memory retrieval, where memory traces created by laterally presented stimuli are more easily accessible in the contralateral hemisphere [52].

Figure 5. Phase Amplitude Coupling during Unilateral Odor Stimulation in Sleep

(A) Locking of spindle amplitude peaks around SO up-state phases in frontal EEG electrodes across experiments (TMR experiment, dark circles; control experiment, light circles) and hemispheres (cued hemisphere, purple circles; uncued hemisphere, gray circles). Circles denote preferred phase for individual subjects. Green bars denote the histogram of preferred phases pooled across experiments and hemispheres. Red line marks the grand average phase (angle) and vector (radius).

(B) Mean normalized preferred phases in the cued (purple) and uncued (gray) hemisphere during the TMR experiment. Circles connected by lines denote individual participants. Full/dashed lines mark later/earlier preferred phase in the cued compared to the uncued hemisphere, respectively. Note that, for most participants (15/19), sleep spindle amplitude in the cued hemisphere peaks later than in the uncued hemisphere (**p < 0.01).

(C) Same as in (B) for the control experiment (learning without contextual odor and unilateral olfactory stimulation during sleep). There are no significant differences between the normalized preferred phases across hemispheres.

See also Figure S5 and Data S1.
experiment also showed that lateralized activity related to individual memories associated with left/right hand movements can be decoded from sleep EEG [53]. Thus, lateralized EEG activity upon learning indicates memory processes that are predominantly unihemispheric.

How do our results relate to previous work on TMR and local sleep oscillations? The memory effects observed here with local TMR are in line with TMR studies comparing contextual odor and vehicle cueing in sleep [10, 54]. In those studies, cued items show similar memory performance before and after sleep, whereas uncued items exhibit significant deterioration. Importantly, our local TMR demonstrates such differences during the same sleep interval, thereby indicating that TMR acts locally on select engram representations. A previous study by Cox et al. [29] tried to modulate memories in one hemisphere alone. Different odors were associated during learning with words presented in either left or right visual fields. The odors served as cues in separate sleep sessions performed in two subject groups that each was exposed (bilaterally) to a single odor in sleep. Although the study revealed differential activity in occipital sleep spindles across hemispheres, differential memory effects were not observed. Our local TMR enabled a within-session comparison that likely increased sensitivity and overcame variability across individuals and sleep sessions. Furthermore, using separated anatomical paths (rather than two different odors) may have contributed to revealing significant differences in memory for cued versus uncued items. Another approach to modulate consolidation of specific human memories in a local manner is to perform closed-loop stimulation during sleep that is time locked with regional sleep oscillations [55]. Such stimulation can increase spindle activity locally and enhance motor memory [56] or disrupt SW power locally and interfere with motor learning [57]. These studies compared brain activity across two different sessions, where considerable variability exists in sleep, brain activity, and memory processing. Our method goes beyond global sleep factors by selectively modulating local oscillations and improving memory of corresponding items. Decoding the content of reactivated memories in sleep from local components of EEG also supports the notion of local processes in memory reactivation [58]. Wang et al. [53] successfully trained a classifier on sleep EEG to discriminate between reactivation of memories related to left or right spatial locations, associated with corresponding hand movements. Another fMRI study showed odor-TMR-evoked representation of category-specific memories in category-sensitive cortical regions [59]. Our study is consistent with such local memory reactivation and extends it by using separate anatomical paths to elicit local processing: unilateral olfactory stimulation allows to experimentally control the areas receiving the cues and compare them with homolog areas processing parallel memories that did not receive the memory cues, thereby isolating and investigating the memory reactivation process in sleep in more detail.

In terms of SW power, the local TMR effects on cortical sleep oscillations extend previous studies showing that olfactory stimulation during sleep enhances SW power [23, 54]. We found that SW power increase was lower in the cued hemisphere and negatively correlates with memory for cued items (but not for uncued items). The processes mediating smaller elevation in SW power in the cued hemisphere remain unclear, but one possible explanation could be that unilateral odor stimulation elicits selective processing in the cued hemisphere associated with EEG desynchronization and lower SW power. In other words, activity in the cued hemisphere may represent a superimposition of global SW power increases and local SW power reduction. Accordingly, a lower SW power increase that correlates with better memory for cued stimuli may reflect a “penetration” of the odor stimulus that efficiently modulates ongoing activities in the cued hemisphere.

The mechanisms mediating the memory benefits of TMR are not fully established but may involve sensory-triggered hippocampal reactivation and hippocampal-neocortical dialog [1, 3], as recently reported in rodent studies [60]. Our results support the notion that temporal coupling between sleep spindles and SO up-states is a useful non-invasive proxy for effective sleep-dependent memory consolidation [1, 4, 43, 61–64]. We find that local TMR causally influences SO-spindle coupling: in the cued hemisphere, spindle amplitude peaks later and closer to the SO peak (up-state) than in the uncued hemisphere, highlighting the regional quality of sleep oscillations and their relation to memory consolidation [27, 29, 30, 33, 55, 65, 66]. Peaking of spindles before the SO peak is associated with impaired sleep-dependent memory consolidation [43], consistent with our findings that spindles in the uncued hemisphere peaked before the SO peak, while memories for uncued words did not benefit from sleep.

In terms of perception of odors and their intensities, post-sleep questionnaires verified there was no explicit awareness of odor cueing, as previously reported [10]. Nevertheless, although apparently not consciously perceived, the number of odor stimulation epochs during N2 sleep significantly correlated with subsequent subjective ratings of odor intensity in the TMR experiment. Although this novel surprising phenomenon is difficult to interpret, the fact that such correlation was not observed in the control experiment (identical stimulation during sleep) may hint it is not a purely physiological effect related to exposure of receptors to the odor. Rather, memory-relevant odor cueing during sleep may covertly affect stimulus representation beyond its effects on memory consolidation. An alternative explanation could be that olfactory stimulation during sleep TMR is the second exposure to the odor (rather than the first exposure in the control experiment). Indeed, repeated exposure to odor lowers the detection threshold for some odorants [67, 68], and similar changes in perceptual threshold could underlie the effect we observe here.

In summary, we report here a novel non-invasive technique for local TMR of specific regional memories, opening new avenues for sleep and memory research as well as potential clinical applications. For example, unilateral sleep TMR could be used to modulate different components of traumatic memories in post-traumatic stress disorder (PTSD) that are lateralized [69] or assist rehabilitation in individuals with lateralized brain damage, such as unilateral stroke.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.01.091.

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AUTHOR CONTRIBUTIONS

E.B. conceived and designed experiments with supervision from Y.D. and N.S.; E.B., E.L., and N.S. designed and built the experimental setup; E.B. and E.H. collected data; E.B. analyzed data with the help of A.A., A.M., and O.P. and supervision from Y.N.; E.B. and Y.N. wrote the manuscript; R.P. supervised the control experiment; and all authors provided ongoing critical review of results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


STAR METHODS

KEY RESOURCES TABLE

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LEAD CONTACT AND MATERIALS AVAILABILITY

This study did not generate new unique reagents. All EEG, behavioral data, and software codes generated in this study are available from the Lead Contact (Yuval Nir, yuvalnir.tau@gmail.com) without restriction.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Participants
Thirty-two healthy participants took part in the main TMR experiment (18 women, mean age 27.87 ± 3.8 years, range 21-46), and an additional thirty-two healthy participants cohort participated in the control experiment (17 women, mean age 27.28 ± 4.71 years, range 21-41). All participants in both experiments reported no history of psychiatric, neurological, sleep or respiratory disorders. All were good habitual sleepers and native Hebrew speakers. The participants provided written informed consent to the procedures. The main TMR experiment was approved by the committee for protection of human participants at the Sleep Disorders Laboratory, Loewenstein Rehabilitation Hospital, Raanana. The control experiment was approved by the IRB (institutional review board) of the Weizmann Institute of Science, Rehovot. Participants received monetary compensation for their participation. On the day of nap experiments, participants were instructed to wake up no later than 8.30 AM and to eat their lunch before the experimental session began in the early afternoon. They were further instructed to limit their caffeinated beverages to one, and to avoid ingesting alcohol during the experimental day. Exclusion criteria were (i) insufficient (< 20 min) time in NREM sleep (10 participants were excluded from main TMR experiment; 11 participants were excluded from control experiment), or (ii) technical problems either in the odor delivery system (2 participants excluded from main TMR experiment; 1 participant excluded from control experiment) or with behavioral memory data (one participant excluded in main TMR experiment due to technical failure, and one participant excluded in control experiment as he fell asleep during the memory task). Subsequent analysis was performed on the remaining participants (Main TMR experiment: n = 19, 10 women, mean ± SD age = 27.4 ± 2.9 years, range 21-30; Control experiment: n = 19, 10 women, mean ± SD age = 27.6 ± 4.3 years, range 21-38).

METHOD DETAILS

Experimental procedures

Main TMR experiment
An overview of the experimental procedure for the targeted memory reactivation (TMR) experiment is presented in Figures 1A and 1B. Participants arrived at the lab in the early afternoon (between 12:00 and 14:00). The experimental room was coated with stainless steel to prevent ambient odor adhesion. In addition, a high-efficiency air filter further assured an odor-free environment. Next, the participants performed the training session of the spatial memory task while bilateral odor stimulation was delivered through a nasal mask covering both nostrils (Figure 1B). Training was followed (~5 min later) by a memory retrieval test without an odor mask (Figure 1B). Next, after fitting of the polysomnographic electrodes and a separate nostril mask, participants were left in bed undisturbed to sleep for two hours (Figures 1B and 1E). Throughout the nap, participants were monitored online from the adjacent control room through a one-sided window, and polysomnographic measures were inspected online at all times (Figure S1A). During NREM sleep, a rose odor was presented to one nostril (left versus right nostril counterbalanced across participants, n = 10 versus 9, respectively). Upon awakening and following a short recovery (~15 min), participants performed the post-sleep memory retrieval test. This test was followed by intensity rating of the same odor used in the task and sleep. Total duration of the experimental session was approximately 4.5 h.
Control experiment

An additional control group underwent a similar procedure to the main experiment, but without contextual olfactory stimulation during the learning task (Figure 1G). Unilateral olfactory stimulation was presented in 30 s odor cues during NREM sleep as in the TMR experiment. In addition, EEG was recorded during the initial learning task using 6 scalp derivations: F3, F4, C3, C4, PO3, and PO4 according to the 10-20 system. Two EOG electrodes, one below and one to the side of the eye recorded blinks and movements to facilitate removal of artifacts. Unilateral olfactory cueing in sleep was counterbalanced across participants (left nostril: n = 9, right nostril: n = 10). For analyzing ERP laterality during learning in wakefulness we used all 30 participants (16 women, mean age 26.96 ± 4.064 years range 20–38), as the minimal NREM sleep criterion was not relevant here.

Memory task

The memory task in both experiments, as well as the retrieval test and the distraction task were implemented in MATLAB using Psychophysics toolbox extensions [70–72]. The memory task was adapted from Cox et al. [29] with minor changes.

Learning phase

Each trial started with a fixation period with a white cross centered on a black screen, and eight targets appearing as small gray octagons with green borders arranged in circle around it (for either 200 ms, 400 ms or 600 ms, pseudorandom). Next, a word appeared in white at the center of the screen (800 ms) followed by enlargement of one of the targets to medium size (400 ms). Then, the word moved toward that target as it gradually decrease in size and the target gradually increase to large size (150 ms). Finally, the word appeared clearly inside the large target octagon (400 ms) before the next trial started. The participants were instructed to memorize the associations between the words and the target locations (Figure 1C). Each target was associated to the same number of words. Word stimuli consisted of common Hebrew concrete nouns (3–5 letters) obtained from stimuli in [76] and their frequency was controlled for in The Word-Frequency Database For Printed Hebrew [77]. In the TMR experiment thirteen participants memorized the word-location associations of 32 words, and six participants memorized 48 associations. All results were derived after collapsing these two groups together, reporting the percent of remembered associations out of all learned associations. All participants in the control experiment memorized 32 word-location associations. The targets were arranged in a circle on the screen, with four targets located in the right visual field and four targets in the left visual field, to elicit processing in the left and right hemispheres, respectively (Figure 1C). Participants were instructed to fixate on a white fixation cross in the center of the screen at all times. The circular targets arrangement was used to render an implicit left/right division of the targets that was not conveyed to the participants. Words appeared in two blocks of 16 words each (24 for participants that learned 48 associations), and repeated in six learning rounds. The word order, the word-target associations, and the word-block assignments were randomized across participants, with one restriction: the same target location could not appear twice consecutively, to limit the formation of cognitive links between words associated with the same location. Each block was followed by a non-hippocampal “distraction” task (counting back out loud in steps of three starting from a three digit number appearing on the screen) as in [29], to minimize active memorizing and preserve the memory to be as visual as possible. Each learning round was followed by a one-minute brake. The number of learning rounds was determined following a pilot experiment (n = 28 participants, below) to form a strong, yet imperfect memory of the words, aiming for success rate around 60% before sleep (chance level is 12.5%). The pilot experiment used eye tracking (recorded at 500Hz, Eyelink-1000, SR Research) to verify the ability of participants to focus their eyes on the fixation cross, and not let their gaze follow the moving word. (Figure S2). In the main TMR experiment only, a rose scent was delivered to both nostrils via a nasal mask throughout the learning blocks (not during the distraction task blocks and breaks) (Figure 1B). After each learning block, participants reported whether they could smell the odor during the task or not, by pressing on one out of two keyboard buttons (on average participants reported they smelled the odor in 93.3% ± 0.12 (mean ± SD) of the 12 blocks).

Memory test

In both experiments, memory was first tested immediately following the learning session (pre-sleep memory test), and again later within ~15 min of the end of the nap (post-sleep memory test). Each test trial (Figure 1D) started with presentation of all eight targets together with a white central fixation cross (for either 200ms 400ms, or 600ms, pseudorandom). Then, a word was presented in the middle of the screen (2500ms) Next, a white point cursor replaced the word and the participants could move it using the computer touchpad toward the target they thought was associated with this word in the encoding phase. When the cursor was close to a target, the octagon was enlarged to medium size, and after target selection with the touchpad button press, the octagon grew further to large size and remained that way for 2500ms until the next trial started. The participants were allowed 10 s to select a target and instructed to guess the correct target if they could not remember. After 10 s, if none of the targets were selected, the next trial began (Figure 1D). No feedback was provided regarding accuracy. Words were shown sequentially in a random order. Odors were not delivered during the post-sleep memory test (Figures 1A and 1B).

Olfactory stimulation

A computer-controlled air-dilution olfactometer [78, 79] was located in an adjacent room, with tubes entering the experimental room through waveguides to prevent potential non-olfactory signals from the olfactometer body. These tubes ended in a small nasal mask (different masks for wake and sleep, as described below). The olfactometer delivered a constant flow of clean air, at a rate of 5 liter per minute (lpm). The airflow to the nose was constantly vacuumed away at the same rate, preventing accumulation or lingering of odor. Odorant pulses were embedded in the flow at certain times of the experiment. High temporal resolution of the stimulus was achieved using a railroad manifold that enabled switching between odor and clean air close to the nasal mask as described in [78]. The odorant...
(Phenyl-ethyl alcohol, CAS Number 60-12-8 Sigma-Aldrich, generally perceived as “rose”) was chosen because it has been shown to minimally activate trigeminal pathways [80]. This odorant has been used successfully as a stimulus in a number of sleep experiments [10, 81]. Task mask (used only in the TMR experiment): The mask (Respironics, contour deluxe SU) covered both nostrils (Figure 1B, left). Odor and clean air stimulation enter the mask from the olfactometer via a Teflon tube. A vacuum tube line evacuated the air, and two small probes were attached to spirometer for measuring respiration. Nasal sleep mask (used both in both the TMR and control experiments): The sleep mask was designed and built in-house especially for this experiment. Its uniqueness is in its complete separation of the olfactory environment between nostrils. The nasal part comprised of a silicone nasal pillow (ResMed, Swift FX Nasal Pillow CPAP Mask). Two stainless steel tubes embedded in Teflon adapters were inserted to holes drilled in the nasal pillow to separate the airflows to each nostril. These inlets are connected to wide plastic pipelines from a BIODEX Disposable Xenon-133 Rebreathing Systems. These pipelines open to room air on the other side and odor air and vacuum tubes within them control the composition of air inside. The participant breathes passively the air in the void within these pipelines. Air tube in one pipeline delivered constant flow of clean air while the air tube on the other pipeline delivered clean air/odor stimulation according to the operator’s instructions. Vacuum lines evacuated the air in the pipelines. Two spirometer probes, one in each pipeline, measures respiration separately from each nostril. Tubes coming out of the mask were suspended from a rail close to the ceiling to prevent discomfort in the sleeping participant due to weight of gear (Figure S3C).

Odor cueing in sleep

Odor cueing in sleep was performed both in the TMR and in the control experiment. Through all sleep stages, the experimenter monitored the polysomnographic measures to identify the sleep stages and deliver odor stimulation accordingly. Odor stimulation started after at least 10 min of sleep and for as long as the participant remained in NREM sleep. The stimulation was stopped upon any polysomnographic signs of arousal, awakening, or REM sleep. Stimulation followed an alternating 30 s-on/30 s-off pattern (referred to as ‘odor-on’ and ‘odor-off’ periods throughout) to reduce habituation. Alternating between these phases was done without any visual, auditory, temperature or other non-olfactory hints that could be perceived by participants.

Polysomnography and Sleep Scoring

Physiological measurements were acquired during sleep with a PowerLab Monitoring System (ML880 ADInstruments, Bella Vista, NSW, Australia) at a sampling rate of 1 KHz with a 50Hz notch filter. Electroencephalogram (EEG) was obtained with four circular electrodes located at positions C3, C4, F3 and F4 according to the 10-20 system, referenced to electrodes placed on the contralateral mastoids A2 and A1. Electrooculogram (EOG) was obtained with two circular Ag/AgCl conductive adhesive electrodes, placed 1 cm above or below and laterally of each eye and referenced to contralateral mastoid electrodes. Electromyogram (EMG) was obtained with two circular Ag/AgCl conductive adhesive electrodes, located bilaterally adjacent to the submentalis muscles. EEG, EOG and EMG signals were pre-amplified (Octal Bio Amp ML138, ADInstruments). Electrocardiogram was obtained with two circular Ag/AgCl conductive adhesive electrodes, placed on left and right sides of the abdomen and referenced to a ground electrode placed on the left foot. Nasal respiration was measured separately from each nostril using two spirometers (FE141, ADInstruments). A representative example of polysomnography data is presented in Figure S1D. Sleep stages were visually scored offline by two independent scorers according to the 2012 American Academy of Sleep Medicine (AASM) Manual [82]. Sleep parameters, including sleep latency, total sleep time and time spent in different sleep stages, were calculated for both experimental and control group (Table S1).

EEG Data Analysis

For all EEG analyses, custom MATLAB scripts were combined with functions from EEGLAB toolbox [73] and MATLAB’s signal processing toolbox. To remove noise for power analysis, the EEG signal was first filtered with a FIR high-pass filter at 0.5Hz using a Hamming window and transition band of 0.5Hz. The signal was then filtered with FIR low-pass filter at 30Hz to remove high-frequency noise with a Hamming window and transition band of 2Hz. For PAC analysis we avoided filtering, to minimize time distortion in the signal. After filtering, the signal was down-sampled to 250Hz and divided to epochs of REM sleep and NREM sleep. Noisy channels and epochs were removed from analysis (TMR experiment: two channels from two individuals and 43.9%/28.2%/3.9% epochs from three other individuals; Control experiment: 3.9% epochs from one individual). All the ensuing EEG analyses (main text and Data S1) were conducted on NREM epochs after these pre-processing steps.

We normalized the power in each ‘odor-on’ 30 s period to the preceding ‘odor-off’ 30 s period (as explained in the following paragraph), so that odor-free epochs were used as baseline to calculate power changes between the hemispheres. To ensure that the differences we found between hemispheres are not a result of baseline differences, we first compared the last 25 s of ‘odor-off’ periods power between the hemispheres using Wilcoxon signed rank tests in the following frequency bands: SW (0.5-4Hz), SO (0.5-1Hz), delta(1-4 Hz) and spindles (12-16Hz), both in frontal and central electrodes. None of these comparisons showed a significance difference between hemispheres (all p > 0.2).

Dynamic spectral analysis using event-related spectral perturbation (ERSP, Figures 4A, 4E, S4A, and S4E) was implemented as follows: For each channel separately, we extracted 55 s epochs around the odor cues (25 s ‘odor-off’ baseline period followed by 30 s ‘odor-on’ period, avoiding the few seconds following odor termination to allow activity to return to baseline levels). The signal
wa was divided to 0.5 Hz frequency bins (59 bins 0.5-30Hz) using FIR filter and Hamming window. For each frequency bin separately, we performed a Hilbert transformation and then normalized to the preceding 25 s baseline:

\[ P_{k}(f, t) = \frac{|H_{k}(f, t)|^2 - \mu_{0}(f, k)}{\sigma_{0}(f, k)} \]

Where \( H_{k}(f, t) \) is the Hilbert transformation for each time point \( t \) in frequency bin \( f \), and odor cue \( k \). \( \mu_{0}(f, k) \) is the mean baseline ("odor-off" period) spectral estimates for odor cue \( k \) and frequency bin \( f \), and \( \sigma_{0}(f, k) \) is the spectral estimate SD for the baseline period of odor cue \( k \) at frequency bin \( f \) [83]. Then, for each participant separately, we took the median normalized power across all odor cues. For visualization, averaged ERSP across all participants and trials are shown in Figures 4A, 4E, S4A, and S4E. To further quantify the modulation in power as a result of odor cues, and to compare this modulation between the hemispheres, we evaluated the power in the frequency bands of interest: SW range (0.5-4Hz), SO range (0.5-1Hz), delta (1-4Hz), and spindle range (12-16Hz). In addition, we used non-parametric cluster-based permutation testing using FieldTrip toolbox [74] across time-frequency bins (see statistical analysis section [84]).

**Detection of slow oscillations (SO) events**

Individual SO events were detected automatically (within the artifact-free NREM signal) using custom script based on their spectral content and duration, using established detection algorithm [33, 43, 85]. In each channel separately, the EEG signal was band-pass filtered offline to the SO frequency range (0.5-1 Hz) using two-way least-squares FIR filtering. Individual half-waves were detected as negative deflections between two zero crossings. Only waves which consecutive zero crossings separated by 0.25 to 1.0 s were considered for the next stage. Then we extracted positive and negative peak amplitudes. Finally, we marked the 15% highest amplitude (negative to positive peak) events as SO events. We defined SO events as ± 2.5 s from the negative peak.

**Detection of sleep spindle events**

For detection of sleep spindles and PAC analysis in relation to SO, we used unfiltered raw data to avoid potential phase distortion that could affect PAC analysis. Other preprocessing steps were identical. Discrete spindles were detected automatically in artifact-free NREM data using a MATLAB custom script based on methods described previously [29, 33, 54], with minor adaptations. First, the NREM EEG signal was bandpass-filtered in the spindle range (12-16Hz), using a zero-phase two way least-squares FIR filter with transition band width of 2Hz. The signal envelope was calculated with a Hilbert transform. The amplitude was further smoothed using a Gaussian kernel (\( \sigma = 40 \)ms) to avoid multiple crossings of thresholds within the same spindle event. Events were detected when the envelope amplitude crossed the detection threshold of mean + 3 SD, computed for each electrode and each participant separately, across all the NREM sleep signal. Multiple events detected within 1 s were merged. The event start/end times were determined when the signal crossed the threshold mean + 1 SD of spindle power across NREM sleep. Events with duration between 0.5 s and 2 s were considered for further analysis. To further reduce the probability of detecting spurious spindles, spectral power of the detected events was calculated using a Fast Fourier Transform (FFT) on the noise-free raw signal. Only events with peak spectral-power within the spindle-frequency range (12-16Hz) compared to adjacent frequency bands (7-9Hz and 18-20Hz) were considered, to avoid events with broadband power increases.

**SO-spindle phase amplitude coupling**

For detecting the preferred SO phase at which sleep spindles occurred, we used the subset of SO events detected (above) that occurred during unilateral odor cueing (‘odor-on’ periods). PAC calculation was implemented according to [43]. We filtered (two-way least-squares FIR filtering) the trough-locked data first to the SO component (0.5-1Hz), Hilbert transformed and extracted the instantaneous phase. Then we filtered (two-way least-squares FIR filtering) the same trough-locked data to the spindle band (12-16Hz), applied Hilbert transform and extracted the instantaneous amplitude. We considered the time range from -2 to 2 s to avoid filter edge artifacts. For every subject, channel, and event, we now detected the maximal spindle amplitude and corresponding SO phase angle. For each channel of each subject we calculated the mean circular direction using the CircStat toolbox [75] to determine the ‘preferred-phase’.

**QUANTITATIVE AND STATISTICAL ANALYSIS**

Statistical analyses were performed within-subjects (\( n = 19 \) subjects both in TMR and control experiments) using custom MATLAB scripts. For behavioral data and for PAC, both parametric and nonparametric statistics were used. Extreme scores of one participant in the control experiment shifted the distribution away from normality, hence we report the parametric analysis of the control experiment without this participant while non-parametric analysis includes him. Change in memory performance over sleep was assessed with a two-way repeated-measures ANOVA using time (pre-sleep/post-sleep) and cueing (cued/uncued) as factors, followed by post hoc comparisons using two-tailed paired t tests, as well as Wilcoxon sign-rank tests. Next, individual post-sleep memory performance was normalized to pre-sleep performance (pre-sleep performance was set to 100%), and change in memory for words was compared between sides with a paired t test as well as Wilcoxon sign-rank test. Effect sizes were calculated using Cohen’s d and non-parametric rank-biserial correlation (RBC) [86]. We used both Pearson and Spearman correlations to assess the
correlation between the number of odor stimulation epochs and perceived odor intensity. To verify no change in performance between cueing sides, we performed two-tailed, two samples t tests and Wilcoxon ranked-sum tests (Data S1). Lack of difference in performance between words appearing in left versus right visual fields (Data S1) was confirmed via two-tailed paired t tests as well as Wilcoxon sign-rank tests. Interaction between experiment type (TMR/control) and visual field of the word stimulations (cued/uncued) was tested both parametrically (via two-way, mixed design ANOVA) and non-parametrically (by calculating the individual differences between cued and uncued scores, and compare them between the experiments using Wilcoxon ranked-sum test).

For the ERP analysis, following rejection of noisy trials, we used a custom-made MATLAB script employing a Monte-Carlo permutation test. We quantified ERP laterality (Figure 2) as the RMS-difference between the contralateral and ipsilateral ERPs in four trial intervals: Entire-trial (0-1750 ms), Word-at-center (0-800ms), Word-moving-to-target-location (target-enlargement+word-moving, 800-1350 ms) and Word-at-target-location (1350-1750 ms). To test for statistical significance (Figures 2A and 2B) we compared the real data to 1,000,000 surrogate samples where we calculated the RMS-difference after randomly shuffling the contralateral & ipsilateral labels within-subjects. P value was calculated as the proportion of surrogate samples with larger values. To test if ERP laterality in the last three rounds of the learning task was larger than in the first three learning rounds (Figure 2C) we obtained the ‘ERP laterality’ for the two task halves separately, and calculated their difference. We then compared it to 1,000,000 surrogate samples where we obtained the ERP laterality difference after randomly shuffling the 1st and 2nd halves labels within-subjects. P value was calculated as the proportion of surrogate samples with larger values, multiplied by two (for a two-tailed test). We calculated the t-statistic by subtracting the mean of the surrogate samples from the real data value, then dividing by the standard deviation of the surrogate samples.

For EEG power spectrum analysis, non-parametric statistical tests were used. To test the effect of odor cueing on EEG power we performed one-sample Wilcoxon sign-rank tests on normalized EEG activity during odor cueing. As EEG power was normalized to the ‘odor-off’ baseline in each cueing epoch separately, values different than zero indicate significant odor-induced modulation. Next, we compared the induced EEG power between hemispheres (cued and uncued) separately for SW (0.5-4Hz), SO (0.5-1Hz), delta (1-4Hz) and spindle (12-16Hz) frequency bands, using Wilcoxon tests. Effect sizes were calculated using RBC [86]. We used Spearman correlations to assess the correlation between SW power and memory improvement, difference between correlations was done using one-tailed Fisher r-to-Z transformation. To further explore oscillatory power differences between the hemispheres, a non-parametric permutation test across time-frequency bins using cluster correction, was applied. This approach controls for multiple comparisons by clustering neighboring frequency and time bins that show the same effect [84] EEG statistics were performed on all 30 s ‘odor-on’ normalized periods in the SW frequency band (0.5-4Hz) in bins of 200ms and frequency resolution of 0.5Hz. We used Monte-Carlo permutation method with 1000 randomization iterations for all tests. The permutation p value was obtained by comparing the cluster statistic to the random permutation distribution. Clusters were considered significant at p < 0.05 (two-sided). Interaction between experiment type (TMR/control) and visual field of the word (cued/uncued) was tested for both SW and spindle frequency bands by calculating the individual differences between cued and uncued power elevation during the odor cues, and comparing them via (i) Wilcoxon ranked-sum test and (ii) a non-parametric 2-samples permutation test, using 1000 Monte-Carlo permutations and cluster correction.

In SO-spindle coupling analyses, circular statistics were calculated using the CircStat toolbox [75]. We first validated that the distribution of ‘preferred-phases’ across participants in all channels is not significantly different from von Mises distribution (circular normal distribution equivalent), using Kuper two-samples test [75] for comparing distributions (circular analog of the Kolmogorov-Smirnov test). Then, we tested against uniformity with Rayleigh [75] tests. To statistically compare the odor-induced ‘preferred-phases’ in a pairwise manner across cued and uncued hemisphere data and to minimize inter-subject variability, we normalized the ‘preferred-phase’ in each participant separately by subtracting the average phase of both hemispheres from both the cued and from the uncued hemispheres ‘preferred-phases’. We then tested for equal circular means using parametric Watson-Williams multi-samples test for equal means [75] (circular ANOVA equivalent), and a non-parametric common-median test. To assess interaction between experiment type (TMR/control) and hemisphere (cued/uncued) on PAC we compared the differences between preferred-phases in each hemisphere between experiments using both parametric Watson-Williams and non-parametric common-median tests.

**DATA AND CODE AVAILABILITY**

EEG, behavioral data and codes are available upon request by contacting the Lead Contact, Yuval Nir (yuvalnir.tau@gmail.com).
Supplemental Information

Local Targeted Memory Reactivation in Human Sleep

Ella Bar, Amit Marmelshtein, Anat Arzi, Ofer Perl, Ethan Livne, Eyal Hizmi, Rony Paz, Noam Sobel, Yadin Dudai, and Yuval Nir
Figure S1. Nasal sleep mask and polysomnography. Related to Figure 1. (A-C) Nasal sleep mask. (A) The nasal part: I. Nasal pillow from Swift™ FX Nasal Pillow CPAP Mask. II. Stainless still and Teflon inlets. III. Plastic pipeline from Disposable Xenon Rebreathing Systems Biodex. (B) The tubes construction: IV. The second end of the pipelines is open to room air. V. Clean air/odor line. VI. Vacuum line. VII. Spirometer probe (green and pink Tygon cannulas). (C) Tubes coming out of the mask were hanged from a ceiling rail to prevent discomfort on the sleeping participant due to weight of tubes. (D) Example of 30s polysomnography recording from single participant during NREM sleep. Rows (top to bottom) show odor stimulus (blue; up phase - on, down phase – off), Electrocardiography (ECG, green), right frontal F4 Electroencephalogram (EEG, dark blue), right central C4 EEG (cyan), left frontal F3 EEG (purple), left central C3 EEG (green), Electromyography (EMG, orange), right EOG (light blue), left Electrooculography (EOG, light green), respiration in the stimulated nostril (pink), and respiration in the unstimulated nostril (purple).
Figure S2. Eye tracking during the learning phase of the memory task. Related to Figure 2. Eye movement heat map histograms of individual participants (n=28). Colors (colorbar legend on right) represent the number of fixations the participant made at every bin (250x250 screen pixels). Participants 6, 8, 12, 19, 20 showing two noncontiguous hotspots represent a camera position change in the middle of the experiment due to a change in the position of the eye after the break between the learning rounds.
Figure S3. EEG lateralization during task learning in frontal and parieto-occipital electrodes. Related to Figure 2. (A-D) Frontal electrode EEG. (A) ERP (n=30 participants) time-locked to word presentation during the first three rounds of the learning task for ipsilateral electrodes (pink) and contralateral electrodes (green) to the target at each trial. Shading around ERP traces represents standard error. Yellow highlight marks areas with significant differences (p<0.05, Wilcoxon sign-rank test) between ipsilateral and contralateral ERPs. Time zero marks the beginning of each trial; 0-800ms, a word appears in the center of the screen; 800-1350ms, target enlargement and the word moves toward the target location; 1350-1750ms, the word appears inside the target location. Significance levels between ipsilateral and contralateral ERPs of each time interval are marked in black arrows in bottom. (B) Same as A, but for the last three rounds of the learning task. Note that the early differences between ipsilateral and contralateral ERPs (< 800ms, before words start moving and there is any difference in visual input) are present for late learning rounds but not for early learning rounds. (C) Comparison of ERP laterality (RMS of the difference between the ipsilateral and contralateral ERPs) for early vs. late learning rounds. Black bars denote jackknife estimates of the standard error. (D) Mean difference between ipsilateral and contralateral ERPs in individual subject data for different trial periods (the late phase of word in the center of the screen: 600-800ms, target-enlargement and Word-moving toward the target: 800-1350ms) for early (light green circles) and late (dark green circles) rounds of the learning task. Red bars represent the mean. Note that the difference between the ipsilateral and contralateral ERPs is higher in the late rounds reflecting lateralized memory processes. (E-H) Same as A-D for parieto-occipital electrodes. See also Data S1.
Targeted memory reactivation (TMR) experiment

(A) Time-frequency decomposition of power changes in frontal EEG of the cued (left panel) and uncued (right panel) hemispheres, induced by odor cues during sleep (average of n=644 epochs, and 19 participants). Thick black bar on top represents 30s odor stimulation. Hot colors mark power increases in the SW (0.5-4Hz) and spindle (12-16Hz) frequency ranges (colorbar legend on right, in SD of baseline). Red contours mark significant differences between the hemispheres using cluster-based permutation testing (** p<0.01).

(B) Power changes in frontal EEG for SW (left bar) and spindle (right bar) activities, averaged across both hemispheres. White circles mark data of individual participants. ~ p<0.1, ** p <0.01 compared to baseline.

(C) Average change in SW power

Control experiment

Figure S4. EEG power changes upon unilateral odor stimulation during sleep in frontal electrodes. Related to Figure 4. (A-D) – Results of main TMR experiment. (A) Time-frequency decomposition of power changes in frontal EEG of the cued (left panel) and uncued (right panel) hemispheres, induced by odor cues during sleep (average of n=644 epochs, and 19 participants). Thick black bar on top represents 30s odor stimulation. Hot colors mark power increases in the SW (0.5-4Hz) and spindle (12-16Hz) frequency ranges (colorbar legend on right, in SD of baseline). Red contours mark significant differences between the hemispheres using cluster-based permutation testing (** p<0.01). (B) Power changes in frontal EEG for SW (left bar) and spindle (right bar) activities, averaged across both hemispheres. White circles mark data of individual participants. ~ p<0.1, ** p <0.01 compared to baseline. (C) Average change in SW power
in the cued hemisphere (purple, left) vs. uncued hemisphere (gray, right). Circles connected by lines mark individual subject data. Solid/dashed lines mark lower/higher power in the cued hemisphere, respectively. ~ p <0.1. (D) Scatter plot with regression line of post-sleep memory for cued words (as % of pre-sleep baseline, y-axis) vs. SW power in the cued hemisphere during odor cueing (as SD of baseline, x-axis) reveals significant negative correlation (* p<0.05). (E-H) same analyses as in A-D for the control experiment (n=679 epochs, and 19 participants). Note that in the control experiment olfactory stimulation does not elevate spindle power nor is SW power increase correlated with memory performance (n.s p>0.1). See also Data S1.

Figure S5. Phase amplitude coupling during unilateral odor stimulation in sleep, in central electrodes. Related to Figure 5. (A) Locking of spindles peaks around SO up phases in central electrodes of both TMR (dark circles) and control (light circles) experiments, and cued (purple circles) and uncued (gray circles) hemispheres. Large circles denote the SO phase, small circles denote preferred-phase for individual subjects. Green bars are the angle histogram of preferred-phases from central electrodes from both experiments and hemispheres together. Red line marks the grand average phase (angle) and vector (radius). (B) Mean normalized preferred-phases in the cued (purple) and uncued (gray) hemisphere. Circles connected by lines denotes individual participants. Full/dashed lines marks later/earlier preferred-phase in the cued compared to the uncued hemisphere, respectively. Note that for most participants (15/19) sleep spindle amplitude in the cued hemisphere peaks prior to that in the uncued hemisphere, (~ p<0.1). (C) – Same as in B for the control experiment (learning without contextual odor, and unilateral olfactory stimulation during sleep). There is no difference between the mean normalized preferred-phases (n.s. p>0.1). See also Data S1.
<table>
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<th>TMR Experiment (Mean ± SD)</th>
<th>Control Experiment (Mean ± SD)</th>
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Table S1. Sleep architecture parameters. Related to STAR Methods. Sleep parameters (mean ± SD), and the average number of odor cues for all participants included in the analysis, for both the TMR (N=19) and control (N=19) experiments. No statistical differences were observed between the two experiments for any of the above parameters.